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**1st INTERNATIONAL CONFERENCE OF THE WÜRZBURG INITIATIVE 3R
(WI3R) – JUNE 5-7, 2024**

PROGRAM & ABSTRACT BOOK

VENUE:

Rudolf Virchow Center (RVZ)

University of Würzburg, Josef-Schneider-Str. 2, House D15, Würzburg, Germany

[WI3R Symposium 2024 \(fourwaves.com\)](https://www.fourwaves.com)

This symposium is kindly supported by:

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<https://www.dfg.de/en>



DFG - Research training group

[GRK 2157 - Graduiertenkolleg 2157 \(uni-wuerzburg.de\)](https://www.uni-wuerzburg.de/grk2157)



DFG - Collaborative Research Centre

[SFB / TRR 225 Biofabrication Würzburg Erlangen Bayreuth](https://www.sfb225-biofab.de)



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Editorial Deadline: 30.05.2024

GENERAL INFORMATION

REGISTRATION

Your registration badge ensures admission to standard conference events. Standard and student registrants receive access to the following:

Full access to conference spaces
Conference materials
Coffee breaks
Lunches

The registration of one of the authors is necessary for oral and/or poster presentation. A single author can present maximum two posters, or two oral presentations or one poster and one oral presentation.

Registration fees do not include the access to Thursday Conference Dinner.

Registration costs are available on the conference website:

[WI3R Symposium 2024 \(fourwaves.com\)](https://www.fourwaves.com/WI3R-Symposium-2024)

REGISTRATION HOURS

The registration desk will be located at the entrance of the RVZ, at the following times:

5.06.2024: 2 – 6 pm (Selected young Investigators and Sponsors ONLY)

6.06.2024: 9 – 6.30 pm (all participants)

7.06.2024: 9 – 3.30 pm (all participants)

PRESENTATION UPLOAD

Speakers should upload their presentations beside the registration desk in the foyer. No other means of uploading are foreseen.

BADGES

All attendees must wear registration badges at all times during the congress to ensure admission to events included in the paid fee, such as technical sessions, exhibition and receptions.

LANGUAGE

The official conference language is English (except DFG prize ceremony: German). No simultaneous translation is provided.

WLAN

You can use the SSID@Bayern-WLAN, please follow the instructions to connect.

APPROVED LITERATURE TABLE AND JOB BOARD

Attendees and peer organizations may place company information, job postings, social flyers, or other printed items appropriate for the conference audience at their booths or at the tables in the Exhibition area.

POSTERS

Poster presentations will be held in the poster area located in the RVZ. Poster presenters

should set-up, present and remove their posters.

TIMEZONES

Unless otherwise noted, all times for this conference and related events will take place in the local time zone, Central European Summer Time (CEST, UCT-2).

CURRENCY

All meeting fees are expressed in EURO.

COMMITTEES

Scientific Board

- Antje Appelt-Menzel, Chair Tissue Engineering and Regenerative Medicine TERM, University Hospital Würzburg
- Andrea Ewald, Department for Functional Materials in Medicine and Dentistry, University Hospital Würzburg
- Florian Groeber-Becker, Fraunhofer Translational Center Regenerative Therapies TLC-RT, Würzburg
- Marco Metzger, Fraunhofer Translational Center Regenerative Therapies TLC-RT, Würzburg
- Amelie Reigl, Fraunhofer Translational Center Regenerative Therapies TLC-RT, Würzburg
- Thomas Rudel, Department of Microbiology, University of Würzburg

Local Organizing Committee

- Landing Page/Poster: Özlem Elbert, Marco Metzger, Amelie Reigl
- Sponsoring and Invoicing: Özlem Elbert, Sibylle Schmidt
- Catering, support and social program: Özlem Elbert, Marco Metzger; Young Investigator Meeting: Constantin Berger, Thea Bruchhardt, Paula Büttner, Maria Prates Guedes, Sven Heilig, Helene Hemmer, Tobias Köhler, Camilla Mussoni, Xuen Ng, Amelie Reigl, Jonas Röder; Fraunhofer ISC Band: Lisa Brändler, Gerhard Domann, Joachim Kalinowski
- Speaker invitation and program organization: Antje Appelt-Menzel, Paula Büttner, Özlem Elbert, Kathrin Geyer-Wilkinson, Eva Hilpert, Florian Groeber-Becker, Jürgen Groll, Christian Lotz, Sanjana Mathew-Schmitt, Marco Metzger, Camilla Mussoni, Thomas Rudel, Rachel Wisshofer, Maximiliane Wußmann, Daniela Zdziebło

WELCOME TO WÜRZBURG

The organizing committee is happy to welcome you to the **1st International Conference of the Würzburg Initiative 3R (WI3R)**! The event is organized by the Fraunhofer TLC-RT, supported by the German Research Foundation (DFG) **funded research clusters** SFB/TRR 225 ("From the fundamentals of biofabrication towards functional tissue models"), GRK2157 (3D-Infect), SFB 1583 (Decide) and the Chair Tissue Engineering & Regenerative Medicine (TERM) of University Hospital (UKW) and University of Würzburg, respectively as a joint event. The Fraunhofer TLC-RT as well as TERM (UKW) develop new cell-based tissue models and test systems, scalable manufacture processes, prototypes and biological vascularized implants, therefore contributing to the 3R principles. The SFB/TRR 225 Collaborative Research Centre is interested in exploring the fundamentals of biofabrication and its systematic exploitation with the aim to generate functional human tissue models. This holds the possibility of **automated production** of functional tissue models that would be invaluable as **animal test replacements**, for research on new drug compounds and cancer therapy, and as option for regenerative therapy. The GRK2157 and SFB 1583 networks focus on the development of human 3D tissue models for infection research aiming to identify molecular mechanisms within the host that control the course of infectious diseases. For more information please visit the respective webpages.

Within this 1st WI3R symposium the German Research Foundation (DFG) will grant the **20th anniversary of the Ursula M. Händel Animal Welfare Prize**. The Ursula M. Händel Prize recognises scientists who have made exemplary and sustained efforts to improve the welfare of animals in research. In particular, this includes the development of methods that contribute to the reduction, refinement and replacement of animal experiments. The €80,000 Ursula M. Händel Animal Welfare Prize is currently the largest prize of its kind in Germany, awarded every two years.

The objective of this conference is to provide a broad communication platform for this multi-disciplinary community to enable exchange between researchers working in the fields of 3R research to identify synergies and boost new research ideas and cooperations.

We want to highlight that the symposium is supported by PhD students from the Fraunhofer TLC-RT, the University Hospital chair TERM, the SFB/TRR 225 and the GRK2157 in a joined effort and want to thank the students for the fantastic job they have done. We furthermore thank the speakers for their commitment and contributions and are confident that the scientific program composed of 20 lectures and 80 posters will stimulate excellent scientific discussions.

Würzburg is a historic city with a rich culture and the renowned residence palace, a UNESCO world cultural heritage and one of the most important baroque palaces in Europe. And it is also a lively University city with a famous local wine culture and lovely country sides.

We are looking forward to a conference with exciting science, stimulating discussions and exchange and multiple opportunities to initiate collaborations!

The WI3R organizing committee

VENUE

As a center of science, research and home to one of **Germany's oldest and most renowned universities, Würzburg** has a long-established tradition of holding international meetings as well as conferences. Award winning museums, world-class wineries, and a wide range of cultural and music festivals allow for attractive social programs. Just an hour's drive from Frankfurt airport, Würzburg sits at the crossroads of numerous German traffic routes and is easily reached from everywhere.

Würzburg is full of intriguing contrasts: progress and tradition, a big-city buzz and countryside charm. Surrounded by vineyards, the city exudes an almost Mediterranean air. Würzburg's most outstanding architectural treasure, the Residence Palace, has been listed as a UNESCO World Heritage Site since 1981 and doubles as a MICE venue of exceptional character. With 4.700 hotel beds and a vibrant nightlife fuelled by the city's large student population, Würzburg is the ideal location for successful small to mid-sized meetings and events.



The Rudolf Virchow Center (RVZ) is the DFG Research Center for Integrative and Translational Bioimaging of the University of Würzburg. It was started in 2001 as one of three German Centers of Excellence funded by the German Research Foundation, DFG. The center derives its name from the pathologist Rudolf Virchow, who was a professor in Würzburg from 1849 to 1856 and was the first to postulate that diseases originated in dysfunctions of cells. Researchers at the Rudolf Virchow Center aim to trace diseases back to dysfunctions of proteins. These are called target proteins because they may serve as targets for diagnostic tools or for therapeutic drugs. The Rudolf Virchow Center also organizes a graduate program and several undergraduate programs in biomedicine and experimental medicine. Its Public Science Center offers courses for children and high school students and public events (Source: https://en.wikipedia.org/wiki/Rudolf_Virchow_Center).

SOCIAL EVENTS

Delegates are welcome to participate in social events (registration needed for Symposium dinner:

Get together of Young Investigators at MAM-MAM Burger restaurant with Pub Quiz: June 5, 2024 – 7:00 pm (public event)

At the first day the young investigators will meet in the local MAM-MAM burger restaurant. Everybody is welcome to join. The MAM-MAM is located within the old town of Würzburg. Three vegan and two vegetarian burgers are offered with different toppings, which are also marked accordingly on the menu.

<https://www.mam-mam-burger.de/>

Address: Blasiusgasse 3, 97070 Würzburg

Symposium Dinner: Thursday, June 6, 2024 – 7:30 pm



Our Symposium Dinner will take place at the Restaurant **Bürgerspital Weinstuben**. "Tradition meets modernity" is the credo of the restaurant, which you can experience both culinary and spatially. In the old wine tavern, for example, typical elements of a Franconian wine tavern dominate - rustic, cosy and authentic. The highlight there is the wine barrel, which seats four people for eating and drinking. In the arcades or the "*Alte Weinstube*", the ambience

is modern, upscale and stylish, combining the old architecture with contemporary elements. Take a look around on the virtual tour or browse through the picture galleries. The beautiful courtyard is always open, weather permitting. But take a look for yourself! From RVZ you can use Tram line 1 or 5, exit at 'Dom' station. A map of RVZ area and Würzburg city can be found in the appendix on pages 101-103. Please find more information about the restaurant here:

<https://www.buergerspital-weinstuben.de/>

Address: Theater Strasse 19, 97070 Würzburg

SCIENTIFIC PROGRAM

Wednesday, June 5th, 2024 (internal non-PI meeting)

2:00 pm	Arrival and attachment of posters
3:00 - 6:00 pm	Young Investigator meeting (RVZ lecture Hall) Ice Breaker – Presentations - Trouble Shooting Session – Poster Sessions Flexible Coffee breaks
From 7:00 pm	Get together at MAM-MAM Burger restaurant with Pub Quiz

Thursday, June 6th, 2024

9:00 – 10:00 am	Arrival of external participants and attachment of posters
10:00 – 10:15 am	Welcome and Introduction (RVZ lecture Hall) Florian Groeber-Becker and Thomas Rudel (WI3R Representatives) Sandra Vorlova (Representative of the city of Würzburg) <u>Keynote lecture:</u>
10:15 – 11:00 am	<u>Winfried Neuhaus</u> , University Vienna and Austrian Institute of Technology, Vienna (Austria); 3Rs - past and progress <i>Session I: From bioprinting towards functional tissues (SFB/TRR 225 - Biofabrication) Chair: Sahar Salehi-Müller / Matthias Ryma</i>
11:00 – 11:30 am	<u>Matthias Lütolf</u> , Swiss Federal Institute of Technology (EPFL), Lausanne (Switzerland); Bioengineering next-generation organoids (digital)
11:30 – 12:00 am	<u>Giovanni Vozzi</u> , Faculty of Engineering, University of Pisa (Italy); Biofabrication technologies for functional tissues
12:00 – 12:15 pm	<u>Louise Breideband</u> , Buchman Institute for Molecular Life Sciences (BMLS), Goethe-University Frankfurt; Laser patterning bioprinting using a light sheet-based system equipped with light sheet imaging produces long-term viable full-thickness skin constructs (selected talk #79)
12:15 – 1:15 pm	Lunch Break / Poster Session <i>Session II: 3D infection models (GRK/RTG 2157-3D Infect and SFB 1583-Decide) Chair: Sina Bartfeld / Thea Bruchhardt</i>
1:15 – 1:45 pm	<u>Woojung Shin</u> , University Medical Center Utrecht, Utrecht (Netherlands); Alternative engineered organ model to investigate host-microbiome crosstalk (digital)

- 1:45 – 2:15 pm Larry Schlesinger, Texas Biomedical Research Institute, San Antonio (USA); Human lung biology models to study tuberculosis and host-directed therapy
- 2:15 – 2:30 pm Spyridon Damigos, University Hospital Würzburg, Würzburg; Recapitulating S. Typhimurium infection in a primary human intestinal tissue-macrophage co-culture model (selected talk #62)
- 2:30 – 3:00 pm ***Session III: 3D models in oncology***
Chair: Gudrun Dandekar / Maximiliane Wußmann
Henner Farin, Georg-Speyer-Haus, Frankfurt; Patient-derived Organoid-Stroma Biobank to study (immuno-)therapy responses in colorectal cancer
- 3:00 – 3:30 pm Katrin Mestermann, University Hospital Würzburg, Würzburg; From Solo to Duett - addressing unmet clinical needs through CAR-Immune cell-composition
- 3:30 – 3:45 pm Jens Kurreck, Technical University Berlin, Berlin; Vascularized metastasis model produced by sacrificial-free direct ink writing (selected talk #25)

3:45 – 4:15 pm ***Coffee Break / Poster Session***

4:15 – 6:30 pm **20th anniversary award ceremony of Ursula M. Händel prize (DFG-funded)**

Music: "Autumn Leaves" - Joseph Kosma

Award ceremony:

Prof. Florian Groeber-Becker (Fraunhofer ISC) and Prof. Peter Seeberger (Vice-president DFG) "**Welcome and introduction to Ursula M. Händel prize celebration**".

Prof. Peter Loskill (W3-Professor for Organ-on-Chip-Research at the Eberhard Karls University Tübingen) and Dr. Silke Riegger (Head of the 3R-Center Business Unit for *In Vitro* Models and Alternatives to Animal Testing at University Tübingen) "**Presentation of the 2024 Prize Awardees**".

Prof. Petra Dersch (Member of the Senate and Permanent Senate Commission on Animal Research of DFG) and Prof. Peter Seeberger (Vice-president DFG) "**Laudatio and Award Presentation**".

Music: "My Romance" - Richard Rodgers

Keynote lecture:

Prof. Christopher Baum (Berlin Institute of Health (BIH), Berlin & Ursula M. Händel-Prize Awardee 2009) "**Tierversuche und alternative Methoden zwischen Erkenntnisgewinn und Risikominimierung**".

Podium discussion with previous Awardees:

Prof. Christopher Baum (Berlin Institute of Health (BIH)), Awardee 2009; Prof. Thomas Eschenhagen (University Hospital Hamburg-Eppendorf, Awardee 2011); Dr. Antje Appelt-Menzel for WI3R (Fraunhofer-Institute for Silicate Research ISC and University Hospital Würzburg, Awardee 2022); Prof. Peter

Loskill & Dr. Silke Riegger (University Tübingen, Awardees 2024); Prof. Brigitte Vollmar (Chair of the Permanent Senate Commission on Animal Research of DFG University Rostock and Chair of DFG Prize-Jury).

Music: "The days of wine and roses" - Henry Mancini

Moderation: Cornelia Lossau (DFG)

Music (Piano): Sebastian Tobias Kuhn ("Streetresonance")

7:30 – 11:00 pm

Symposium Gala Dinner (Bürgerspital Weinstuben, Theaterstraße 19, Würzburg)

Friday, June 7, 2024

9:00 – 10:00 am

Podium discussion: NAMs in 2024, hurdles and challenges:

Ulrich Dirnagl (Berlin Institute of Health, Berlin), Julia Schüler (Charles River Discovery Research Services, Freiburg), Marian Raschke (Bayer AG, Berlin), Dilyana Filipova (Doctors Against Animal Experiments, Cologne), Moderation: Florian Groeber-Becker (Fraunhofer ISC, Würzburg)

Session IV: Disease modeling and emerging technologies

Chair: Antje Appelt-Menzel / Winfried Neuhaus

10:00 – 10:30 am

Valentin Wegner, Jena University Hospital, Jena; Organ-on-chip - emerging in vitro models in dissecting host-pathogen interaction

10:30 – 10:45 am

Fulya Ersoy, University of Tübingen, Tübingen; Monitoring Neurosphere electrophysiological activity using a novel NeuroMPS with integrated microelectrodes (selected talk #30)

10:45 – 11:00 am

Jan Hansmann, Fraunhofer ISC, Würzburg and University of Applied Sciences Würzburg-Schweinfurt; Automation and Artificial Intelligence in the context of 3R (selected talk #26)

11:00 – 11:15 am

Alessia Moruzzi, NMI Natural and Medical Sciences Institute, Reutlingen, SpheroFlow: a user-friendly Heart-on-Chip integrating hiPSC-derived cardiac 3D microtissues amenable for multi-parametric non-invasive monitoring (selected talk #66)

11:15 – 11:30 am

Coffee Break

Session V: Regulatory acceptance of NAMs

Chair: Christian Lotz / Michael Oelgeschläger

11:30 – 12:00 am	<u>Michael Oelgeschläger</u> , German Federal Institute for Risk Assessment (BfR), Berlin; Development and standardisation of (complex) in vitro test methods: challenges and concepts
12:00 – 12:30 am	<u>Katharina Koch</u> , DNTOX GmbH and Leibniz Research Institute for Environmental Medicine, Düsseldorf; In vitro tests to replace Developmental Neurotoxicity (DNT) studies
12:30 – 12:45 am	<u>Svenja Wingerter</u> , Eberhard Karls University Tübingen, Tübingen; Integration of biological membranes into Organ-on-Chip systems to model age-related macular degeneration (selected talk #72)
12:45 – 1:45 pm	<i>Lunch Break / Poster Session</i> <i>Session VI: 3R centres and implementation</i> <i>Chair: Silke Riegger / Marco Metzger</i>
1:45 – 2:15 pm	<u>Jennifer Rosowski</u> , Charité - Universitätsmedizin Berlin Campus Virchow Klinikum, Berlin Charité 3R
2:15 – 2:45 pm	<u>Rene Tolba</u> , RWTH Aachen University, Aachen, 3R Competence Network NRW
2:45 – 3:00 pm	<u>Florian Groeber-Becker</u> , Fraunhofer ISC, Würzburg, Würzburg Initiative 3R - WI3R
3:00 – 3:30 pm	<i>Prizes</i> (best talk & best poster, travel awards) and final remarks
<i>-End of symposium-</i>	

POSTER ABSTRACTS

84 - Endothelialized perfusable microvascular networks for biofabrication of standardised in vitro tissue models

Robin Dai¹

¹ Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine, University Hospital Erlangen

Introduction: Despite the considerable advances in biofabrication, engineering of larger 3D tissue constructs has been restricted by the diffusion limit of oxygen and nutrients. The subproject B02 of the SFB/TRR 225 produces perfusable and fully endothelialized microvascular networks aiming at biofabrication of tissue models with improved oxygen and nutrient transport that could replace animal testing.

Our goal is to adapt the microscopy-compliant vascularized constructs to tissue-specific requirements to enable investigations of e.g. muscle tissue, glomeruli and tumours in physiological-like conditions and disease models.

Methods: Melt Electrowriting (MEW) was used to produce sacrificial structures of thermoresponsive poly(2-oxazoline) (POx), which were then embedded in hydrogels and subsequently removed to create microchannels. Endothelialization was achieved using human umbilical vein endothelial cells and validated using fluorescent staining. Barrier assay and response to stimuli were tested to verify the functionality of the endothelium. Migration of cells in the surrounding hydrogels was investigated.

Results: We have successfully created reproducible sacrificial POx scaffolds with biomimetic bifurcating geometries. Using dedicated microscopy-compliant bioreactors developed within the project, POx scaffolds were embedded in a supporting hydrogel and removed by lowering the temperature, which resulted in a formation of bifurcating channel. Upon subsequent endothelial cell seeding, a functional endothelial monolayer was achieved in the channels within 5 days [Ryma et al. Adv Mater 2022]. We have also been able to produce more complex POx scaffolds and a unidirectional flow bioreactor setup to closer mimic physiological conditions. Different hydrogel types have been tested as surrounding matrix that should be compatible with tissue-specific cells and enable cell motility and self-assembly of tissue structures, while supporting the lumen structure stability to maintain perfusability in the bioreactor.

Conclusions and Outlook: The microscopy-compliant vascularised 3D model system developed in B02 project contains functional endothelium and can be adapted to different tissue types to serve as an alternative to animal experiment for e.g. tissue formation, disease modelling or drug screening studies.

In the future, more complex vascular geometries with dedicated bioreactors, advanced cell adhesion and angiogenesis studies, as well as printing of tumour spheroids within the model are planned.

83 - Tailoring 3D printability and cell-material-interaction of alginate-based-bioinks utilizing microspheres for pre-crosslinking

Jonas Röder¹, Dudnik Eva¹, Aldo R. Boccaccini¹

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In this project the pre-crosslinking of alginate based bioinks for 3D bioprinting was investigated following a previous approach developed by the authors [1]. In particular, we investigated how the 3D printability is influenced by the addition of different kinds and amounts of micro particles. Micro particles were produced in a regular, spherical shape to rule out effects of particle morphology. Microspheres with polymer content between 0.5% and 2.0% (wt%) were produced with sizes ranging from 250 to 400 µm. These spheres were immersed in calcium chloride solution to soak for 24h but could be stored in the solution for up to 6 weeks without meaningful degradation. Calcium loaded microspheres were dispersed in gelatin at different weight percentages (ranging from 5% to 12.5%) and subsequently mixed with oxidized alginate for 24 hours. After 24 h an increase in printability and shape fidelity was observed. This effect was also confirmed by rheological measurements. The addition of microspheres and the resulting pre-crosslinking further changed the degradation behavior of the bioinks after the final crosslinking with more calcium und microbial transglutaminase. Degradation was slowed down compared to the reference and swelling during the first day of incubation increased. In regards of the formed polymer network, we were able to confirm the dissolution or incorporation of the spheres into the network of fully crosslinked samples. In cell studies with BJ-1 human fibroblasts we found elongated cells after 7 days and a conducted WST-8 assay confirmed a slight increase in cell number, which can be explained by a reduction of polymer network density due to the pre-crosslinking, allowing for higher cell migration and proliferation. Overall it can be stated that the present attempt on refining the pre-crosslinking method based on calcium loaded microspheres was successful and generated bioinks that displayed superior printability while maintaining desirable degradation and cell-material-interaction.

References

[1] J. Hazur, J. Röder, et al, Pre-Crosslinking with Hydrogel Microparticles Enhances the Printability of Alginate-Based Inks. *Macromol. Mater. Eng.* 2023, 308, 2200675.
<https://doi.org/10.1002/mame.202200675>

Acknowledgments

This project was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Projektnummer 326998133—TRR 225 (subprojects A01).

82 - HLA Diversity and Compatibility in Immunocompetent Human Organ-on-Chip Tissue Models

Julia Roosz¹, Cara Buchanan², Lena Christ¹, Claudia Teufel³, Annie Moisan², Tengku Ibrahim Maulana³, Peter Loskill³

¹NMI Natural and Medical Sciences Institute, ²Wellcome Leap, Culver City, CA, USA,

³Eberhard Karls University Tübingen, Tübingen, Germany

In recent years, the need for alternatives to animal models has led to the development of immunocompetent human organ-on-chip tissue models to overcome the poor transferability of animals to humans in pharmaceutical research [1]. This rising development of immunocompetent human tissue models has been met with the challenge of sourcing appropriately Human Leukocyte Antigen (HLA)-matched immune cells. Compatibility of different donor-derived cell sources within such models is often overlooked, leading to potential alloreactivity that can obscure interpretation of the true immune response. Moreover, despite the importance of representing immune diversity and specificity in models of drug efficacy and safety, this is rarely accounted for in preclinical in vitro testing. As a result, reports on inter-individual variabilities amongst drug responders and non-responders, and population-specific drug-induced immune-related adverse events (irAEs) are only generated when the drug has entered clinical trials and reached the market.

Here, we provide an overview of the vast haplotype diversity and limited population overlap of the human HLA system based on publicly available allele frequency databases [2]. We give a perspective on how this information, together with routine adoption of next generation sequencing (NGS)-based HLA typing, can guide experimental work towards building more predictive human tissue models. This includes a program-wide initiative to sequence sample HLA genes, create a repository of results, and pro-actively guide the choice of cell sources in our multi-cellular tissue models. We also present experimental evidence from a panel of HLA-typed donor-derived human T cells co-cultured with different donor-derived human dermal microvascular endothelial cells (mVECs) in an endothelialized chip screen. Treated and untreated T cells were analyzed by FACS and cytokine secretion to correlate T cell haplotype and degree of mismatch with the endothelium on immune response. Together, this highlights the important need to consider HLA matching and diversity in preclinical test systems, and future work may further help the informed selection of donors/cell lines that best recapitulate authentic and potentially haplotype-specific immune responses.

81 - Development of a human organoid model to study UPEC infections in the prostate epithelium

Kimberly Klapproth¹, Maria Guedes¹, Charis Kalogirou², Carmen Aguilar¹

¹Institute of Molecular Infection Biology (IMIB) University of Würzburg, ²Klinik und Poliklinik für Urologie und Kinderurologie, Uniklinikum Würzburg

Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are among the most common and recurrent infections worldwide. While UPEC typically targets the bladder epithelium, in males, it can also migrate and infect the prostate, resulting in bacterial prostatitis. Treating this infection is challenging and often requires longer and more intensive courses of antibiotics. Despite its prevalence, UPEC pathogenesis in the prostate remains poorly understood, partly due to the lack of in vitro models mimicking the prostate epithelium. Female mice are often used as models for UTIs; however, infecting male mice poses challenges due to the anatomical structure of male organs. To reduce the use of animal models while improving in vitro models, we have developed an advanced model of the prostate epithelium based on mouse prostate organoids. This model has enabled us to decipher the initial steps of infection and identify specific bacterial and host requirements for the infection to succeed. Now, in my PhD project, I plan to translate this knowledge into a human prostate model using human adult stem cell-derived prostate organoids. These organoids are generated from human tissue biopsies and expanded in three dimensions. Subsequently, the organoids are seeded onto 2D surfaces where they can differentiate into basal or luminal cells as observed in human prostate epithelium. To support proper cell differentiation, we have evaluated the impact of different growth factors using immunofluorescence and protein analysis of well-characterized cell markers (e.g., KRT14, KRT5, ZO1, ACP3, PSA). Following fine-tuning of the differentiation conditions, this model will be used to identify new host factors controlling the infection by UPEC in these cells. Importantly, targeting these host factors with new or already existing drugs could lead to the development of novel host-based treatment approaches.

80 - Investigating the Effect of the RSV A-ON1-defining Nucleotide Duplication on Infection in primary Human Respiratory Epithelial Cells

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RSV causes frequent hospitalization due to lower respiratory infections in infants and the elderly population. The viral glycoprotein G and Fusion protein F play a key role in attachment and entry to host cell and are major antigens. RSV is classified into subgroups A and B and further categorized into genotypes based on the G ectodomain sequence. Both are found to co-circulate in the human population. In 1998, a new RSV B genotype, BA, was identified, featuring a 60-nucleotide duplication in the G ectodomain. Over a decade, this genotype and its descendants gradually replaced other B genotypes, becoming globally dominant. A similar 72-nt duplication in the year 2010-11 of RSV A, known as ON1, emerged and swiftly became dominant in the subsequent season. We hypothesized that these duplications may provide a selective advantage and contribute to replicative fitness. Henceforth this study aims to analyze and compare RSV infections in human respiratory epithelial cells. To address this, we generated a recombinant RSV (rRSV) of the prototypic strain A2, imitating the duplication event of 72-nt in genotype ON1. Initially, viral replication of parental rRSV A2 and mutant rRSV A2 G₊₇₂ was observed in conventional cell lines like HEp-2 and Calu-3 and in well-differentiated human tracheal epithelial cells (hTEC). We generated rRSV A2G₊₇₂ with the expression of a larger G protein compared to the parental virus. Surprisingly, replication of rRSV A2 G₊₇₂ was found to be somewhat attenuated in Calu-3 cells and showed no difference over recombinant wild type in HEp-2 cells. Confocal microscopy images depicted RSV infection within ciliated cells of human tracheal epithelial cells. Both recombinant viruses were productively released to the apical side of the epithelial layer. Electron microscopic images also showed filamentous virus particles budding from epithelial cells. Despite similar immunostaining results, the initial electron microscopy results suggested more cellular damage from rRSV A2G₊₇₂ infection. In summary, the findings indicate a rather attenuated replication of rRSV A2G₊₇₂ in standard cell lines, while successful infection and replication of recombinant viruses in hTECs. Infection is limited to ciliated cells for both parental and mutant viruses, leading to cilia reduction.

79 - Laser patterning bioprinting using a light sheet-based system equipped with light sheet imaging produces long-term viable full-thickness skin constructs

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Light-based 3D bioprinting is emerging as a key technique in tissue engineering and regenerative medicine, allowing to fabricate complex, functional three-dimensional (3D) tissue constructs with precise control over cellular organization and spatial distribution. We introduce a new 3D bioprinter that combines live imaging of the bioprinted tissue with high resolution and high-speed capabilities. The printer uses a light sheet illumination to photocrosslink polymers into hydrogels at a printing speed of up to 0.66 mm³/s with a resolution of 15.7 µm. To characterize the resolution and design capabilities of the light sheet bioprinter, a range of structures were printed free-floating or on surfaces. The light sheet bioprinter reads standard G-code files with little adaptations, hence any 3D structure can be bioprinted.

A significant advancement of this bioprinter is its ability to monitor cells and hydrogel during crosslinking, which enables real-time assessment of the 3D-bioprinted structure's quality. First, fluorescence recovery after photobleaching (FRAP) was carried out to determine the crosslinked hydrogel's properties. Then, using photocrosslinkable hydrogels and human Hs27 and HaCaT cells, skin constructs were bioengineered.

Fibroblast cells (Hs27) were encapsulated using this method, and the viability was measured directly after bioprinting and seven days after encapsulation, which was found to be high (83% ± 4.34%). Furthermore, a full-thickness skin construct (containing Hs27 and HaCaT cells) was bioprinted and maintained in culture for 6 weeks, after which it was imaged, showing the presence of skin markers. This demonstrated the long-term viability and physiological relevance of the bioprinted tissue in terms of stratification and gene expression.

This novel light based bioprinting technique enables fast and high resolution biofabrication, coupled with the possibility to image cells and hydrogel before, during and after the bioprinting procedure with light sheet fluorescence imaging. Producing physiologically relevant tissues helps reducing the need for animal testing in the drug discovery process, aiming ultimately to replace them.

78 - Profiling of Age-Dependent Mucociliary Differentiation of Human Nasal Airway Epithelial Cells cultured at Air-Liquid Interface

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The human respiratory system plays a critical role in the defense against airborne pathogens and the maintenance of homeostasis. Viral respiratory infections exhibit age-related differences in susceptibility and outcome. Little is known about the cellular and molecular mechanisms underlying this age-related susceptibility. The differentiation of human primary airway epithelial cells at the air-liquid interface has been shown to represent a valuable *in vitro* 3D model allowing studies of respiratory epithelial physiology and infection dynamics (hAEC-tissue model). However, the age-specific differentiation of hAECs in ALI cultures has not yet been characterized in-depth. In particular, it is unclear how well the age signature of the donor remains preserved.

In this project, we compare hAECs from term neonates and healthy adult donors obtained by nasal brushing and investigate their differentiation dynamics and characteristics when grown at ALI. Specifically, we study proliferation and cell death kinetics, differentiation of cell subsets, cell metabolism, barrier function and production of cytokines and growth factors.

Our preliminary findings show no major differences in proliferation and cell death kinetics and epithelial barrier function between neonatal and adult hAEC-tissue models. However, the proportion of secretory cells in mature hAEC-tissue models tends to be higher in differentiated adult compared to neonatal models. In contrast, adult models have a lower energy metabolism with lower rates of glycolysis as well as OXPHOS compared to neonatal hAEC-tissue models. Significant differences can also be observed in the baseline production of cytokines and growth factors generally differing in an inverse manner between adult and neonatal hAEC-tissue models.

This study contributes to the understanding of how hnAECs mature *ex vivo* and how well the *in vivo* phenotype with a focus on the donor's age is preserved. By identifying age-specific features influencing the differentiation we aim to reveal underlying mechanisms driving susceptibility to respiratory infections across different age groups.

77 - Advanced Tissue Modeling: Magnetic Fibrillar Fragments for Enhanced Cell Alignment in Vascularized 3D Hydrogel Tissue Models

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Achieving precise cell alignment within 3D tissue constructs plays an important role in mimicking native tissue architecture and function *in vitro*. Nanofibrillar fragments with integrated iron particles enable magnetic manipulation of the scaffold. These fragments can be embedded into hydrogel systems and orientated by external magnetic fields, which allow precise positioning and orientation of these fibers. This is a promising strategy for improving cell alignment and adhesion in 3D hydrogel systems and for achieving control over cellular orientation, mimicking the structural organization found in native tissues. The synergistic integration of magnetic guidance of nanofibrillar fragments and vascularization, using sacrificial scaffold-based channel generation, in a double-hierarchical 3D hydrogel system shows great potential for developing advanced tissue models.

To achieve this goal, we created scaffolds using Melt Electrospinning (MEF) with blends composed of 30% PCL and 70% PVAc and incorporated iron particles. Selective dissolution of the PVAc using 70% EtOH resulted in nanofibrillar PCL scaffolds with embedded particles. Although iron particles were added to the thermoplastic blend before printing, nearly all particles were retained in the PCL fraction, which was confirmed by gravimetric and thermogravimetric analyses. Scaffolds were furthermore analyzed regarding their surface morphology and particle distribution using scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX), confirming an even distribution of particles in the scaffolds. Additionally, focused ion beam (FIB) was used to determine the presence of the particles inside fibrils. Moreover, our scaffolds support cell adhesion, elongation, and proliferation, which was confirmed by live/dead stainings of cultured C2C12 mouse myoblast cells. In the following experiments, the scaffolds were laser-cut into fragments of different lengths and embedded in a vascularized 3D hydrogel system to precisely position the fragments with external magnetic forces to support cellular alignment inside the 3D hydrogel. By combining two advanced technologies, namely MEF with particles and sacrificial template technology, inside 3D hydrogels, we aim to achieve precise cellular alignment and create double-hierarchical tissue constructs that closely resemble native tissue organization. This advancement opens new avenues for the development of sophisticated tissue models with enhanced functionality and structural fidelity.

76 - Dynamic *in vitro* and *ex vivo* models on-a-chip: Bridging the gap between high throughput screening and systemic studies *in vivo*

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Both in the field of drug development and in risk assessment of chemicals, the need for new *in vivo*-like and animal-free approaches for chemical and pharmaceutical safety assessment is increasing. Flexible and easy-to-use microfluidic systems, combined with cell models, help to close the existing gap between high throughput screening models and systemic studies *in vivo*.

The developed microfluidic platform consists of flow modules that are operated in combination with miniaturized incubator microscopes and sensor systems. The structure of the fluidic modules and the experimental set-up can be adapted to the respective scientific problem.

Our presented fluidic system is therefore suitable for various fields of application:

- (a) the dynamic cultivation of 2D cell cultures (e.g., barrier models such as intestine or blood-brain barrier) [1], 3D cell cultures (e.g., organoids such as liver or intestine) [2], and air-liquid interface models (e.g., lung),
- (b) a mucus chip test platform for testing the permeation of drugs and chemicals across the buccal and intestinal mucus barrier [3],
- (c) a combination of mucus model, 2D model and/or 3D models,
- (d) analysis of individual cells. The flexible technology enables the development of various array structures in the used chip membrane for positioning of single cells and their individual analysis.

In addition to the simple cultivation of cell models outside an incubator, the system allows a combined inline analysis of the cells (live imaging, electrical measurements, sensor technology).

These flexible devices, combined with advanced *in vitro* models such as hiPSC-based organoids, can make significant contributions in terms of the 3R concept in the regulatory area of safety assessment as well as in application-oriented and basic research. And thus, they have a great potential to close the gap between standardized *in vitro* tests and subsequent *ex vivo* and *in vivo* studies.

[1] Kohl et al. 2021. Microfluidic In Vitro Platform for (Nano)Safety and (Nano)Drug Efficiency Screening, DOI:10.1002/sml.202006012

[2] Elberskirch et al. 2021. Microfluidic 3D intestine tumor spheroid model for efficient *in vitro* investigation of nanoparticulate formulations, 2021, DOI:10.1016/j.jddst.2021.102496

[3] Elberskirch et al. 2019. A novel microfluidic mucus-chip for studying the permeation of compounds over the mucus barrier, DOI:10.1016/j.jddst.2019.101248

75 - Bioactive Hydroxyapatite Coating on Collagen-Mimetic Nanofibrils for Bone Tissue Engineering

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Mineralized collagen fibrils play a pivotal role in bone tissue engineering due to their unique structural and functional properties. Collagen, the main component of bone matrix, provides the framework for tissue formation, while mineralization enhances mechanical strength and stability. Hydroxyapatite (HA) as the main inorganic component of bone is a highly suitable material due to its excellent biocompatibility and bioactivity. We therefore aim to create a biomimetic composite material that closely mimics the composition and architecture of natural bone tissue, favoring cell attachment, proliferation, and differentiation based on mineralized collagen-mimetic nanofibrils (CMNFs) with incorporated HA particles.

CMNFs were produced using Melt Electrospinning (MEF) of 30 wt% PCL and 70 wt% PVAc with and without addition of HA or nano HA (nHA) to the blend, followed by selective dissolution of the PVAc with 70% EtOH to obtain nanofibrillar microbundles with embedded particles. CMNFs were characterized in detail by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) regarding surface morphology, particle content and distribution. Gravimetric and thermogravimetric analysis was carried out to estimate the particle content. To obtain biomineralized scaffolds, these particle-containing fibrillar scaffolds were immersed in simulated body fluid (SBF) solution and compared to a standard HA-coating protocol with polydopamine, gelatin and SBF. Moreover, bioactivity was investigated over a time period of 7 days and bone marrow-derived mesenchymal stem cells (bmMSCs) were seeded on CMNFs and analyzed for cellular adhesion and proliferation.

The presence and distribution of HA particles was confirmed by SEM and EDX analysis, showing an even distribution throughout the scaffolds. Due to their small size, nHA could not be tracked by SEM and EDX. However, gravimetric analysis confirmed the presence of particles in the fibrils. Interestingly, nHA-containing scaffolds in SBF showed a very uniform growth of HA crystals than scaffolds containing HA and outperformed the standard HA coating protocol. Cell adhesion, alignment and proliferation was confirmed by live/dead staining.

Overall, the results not only demonstrate good cellular adhesion, alignment and proliferation but also the successful fabrication of mineralized CMNFs and their superior performance in promoting HA crystal growth compared to traditional coating protocols.

74 - Printable Methacrylated Collagen Type I-based Hydrogels for Studying Breast Cancer Cell Migration in a 3D Environment

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Bioprinting allows for the engineering of sophisticated in-vitro 3D tumor-stroma models that mimic the naturally occurring tumor microenvironment. Such models can be used, e.g., to investigate tumor invasion and metastasis. However, there is still a lack of well-defined bioinks that enable tumor cell migration, which represents a prerequisite for realistic 3D tumor-stroma models. Therefore, in this study, we developed a bioink consisting of ECM-derived biopolymers, namely methacrylated collagen type I (ColMA) and thiolated hyaluronic acid (HA-SH), that is printable via extrusion-based bioprinting and supports tumor cell migration.

We tested different formulations of ColMA, HA-SH, and LAP regarding their printability, rheological and mechanical properties. Additionally, we examined the ultrastructure of the polymerized bioink using confocal reflection microscopy and scanning electron microscopy. We then studied the invasion of cells from MDA-MB 231 breast carcinoma spheroids into the surrounding matrix, and the effect of Rho-associated protein kinase (ROCK) and/or broad-spectrum matrix metalloproteinase (MMP) inhibitors on cell invasiveness.

Bioinks were printable over a range of ColMA concentrations (1.5 - 5.5 mg/ml), however ColMA concentrations >2.5 mg/ml and the addition of high molecular weight HA-SH were necessary for the light-induced radical polymerization and thus for the sol-gel transition. The printed hydrogel displayed a fibrous morphology formed by collagen fibril networks with an average pore size of 10 μm . Cell viability was ~90 % after printing and photo-initiated crosslinking, independent of the nozzle diameter (150 - 330 μm). Within 24 h after printing, single cells spread and migrated radially out from the spheroids into the surrounding matrix. With increasing ColMA concentrations, the migration speed decreased. MMP inhibition almost completely suppressed cell migration, whereas ROCK inhibition led to changes in cell morphology.

In summary, the tumor invasion behavior in our newly developed printable ColMA/HA-SH bioink was comparable to that in pure non-printable collagen gels, which are currently regarded as the gold standard for generating in-vitro 3D tumor-stroma models. Ongoing studies aim to utilize the new bioink in 3D bioprinted models in combination with a stromal adipose compartment to investigate different aspects of breast cancer cell migration within an intricate 3D microenvironment, such as stromal cell-induced stimulation of tumor cell migration.

73 - Infection of human respiratory tissue models with *Staphylococcus aureus* and influenza A virus

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Human airway tissue models (hATMs) have emerged as valuable tools for studying host-pathogen interactions involving airborne bacteria and viruses. Our human 3D hATMs reflect the architecture of the conducting airways in humans. The existing model is comprised of fibroblast-loaded connective tissue and a respiratory epithelial layer, these hATMs show the mucociliary phenotype, facilitating mucus transport, which is an important factor for pathogen clearance.

Until recently, our respiratory tissue models were built on a 3D scaffold derived from a decellularized porcine small intestine. In order to replace the associated animal experiments, this study aimed to substitute the biological scaffold with a synthetic polyamide 6 (PA6) matrix. We evaluated the suitability of these PA6-based models for investigating viral and bacterial infections.

To address this objective, human respiratory fibroblasts and epithelial cells were seeded onto both scaffold types, and the morphology of the models was analyzed using histological techniques. Additionally, we measured the epithelial barrier and conducted RT-qPCR and immunofluorescent staining to study the infection of the tissue models with influenza A virus (IAV). Furthermore, we investigated the infection of PA6-based hATMs with *Staphylococcus aureus* using CFU assays and histological analysis.

Comparative analyses of respiratory tissue models based on the biological and synthetic scaffold revealed no apparent differences concerning tissue model morphology, cell type composition, epithelial barrier properties, and susceptibility to IAV infection. Furthermore, PA6-based hATMs were successfully infected with *S. aureus*.

These findings indicate that PA6 fibers are suitable scaffolds for constructing human respiratory tissue models and can replace biological scaffolds and associated animal experiments. The advanced features of these models allow for the simulation of infection dynamics and the study of pathogen interactions with human tissue *in vitro*, offering a promising platform for preclinical research and the investigation of infection mechanisms.

72 - Integration of biological membranes into Organ-on-Chip systems to model age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world and its incidence is expected to rise due to population ageing. The disease is characterized by a progressive destruction of the macula, and in the diseases advanced stages by choroidal neovascularization or geographic atrophy. Animal models have provided some insights into the underlying mechanisms of AMD but cannot shine light on the complex mechanisms happening on a molecular level. To understand the disease in detail, more physiologically relevant human models are required. The emergence of human induced pluripotent stem cell (hiPSC) derived retinal organoids (ROs) provided a crucial step to tackle this challenge, especially when combined with Organ-on-Chip technology as complex Retina-on-Chip (RoC) models. However, these models use plastic membranes to separate compartments and lack a physiological Bruch's membrane (BrM) and the choriocapillaris, which are important to understand the consequences of extracellular matrix (ECM) remodeling in the progression of the disease.

In this project, a new generation of a polydimethylsiloxane-free RoC platform amenable for the integration of biological membranes has been developed. Multiple membrane sources have been tested, including BrM obtained from porcine eyes, amniotic membranes from human placenta, and commercially available collagen membranes. Tailored protocols for isolation, decellularization and drying for preprocessing of the membranes before integration in the chip were developed. Since traditional microfabrication methods require high temperatures that are not suitable for biological membranes, a styrene-ethylene-butylene-styrene/poly(methyl methacrylate) hybrid approach was established that enables chip bonding at 37°C. hiPSC derived retinal pigment epithelial (RPE) cells were cultured in the chip system forming a viable and compact layer on top of the membranes. To recreate the full complexity of retinal tissue, ROs were added enabling direct interaction of photoreceptors and RPE. Furthermore, endothelial cells could be seeded into the perfusion channel, lining the choriocapillaris side of the membrane.

The novel model enables us to study ECM remodeling and molecular alterations in retinal tissue during AMD development. Moreover, the concepts and microfabrication methods established are not only suitable for retinal models, but can be transferred to other tissue and disease models that require biological membranes.

71 - The Effect of Cell-Imprinted PDMS Substrates on MCF7 Cells Response to Doxorubicin

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Breast cancer, a widely spread cancer among women, may be correlated with the uncontrolled growth and distribution of cells, ultimately resulting in malignancy. It is imperative to determine suitable dosages of chemotherapy medications and nanocarriers for effective cancer therapy. The study aimed to evaluate the effectiveness of using cell imprinting with PDMS for MCF7 cell culture. Findings suggest that MCF7 cells demonstrate increased vulnerability to apoptotic cell death on structured PDMS surfaces compared to flat surfaces. The determination of the IC₅₀ of doxorubicin was carried out through the Resazurin cell viability assay, indicating that MCF7 cells exhibit a decreased IC₅₀ on patterned PDMS substrates.

70 - YOLO-based pipeline for the detection and classification of live and dead cells

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Deep learning models have become a valuable tool for innovative and efficient solutions in a wide range of challenges in healthcare and life sciences, facilitating tasks with higher precision and time-efficiency compared to conventional manual procedures. This study demonstrates the utilization of the You Only Look Once (YOLO) deep learning model for automated cell count analysis, showing the potential for incorporating automation and machine learning workflows in life science laboratory settings. Accurate cell counting is essential for generating advanced tissue models, which offer great potential to replace animal testing in research. The framework has been designed for detecting images acquired through microscopy of a Neubauer chamber containing live and dead cells. Images captured by the microscope are stored in multi-layered files. The initial step in the pre-processing pipeline involves identifying the optimal candidate layer in terms of sharpness. Subsequently, a sliding window matching the size of the Neubauer chamber grid is applied to each image to generate cropped slices for counting. Next, these slices are fed into the detection model for the classification and count of live and dead cells. The training dataset is formulated using the Roboflow platform and comprises 7,142 annotated instances of cells, including 5,966 live cells and 1,176 dead cells. We studied how parameters like batch size influenced the algorithm's accuracy, ensuring a thorough evaluation across varying settings. A mean average precision of 0.986, 0.956, 0.951, and 0.919 is achieved for both classes at batch sizes of 8, 16, 32, and 64, respectively. For further validation of the model, we conducted a manual cell count experiment with the assistance of participants skilled in cell counting. This experiment involved comparing the manual and automated cell counts across eight counting grids. In addition, the algorithm was tested on images containing varying numbers of cells, ranging from an average of 45 cells to 400 cells, to study its performance under different cell densities. In summary, our integration of deep learning models for automated analysis in life science labs enhances the standardization and high throughput of the generation of tissue models, which offers alternative testing rather than animal models.

69 - The Evolution of in vitro Skin Models: Bridging Epidermis, Full-Thickness Models, and hiPSC-Derived Organoids

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Mimicking the complex structure of human skin in vitro is a major challenge due to its multi-layered composition of epidermis, dermis, and hypodermis, which houses various cell types and structures such as hair follicles. Traditional 2D cell cultures, while enabling high-throughput rapid testing, are limited in their representation of this complexity. However, with the advent of human induced pluripotent stem cells (hiPSC), it has become possible to generate organoids that closely simulate the intricate nature of human skin. Our laboratory utilizes a range of in vitro skin models, from Reconstructed Human Epidermis (RHE), which mimics the barrier function of the natural epidermis, to more complex Full-Thickness Skin Equivalents (FTSE) and hiPSC-derived skin organoids (SO). These organoids incorporate various skin-specific cell types such as keratinocytes, dermal cells, adipocytes, and complex hair peg formation.

Reconstructed Human Epidermis (RHE): The RHE models are exemplary for testing skin barrier functions and conducting impedance measurements, which are non-invasive techniques. These models are particularly effective for high-throughput screening and rapid testing of epidermal functions. RHE also facilitates the examination of epidermal conditions like epidermolysis bullosa simplex by using patient-derived keratinocytes. Furthermore, RHE is employed in researching epidermal burn wounds, while both RHE and Full-Thickness Skin Equivalents (FTSE) are utilized in wound healing studies. FTSE models are more complex than RHE, incorporating a Type I collagen-based dermal component. They allow for a more in-depth study of the skin's dermal layer and are suitable for research into the underlying mechanisms of skin disorders.

In parallel, the development of hiPSC-derived organoids (SO) mimicking the skin are remarkable in their ability to recapitulate the intricate architecture of the human skin, including skinlayers and the formation of hair-like structures. For example, it's used for cancer modelling, providing a comprehensive simulation of complex skin tissues. In the SO we identified several skin-specific cell types such as keratinocytes (CK5), dermal cells (vimentin), adipocytes (nile red) and complex hairpeg formation (indicated by the expression of CK5, CK17).

These approaches collectively enable comprehensive investigations, by integrating immuncells, into skin functions, drug testing, and understanding the mechanisms behind skin disorders and regenerative processes.

68 - Establishing organotypic slice cultures and dissociated cell cultures of olfactory epithelium to study Borna disease virus 1 infection

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The olfactory system consists of respiratory and olfactory epithelium (OE). Due to its direct connection to the environment it serves as an ideal entry point for neurotropic virus infections, for example borna-disease-virus 1. The olfactory epithelium consists of supporting cells, basal cells, brush cells and olfactory sensory neurons which are directly connected to the brain and are therefore of special interest in case of neurotropic infections. Borna disease virus 1 (BoDV1) leads to fatal non suppurative meningoencephalitis in humans and other dead-end hosts like sheep and horses and a persistent symptomless infection in its reservoir. To study entry routes in the reservoir- and endhost organotypic cultures and dissociated cell cultures are important. In the first step we are developing the cultures with the rat olfactory epithelium to compare them with the olfactory epithelium of *Crocidura leucodon* (bicolored white-toothed shrew) as the reservoir host of BoDV-1. For the dissociated cell cultures, tissue from the nasal cavity is dissected and incubated with enzymes to dissociate. The cells are growing on poly-L-lysine coated glass coverslips in cell culture plates using Neurobasal-Medium A. Culturing tissue with layers of cartilage, bone, connective tissue and neuronal tissue brings its specific needs for nutrition, attachment and connection for example to the brain olfactory bulb. The tissue for the organotypic slice cultures is grown on Millicell cell culture inserts with different mediums such as Neurobasal A and DMEM and different locations of the olfactory system to assess infectious routes. Slices are cut by hand or tissue chopper. The ongoing production of mucin from goblet cells in the respiratory epithelium provides challenges in adequate diffusion of cell culture medium through the membrane to the tissue. Large pieces provide the same challenges for nutrition, but tissue architecture is preserved better to resemble the original tissue. Life/Dead staining is used to assess survival and immunohistochemistry is used to show different cell types and growth. Olfactory marker protein is used to show olfactory neurons. Double staining at different days post infection can be used to show infected cell types.

67 - Advancing 3R Principle in Spinal Cord Injury Research: A Meta-analysis Approach

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To advance the principles of Replacement, Reduction, and Refinement (3Rs) in research, this study employs a systematic review and meta-analysis to investigate treatment strategies for Spinal Cord Injury (SCI), with a specific focus on Chondroitinase ABC (ChABC) and NOgo interventions. SCI presents a significant global healthcare challenge, profoundly impacting motor, sensory, and autonomic functions and often resulting in lifelong disability. Given the complexity of SCI and the urgent need for effective treatments, the exploration of innovative interventions is crucial for enhancing patient outcomes and promoting ethical research practices.

This study systematically reviews the existing literature on ChABC and NOgo treatments in animal models of SCI. By evaluating the efficacy of these interventions in restoring locomotor function post-injury, the research provides valuable insights into their therapeutic potential and their alignment with the principles of Replacement, Reduction, and Refinement. Importantly, the findings of this meta-analysis extend beyond clinical efficacy to address 3R considerations in SCI research.

By highlighting the practical implications for future animal experiments, the study promotes the responsible and ethical treatment of animals in scientific research. Specifically, it underscores the importance of the 3R principles in minimizing animal usage in SCI research. By reviewing evidence and promoting ethical research, this study helps improve SCI treatments while ensuring animal welfare and scientific integrity.

66 - SpheroFlow: a user-friendly Heart-on-Chip integrating hiPSC-derived cardiac 3D microtissues amenable for multi-parametric non-invasive monitoring

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Heart-on-chip systems mimic the structure and biology of cardiac fibers in vitro. They utilize a continuous perfusion system to sustain tissue homeostasis by supplying nutrients and eliminating waste metabolites [1].

Cardiomyocytes exhibit a versatile metabolism, adapting to different substrates like fatty acids, glucose, lactate, and ketone bodies in both physiological (fetal to adult cardiac maturation) and pathological states (ischemia, hypertrophy cardiomyopathy, obesity and diabetes). On-chip metabolic assessments offer a valuable tool for studying these shifts in cardiac tissues under pathophysiological conditions and assessing potential therapeutic interventions.

We have developed a user-friendly heart-on-chip platform featuring dumbbell-shaped tissue chambers and perfusable microchannels [2]. The platform enables facile and robust generation of aligned cardiac microtissues via an innovative loading approach: pre-formed cardiac spheroids are injected via hydrostatic-pressure driven flow and subsequently fuse into 3D uniaxially aligned cardiac fibers, which can be cultured under perfusion for multiple weeks. Utilizing a simple “DIY” pacemaker, the cardiac microtissues can be continuously paced throughout long-term culture.

To enable continuous monitoring of tissue functionality in a non-invasive manner, we implemented three orthogonal approaches: optical flow motion tracking, integrated luminescent oxygen sensors, and Raman microspectroscopy. Leveraging this toolbox of readouts, we could, i.e., visualize tissue changes in O₂ consumption upon electrical stimulation, proportional to the pacing frequency. The higher metabolic rate of cardiac tissues upon pacing was further confirmed by increased O₂ consumption and higher lactate production compared to control tissues over a 10-day period. Moreover, Raman imaging revealed dynamic changes in lipid and protein content of electrically stimulated tissues over time. Lastly, the integrated oxygen sensors even enabled a fully automated on-chip respirometry analysis.

For the future, the user-friendly, integrated heart-on-chip model paves the way for investigations into metabolic changes in chronic heart conditions, to assess efficacy and safety of long-term drug treatments, and to study repeated dose toxicity.

[1] Arslan U, Moruzzi A, Nowacka J, *et al.* (2022) *Materials Today Bio* 14, 100259.[2]
Schneider O., Moruzzi A. *et al.* (2022) *Materials Today Bio* 15, 100280.

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65 - Establishment of an organ-on-a-chip 3D in vitro model of the human blood-brain barrier

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Various methods have been developed to culture brain cells to replicate the brain microenvironment. Simple cultures often fail to replicate important features of the blood-brain barrier (BBB) under physiological conditions, such as shear stress and protein expression modulation. To best replicate the in vivo conditions, organ-on-a-chip was introduced. These chips are microfluidic devices in which tissue can be cultured in a physiologically relevant microenvironment achieved by geometric, mechanical and biochemical factors. The microfluidic devices can be used for imaging in various types of microscopes and for studying healthy and diseased patient-derived tissue.

Here, we used SynBBB microfluidic device to construct a 3D in vitro model of a human BBB. This organ-on-a-chip has a central chamber and flanking microchannels, which allow to create a 3D tissue model of the brain that provides a morphologically and biologically realistic microenvironment close to the in vivo situation. The device enables the measurement of transendothelial electrical resistance (TEER) and real-time monitoring of barrier properties. The three cell types of the neurovascular unit, the microvascular endothelial cells (hCMEC/D3), pericytes and astrocytes were cultured in the microfluidic device. Endothelial cells were cultured in the apical chamber (outer channels), while astrocytes and pericytes were cultured in the basolateral chamber (central chamber). Porous membranes enable communication between the different cell types. Different ratios of pericytes and astrocytes and different flow rates of the cell culture medium were tested. Cell growth and barrier properties were monitored for three weeks using microscopic images and TEER measurement.

The SynBBB microfluidic device appeared to be suitable for 3D culture of neurovascular unit cells for up to three weeks under different shear stress conditions. The endothelial barrier properties remained stable during the culture time in a microfluidic device. In summary, this model can be used for future functional studies and to model various central nervous system diseases in vitro.

64 - Analysis of tumor progression in biofabricated co-culture melanoma models

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Early metastasis from primary tumors and tumor dormancy, in which cancer cells stay in the body for years without progressing, pose significant clinical challenges of melanoma, a highly malignant tumor of melanocytes. The field of biofabrication has become a focus of cancer research due to its ability to enable the reconstruction of the extracellular matrix, allowing a reproducible cell distribution and arrangement. Biofabricated structures hold the possibility to closely resemble the in vivo situation, and can potentially replace a high number of animal experiments. This project aims to create complex melanoma tumor models that can simulate various stages of tumor progression and analyze their effects on cell behavior. A new formulation of 3D-printable pre-crosslinked alginate dialdehyde-gelatin (ADA-GEL) and ink composed of alginate and cellulose nanofibrils (Cellink Bioink, Schmidt 2019) were used as models of progression and dormancy, respectively. Both inks were tested as monoculture biofabricates by printing melanoma cells from different stages of tumor development. Newly, co-culture models were established by adding human adipose-derived stem cells (ADSCs), to mimic the tumor microenvironment more precisely and examine potential influences on tumor progression. Melanoma cells and ADSCs showed a high cell survival rate in both inks over a period of 14-days. To monitor each cell cycle phase within the biofabricates, melanoma cells were also printed as fluorescence ubiquitination cell-cycle indicator (FUCCI) labelled cell lines. First results indicate that melanoma cells exhibited a high proliferation rate in pre-crosslinked ADA-GEL compared to the previously tested ADA-Gel formulation, whereas the same cell lines are not proliferating in Cellink, both as monoculture and co-culture. ADSC seem not to affect the proliferation. Ultimately, these established models offer the possibility of conducting highly complex animal experiments with 3D biofabricates that resemble human pathophysiology.

63 - Development of a single-use silicone flow bioreactor for the dynamic cultivation of 3D corneal in-vitro models

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The cornea, the protective outer layer of the eye, is a major contributor to blindness worldwide. It accounts for 5.1% of cases and is the fourth leading cause of blindness, according to the World Health Organisation. Several preclinical corneal research models are used to develop new drug candidates or to understand pathomechanisms. These include ex vivo models, such as porcine corneal tissue, and in-vitro models of varying complexity, all following the 3Rs principle (reduce, refine, replace). Studies show that dynamic cultivation of porcine ex vivo cornea tissue improves cell number, viability, transparency, and overall tissue quality. However, it is unclear whether this effect can be applied on in-vitro generated 3D models, which are typically cultured in a static environment. In addition, dynamic cultivation has the potential to mimic the eye's natural mechanical barriers, including tear flow, which could ultimately provide a more accurate prediction of drug permeation or wound healing.

For the dynamic cultivation of corneal 3D models, a flow bioreactor was developed using computer aided design (CAD). The bioreactors were manufactured using silicone to facilitate low-cost production and scalability. Negative moulds for silicone casting were 3D printed using stereolithography (SLA). The bioreactor consisted of two bodies with lids. Both parts enclose an SLA-printed chip onto which an insert membrane is mounted. The chip sealed the two silicone parts and provided two closed compartments for apical and basolateral media circuits. To generate an in-vitro reconstructed corneal epithelium (RCE) model, cells of the human immortalised corneal epithelial cell line (hTCEpi) were seeded on a membrane, cultured at an air-liquid interface (ALI) and transferred to the bioreactor at different time points. RCE models cultured in the bioreactor showed a multilayered epithelium with increased cytokeratin 14 (CK14) and Ki67 expression compared to statically cultivated RCE models. Different apical media flow modes allowed the study of intermittent and continuous flow in the dynamic setting. In addition, transparent silicone lids enabled non-invasive morphological assessment through the bioreactor using optical coherence tomography (OCT). Prospectively, this bioreactor could be used for wound healing and permeability studies in a dynamic setting.

62 - Recapitulating *S. Typhimurium* infection in a primary human intestinal tissue-macrophage co-culture model

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Salmonella enterica serovar Typhimurium (STm) is an enteric pathogen that causes site restricted gastroenteritis in humans. In severe cases STm can systematically spread leading to non-typhoidal Salmonellosis (NTS). Infection studies on animal models have shown a differential outcome of STm infection, irrelevant to the human situation, making mouse models, inadequate for translational research. In this work, we applied a top-down tissue engineering approach based on a decellularized pig small intestinal submucosa (SIS), as a 3D matrix, to generate an in vitro model for human-specific intestinal infection studies. The model was setup on custom cell crown-like inserts. Intestinal fibroblasts and monocyte-derived macrophages were embedded in the SIS and followed by the development of a primary enteroid layer. Models were subjected to further infection studies with STm for the time course of 8h without the addition of antibiotics. The differentiation profile of the epithelial layer revealed the presence of GP2 positive M-like cells, indicating the impact of macrophage co-culture on their maturation. GP2 positive cells were in addition found susceptible to early invasion by STm. Phagocytosis of STm by macrophages was determined using light and electron microscopy in the sub-epithelial compartment. Luminal transmigration of macrophages and further uptake were also indicated on late infection. On later timepoints colonization of the lumen accompanied by epithelial cell extrusion were recapitulated in the model. Extruded epithelial cells were shown to provide a reservoir of a re-invasive STm population. In conclusion, our study introduces a novel human intestinal tissue model, utilizing primary cells and incorporating interactive cellular immune components. Demonstrating a robust response to *Salmonella typhimurium* (STm), this model holds promise for investigating a spectrum of enteric pathogen infections.

61 - Exploring immunotherapy resistance in oral cancer using organoid technology

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Oral squamous cell carcinoma (OSCC) is the most common form of head and neck cancer. Its current standard of care involves surgical interventions combined with irradiation and/or chemotherapy. Despite treatment, survival rates remain low, and rates of recurrence or metastasis remain high. Recently, therapy with immune checkpoint inhibitors (ICIs) has been approved to treat recurrent or metastatic OSCC. Nevertheless, ICI treatment only works for 1 out of 5 patients. To explore whether we can model ICI therapy resistance *in vitro*, we established autologous co-cultures of cancer organoids and T cells.

First, we collected tissue and blood samples from patients with primary, recurrent, and metastatic OSCC. We next generated a biobank of patient-derived organoids (PDOs) and isolated peripheral blood mononuclear cells (PBMCs) from the patient material. We then established co-cultures of PDOs and PBMCs to expand tumor-reactive cytotoxic T cells. Using immunophenotyping, we assessed T-cell expansion and anti-tumor reactivity.

Gene-expression profiling and flow cytometry of PDOs revealed patient heterogeneity in IFN- γ response. Although treating PDOs increased the expression of the immunomodulatory molecules (such as HLA-ABC, and PD-L1), metastasis organoids showed significantly lower antigen presentation than primary tumor organoids. Next, we co-cultured PDOs with autologous PBMCs in the presence of interleukin-2. Over two weeks, we found robust immune-cell expansion in the co-culture. Comprehensive immunophenotyping confirmed that CD3⁺ T cells were enriched in the immune-cell fraction. While there was a robust expansion of CD4⁺ T cells in co-cultures with primary tumor organoids, only 3 of 10 PDO lines showed an expansion of cytotoxic CD8a⁺ T cells following ICI treatment, reflecting the heterogeneous response of patients to immunotherapy. We are currently performing organoid-killing assays to validate the tumor-specificity of the expanded T cells. Furthermore, we are assessing which T cell clones are predominant in co-cultures by TCR-sequencing.

By adapting cancer organoids as an immuno-oncological model, we show for the first time that tumor-reactive T cells can be expanded from OSCC *ex vivo*. The model system can ultimately be applied to better understand why treatment fails and predict ICI treatment outcomes.

60 - Impedance-based in vitro analysis of chemical eye irritation

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For the endpoint of eye irritation, new approach methodologies (NAMs) based on ex vivo or in vitro models have already been developed. Promising NAMs exist that can classify a broad range of chemical eye irritants [1]. However, no NAM is still able to replace the Draize eye test by predicting the full range of different chemical categories of eye irritation according to the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) in a single test [2]. The reasons for this are complex, but can display the limited applicability domain of existing NAMs and the lack of suitable analysis methods for tissue recovery. In order to improve the range of applications, the ImAi-test, based on the non-invasive method of impedance spectroscopy, could be integrated as a complementary tool for the development of regulatory acceptable NAMs.

To improve the applicability domain of eye irritation testing, we examined effects of concentration-dependent eye irritation employing a modified protocol of the Organisation for Economic Co-operation and Development (OECD) 492 test guideline [3]. Instead of an MTT-assay, we used non-destructive impedance spectroscopy to analyse reconstructed cornea like models based on primary human cells. By using impedance spectroscopy cell barrier properties are detectable via adjusted frequency ranges. We tested four category 1 substances in three different dilutions: 100%, 5% and 1%. Tracking concentration dependent damage, we measured impedance 6 times over 11 days. The transepithelial electrical resistance at the frequency of 1000 Hz (TEER₁₀₀₀ Hz) decreased to below 10 % in undiluted category 1 chemicals, indicating severe eye damage. However, with the dilution the TEER values increased above 80% of the control indicating a non-irritant and a dose dependent effect. These findings were supported by morphologic analysis of H&E stainings in the tissues, displaying dose dependent damage.

Summarized, impedance spectroscopy indicated substance-related irritation effects of diluted testing substances in our cornea like model. Impedance-based predictions could influence the labelling of formulated consumer goods and further development for NAMs. References

[1] OECD (2022), Test No. 492B [2] UN (2017). United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS). [3] OECD (2019), Test No. 492

59 - Effects of Pilt Knockout on Blood-Brain Barrier Integrity in vitro

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Background:The blood-brain barrier (BBB) plays a critical role in controlling the influx and efflux of biological substances that are essential for brain metabolic activity and neuronal function. The barrier properties of specialized endothelial cells of the BBB depend, among others, on the tight junctions (TJs) between neighboring cells. TJs are dynamic structures composed of a number of transmembrane and membrane-associated proteins arranged in multimolecular complexes and involved in intracellular signaling [1]. Pilt (Protein incorporated later into tight junctions), also known as Tjap1 (tight junction associated protein 1), is expressed at the BBB and incorporated into TJs after the claudin-based junctional strands are formed [2]. We identified Pilt as a direct target of microRNA-212/132 under hypoxic condition [3]. Although the structural composition of TJs complexes has been well described, little is known about the role of Pilt in the properties of the BBB.

Methods:In this study, we generated Pilt knockout (KO) cell lines using genome editing technology. Pilt KO and control cell line were examined by cell viability, tube formation and wound healing assays. To investigate the effects of Pilt on BBB properties in vitro, we measured paracellular permeability, transendothelial electrical resistance (TEER), and performed immunofluorescence staining. To investigate the effects of Pilt on BBB integrity under hypoxic conditions, the cell lines were exposed to oxygen-glucose deprivation/reperfusion (OGD/R) to mimic ischemic conditions in vitro.

Results:Pilt KO cell line showed increased TEER, decreased paracellular permeability, decreased proliferation rate, increased cell migration rate, and altered expression of associated TJ proteins compared the control cell line. OGD exposure resulted in decreased TEER and increased paracellular permeability of the Pilt KO cells, as well as altered expression of junction proteins, transport proteins and inflammatory factors.

Conclusions: Pilt appears to play an important role in regulating the integrity of the BBB.

Reference:[1]Luissint AC, et al. Fluids Barriers CNS. 2012;9(1):23. Published 2012 Nov 9. [2]Kawabe H, et al. J Biol Chem. 2001;276(51):48350-48355. [3]Burek M, et al. Transl Stroke Res. 2019;10(6):672-683.

58 - 3D Adipose Tissue Model with Tunable Fat Content Consisting of Multicellular Spheroids Seeded into Melt Electrowriting (MEW) Scaffolds

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Objectives: Representative in-vitro models of adipose tissue are of key importance to investigate its multifaceted role in metabolism, as well as in the development of metabolic disorders and cancer progression.

In order to closely mimic the dimensionality and the coherence of the native tissue, we developed an advanced 3D model consisting of spheroids in a tailored melt electrowriting (MEW) scaffold, which formed an adipose-tissue like structure and could be long-term cultured. In addition, the triglyceride (TG) content and physiological function of the model were tunable through fatty acid supplementation.

Methodology: Spheroids were placed in MEW boxes, differentiated along the adipogenic lineage, and cultured for up to 10 weeks. Differentiation and stimulated lipid storage after oleic acid (OA) supplementation were assessed via TG quantification and gene expression analyses. Tissue development and ECM production were analyzed by histological/immunohistochemical stainings and scanning electron microscopy (SEM). Physiological function of the adipose model was assessed based on lipolysis and glucose uptake assays.

Results: The adipose model exhibited pronounced adipogenic differentiation indicated by the deposition of large lipid droplets and a high TG/DNA ratio, that increased markedly over time. Analysis of the ECM revealed the formation of a coherent, adipose tissue-specific ECM composition. The structure and phenotype of the fabricated adipose tissue were stable in long-term culture over ten weeks. Supplementation with OA enlarged the average lipid droplet diameter and significantly increased TG content. Furthermore, glycerol secretion upon lipolytic stimulation was boosted. Additional physiological characterization of the adipose model such as response to inflammatory stimuli is currently conducted.

Conclusion: The presented 3D adipose tissue model exhibits a native-like adipose phenotype with mature adipocytes and a tissue-specific coherent ECM, which can be maintained for at least ten weeks. The adipose model is tunable through fatty acid supplementation leading to increased lipid droplet size and TG content and altered physiological functions such as enhanced lipolytic response. The results underline the plasticity of the model and demonstrate its potential as a tool for studies on adipose (patho-)physiology, as well as adipose compartment in complex models to study the interaction between adipose tissue and cancer.

57 - In vitro conjunctiva model and its applications in the 3R context

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The human tear film covers the ocular surface and comprises a protective layer against microorganisms, mechanical stress, and drought. Lacrimal glands secrete the aqueous layer which builds the largest part of the tear film. Meibomian glands secrete lipids on the ocular surface to prevent fast evaporation. Conjunctival mucins build the third layer to smoothen the tear film and protect against adherence of pathogens. If one of those tissues is impaired, the tear film loses stability which can cause Dry Eye Disease (DED). Independent of the underlying cause, DED can cause blurry vision, sensitivity to light or inflammation of the ocular surface. While this disease is still mainly investigated on animal models, complex 3D models are on the rise to reduce and replace animal models. We developed a 3D in vitro conjunctiva model that shows important characteristics of native human tissue such as fibroblasts in a connective tissue equivalent as well as a multi-layer epithelium with goblet cell differentiation. Epithelial cells showed specific marker expression of all known conjunctival mucins. Goblet cells were verified via Alcianblue and MUC5AC staining. We were able to induce inflammation via TNF- α application in our model in order to simulate DED symptoms in vitro. This provides a new platform for drug testing and thus, the possibility to reduce animal experiments. In this context we are developing drug delivery systems based on micelles and hydrogels to treat causes and symptoms of conjunctival syndromes. As a next step, we are developing a co-culture model of conjunctival tissue and meibomian gland organoids to add an additional compartment of the tear film.

56 - Investigation of mitochondrial function in hiPSC-derived neurovascular in vitro models of PARK2 copy number variation carriers with adult ADHD

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Copy number variations (CNVs) in the *PARK2* gene have been shown to be associated with attention deficit hyperactivity disorder (ADHD). Parkin is involved in mitochondrial quality control, indicating that *PARK2* CNVs might impair mitochondrial energy metabolism. Blood-brain barrier (BBB) disruption has been implicated in ADHD, since changes in BBB functionality and metabolic activity can lead to barrier opening and a disrupted microenvironment, disturbing neuronal function.

While treatment with stimulant medication can alleviate symptoms of ADHD, about 50% of adult patients are non- or only partial-responders to pharmacological therapy, demonstrating that development of novel medication is inevitable. According to the 3R principles, an isogenic microfluidic *in vitro* co-culture model of the neurovascular unit (NVU) based on human induced pluripotent stem cell (hiPSC)-derived brain capillary endothelial-like cells (BCECs) and cortical neurons was established to provide a standardized platform for drug screening in ADHD treatment.

To investigate *PARK2* CNV-associated functional and metabolic changes at the NVU, we analyzed BCECs derived from hiPSC lines of ADHD patients with *PARK2* CNVs compared to wildtype carriers with (and without) ADHD. Mono-cultured BCECs of *PARK2* CNV carriers with ADHD did not display signs of disrupted barrier integrity and impaired cellular functionality under baseline conditions. However, preliminary Seahorse extracellular flux analysis revealed that both mitochondrial-related and glycolytic ATP production of BCECs with *PARK2* CNVs was slightly diminished compared to wildtype controls. Within BCECs, mitochondrial oxidative phosphorylation accounted for just over a third of total ATP production, suggesting that BCECs utilize primarily glycolysis for ATP synthesis. Furthermore, co-culture and microfluidic culture conditions were established for future studies.

Compliant with the 3R principles, our work will promote drug screening in ADHD treatment, thereby improving drug-related therapy approaches at the BBB and NVU. Additionally, novel therapeutic approaches will be validated to restore cellular and mitochondrial function.

55 - ReBiA - Robotic enabled Biological Automation

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The increasing global investment in medical research is largely driven by the need to discover and develop specific drugs and therapeutic agents. The majority of the costs associated with their development are incurred during the clinical phase. Typically, only about one in ten drug candidates successfully completes clinical trials, even though they may show promising results in the preclinical phase. The use of in vitro test systems that offer enhanced predictive capabilities early in research could potentially narrow this gap before entering clinical trials. Two-dimensional (2D) cell cultures have facilitated the assessment of drug effects on human cells from target tissues. However, these monolayers do not recapitulate the intricate structure and function of entire organs or tissues and lack critical tissue functions. In response, the focus of tissue engineering has shifted to the creation of three-dimensional (3D) in vitro cell culture systems. These systems use biological or synthetic scaffolds populated with human cells to support tissue-specific structures and functions, closely simulating in vivo conditions and improving predictive accuracy over traditional 2D cultures.

The complexity of these tissue models presents manufacturing challenges. Standardizing these systems for widespread industrial use requires minimizing human intervention, which we believe will leverage their benefits. Recent advances include the development of a robotic system called ReBiA (Robotic enabled biological automation), which increasingly automates cell culture tasks. This system doesn't prioritize high throughput, but instead is designed to quickly adapt to new processes using a dual-arm robot that is compatible with conventional laboratory materials. This versatility is evidenced by data showing successful automation of various tissue model production from Transwell-based skin models to tumor spheroids and intestine organoids in Matrigel over the past 16 months. This system improves the consistency of 2D and 3D tissue engineering by reducing human error and automates the documentation of process parameters - such as pipetting volumes and temperatures - and stores this data for future reference. We are also exploring the integration of metabolic sensors to non-invasively monitor model quality during cultivation. This comprehensive data collection helps predict developmental outcomes at an early stage, potentially saving time, cost and resources.

54 - Breast cancer-on-chip model for efficacy and safety testing of CAR-T cells

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Physiologically relevant human in vitro models that recapitulate the challenges of solid tumors and the tumor microenvironment (TME) are highly desired in the chimeric antigen receptor (CAR)-T cell therapy field. To evaluate CAR-T cell efficacy and safety, we developed a microphysiological breast cancer model on a chip that incorporates relevant TME components and allows constant perfusion of CAR-T cells.

The chip is tailored to compartmentalize tumor aggregates of a triple negative ROR1+ breast cancer cell line MDA-MB-231 or patient-derived tumor organoids (PDOs) obtained from metastatic breast cancers. They were loaded into microfluidic tumor chambers located underneath a medium channel that is lined with primary microvascular endothelial cells. ROR1- or CD19-targeting CAR-T cells or control untransduced T cells from the same donor were constantly perfused (20 µL/h) through the medium channel, where the T cells were able to extravasate from the endothelium and infiltrate the tumor. The platform was utilized to assess on-target on- and off-tumor cytotoxicity of CAR-T cells via quantitative imaging analysis (CAR-T cell recruitment, tumor infiltration, tumor growth), monitor “real-time” cytokine release kinetics (IL-2, IL-6, IL-10, IFN-γ, TNF-α, and granzyme B), and safety intervention by controlling the immune reaction with a pharmacological on/off switch using dasatinib.

Throughout the culture period of 8 days, we found that CAR-T cell infiltration into the tumor aggregates, their persistence, and ability to control tumor growth depended on the tumor's ROR1 expression. Additionally, high levels and release kinetics of inflammatory cytokines typically observed in the clinic to monitor the development of cytokine release syndrome were observed in CAR-T cell condition, which was significantly higher than in control T cell condition at almost all measurement time points. Applying dasatinib during the chip culture enabled a temporal control CAR-T cell response as a safety measure. The ability to investigate the efficacy and safety of CAR-T cell therapy and recapitulate patient heterogeneity in this model may provide new insights into the mechanisms of CAR-T cell-mediated cytotoxicity in a physiologically relevant TME. This, therefore, provides the opportunity for a broad application in the bench-to-bedside translation and acceleration of the preclinical development of novel CAR-T cell products.

53 - Proof of concept for using a human *in vitro* primary mucosal respiratory model for online-generated particle exposure studies

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Introduction New approach methodologies (NAMs) are promising methods to replace animal models in experimental inhalation toxicology. For ultrafine Particles (UFP), WHO primarily bases guidelines on epidemiological data and no recommendations currently exist for UFP. Reliable *in vitro* data would contribute to mechanistic insights and support risk assessment. Such experimental particle toxicology research requires multidisciplinary input to enable particle characterization, suitable biological test-systems, and sophisticated exposure setups. Applying feasible NAMs in this field is essential to provide data of high translational value.

Methods We utilized commercially available membrane supports to culture respiratory mucosa from 5-10 human donors (assay-dependent). Cells were isolated from tissue retrieved during nasal surgery and cultured in the apical (epithelial cells) and basal compartments (fibroblasts) of inserts to generate respiratory mucosal models. The models were transported to an exposure facility. High organic component UFP were generated by a combustion aerosol standard (miniCAST) soot generator and characterized. Cyto- and genotoxicity, oxidative stress, cytokines and epithelial barrier integrity were evaluated 2 hours after exposure.

Results Models differentiated to the mucociliary phenotype over ~45 days. They could successfully be transported and exposed in the exposure facility without significant differences between incubator and clean air-controls. Particles had a peak mobility diameter of ~40-50 nm and contained toxicologically relevant polyaromatic hydrocarbons (PAH) such as benzo[a]pyrene (BaP) in high concentrations. Post-exposure analyses showed no cyto- or genotoxicity after 2 h. Expected high baseline expression of IL-6 and IL-8 was confirmed. A slight tendency towards barrier disturbance was observed in 9 donors and evidence of donor-specific oxidative stress was found.

Discussion Using standardized inserts enabled model establishment and air-liquid interface exposure of primary human respiratory mucosal models to UFP using miniCAST. The strict control of e.g. exposure temperature over 4 h is partly based on the system's geometry, which is compatible with the commercial inserts. Initial results using our model partly differed from in parallel exposed lung cell lines, highlighting the importance of an informed model choice. We suggest that primary human *in vitro* models as NAMs may be more appropriate to address certain questions in particle inhalation research, than animal models or cell lines.

52 - Fluorescence reporter to investigate cellular activity in biofabrication

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A major challenge in biofabrication is to monitor specific cell properties after the printing process and during different maturation steps. As cells are exposed to many different influences such as shear-stress during printing which potentially lead to cell death, reporter systems for cellular activity are established. We are using a protease-sensitive peptide reporter system that can be site-specifically incorporated into alginate-based bioinks and subsequently provide insights into cell function. Protease sensitive sequences are identified using proteomic identification of protease cleavage sites (PICS) assays. Förster resonance energy transfer (FRET) responsive peptides were designed and used as read-out system. This reporter system enables a qualitative and quantitative evaluation of cellular activity in bioprinted alginate or alginate dialdehyde (ADA) constructs. In future, the reporter peptides are adaptable for different bioink platforms.

51 - Advancing Immunological Insights through 3D Modeling of Fibrosis in hiPSC-Derived Skin Organoids

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In the study of skin diseases, mouse models have traditionally been utilized. However, these models present limitations in faithfully recapitulating human skin physiology and pathology, as murine skin differs significantly from human skin in structure, immune response, and wound healing mechanisms, thus hindering translational relevance. Moreover, the reliance on animal models raises ethical concerns and may not fully align with the principles of the 3Rs (replacing, refining, reducing) in animal testing. In response to these challenges, researchers have increasingly turned to *in vitro* models for studying skin biology and diseases. However, conventional models often lack the three-dimensional complexity and dynamic interactions found in native skin, making its accurate replication quite difficult.

Recognizing these limitations, our research utilizes human induced pluripotent stem cells (hiPSCs) to create three-dimensional skin organoids (SO) that closely resemble the architecture of native skin, following the optimized protocol developed by Lee et al.

Within these hiPSC-derived SO, we characterized a spectrum of skin-specific cell types, such as keratinocytes (CK5), dermal cells (vimentin), adipocytes (nile red) and intricate hair peg formation, indicated by the expression patterns of CK5 and CK17. Additionally, we are exploring the induction of skin fibrosis within the SO using transforming growth factor-beta-1 (TGFβ-1) and culturing the organoids for 7 days. Characterization of skin fibrosis involves immunofluorescence staining for α-smooth muscle actin (α-SMA), Ki67, Fibroblast activation protein (FAP) and collagen 1.

Furthermore, *in vitro* models often lack essential immune components necessary for comprehending both physiological and pathological mechanisms. To bridge this gap, we are integrating hiPSC-derived macrophages (RFP-tagged), which were developed in the research group led by Nico Lachmann, into the SO for a period of at least 21 days. By combining these approaches, we aim to elucidate the contribution of macrophages to fibrosis progression. Our study monitors changes in tissue morphology, extracellular matrix deposition, and inflammatory responses in the presence of fibrotic stimuli.

In summary, our research underscores the significance of hiPSC-derived skin organoids in advancing our understanding of immune-related skin disorders, offering a more translational alternative to animal models.

50 - 3D cell culture as useful tool for melanoma research and investigations on tumor therapeutics

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Among the types of skin cancers, melanoma is the most dangerous with estimated 330 000 new cases worldwide in 2022. Early metastasis and dormancy are two particular hallmarks of melanoma that make it so difficult to treat. Even though large progress was made in melanoma research, especially molecular and phenotypical changes in early tumor development still need to be understood. In the context of tumor therapy, new therapeutic options e.g. targeted therapies, such as BRAF inhibition, were established. However, therapy resistance is still a problem. Our investigations in 2D cell culture regarding resistance mechanisms showed that KRAS, which is acting upstream of BRAF, also plays an essential role in melanoma proliferation and therefore may be a therapeutic target.

Although valid research data from cell culture on resistance development in melanoma is existing, we would like to expand and improve this knowledge and established a complex 3D tumor model to investigate tumor cell behavior. The 3D model is based on different biomaterials, in which tumor cells are embedded. These 3D models offer the opportunity to overcome certain drawbacks of the common 2D cell culture but at the same time come closer to animal models or *in vivo* situations.

As it is already known for BRAF inhibitors that resistance can occur, we wanted to mimic this phenomenon in our 3D model. This gives us the opportunity to investigate putative mechanisms that are involved in the occurrence of resistance.

In our experiments, we treated tumor spheroids in alginate with common BRAF inhibitors, Vemurafenib and multikinase inhibitor Sorafenib. FUCI reporter cells were used to monitor the cell cycle during the experiments. Interestingly, the treatment with Vemurafenib led to a stable G1 arrest of the cells, however not final killing of the tumor cells. Sorafenib treatment seemed to lead to complete cell death after 7 days of treatment, nevertheless, we revealed growing tumor cells after stopping treatment at day 7 or even with continuous treatment after 28 days, suggesting that some cells survive the treatment and become resistant. These results show that this is a promising model to study the occurrence of resistance.

49 - Innovative Approaches to Skin Organoid Technology: Automation and Organ-on-a-Chip Integration

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Skin organoids derived from induced human pluripotent stem cells (hiPSC) hold immense promise as in vitro models for studying skin biology and disease. They mimic the complexity of native skin, including the presence of hair follicles and sebaceous glands, and provide a more physiologically relevant environment for research. Despite these benefits, current methods for their differentiation present significant challenges, as do their inside-out orientation and lack of vasculature. To address these challenges, this project proposes automating the skin organoid differentiation process. By utilizing a commercially available liquid handler, we seek to streamline workflows, reduce labor costs, and enhance consistency in differentiation outcomes. Initial results demonstrate successful differentiation, yielding all requisite cell types and structures, albeit with some manual steps remaining despite a notable reduction in hands-on time.

A pivotal aspect of our research involves establishing a static air-liquid interface (ALI) culture for inside-out oriented skin organoids. By culturing the organoids to mimic the *in vivo* environment, we aim to promote the development of a more robust skin barrier. Culture for 28 days at the air-liquid interface already presented a correct orientation. Further maturation of the epidermis was indicated by the expression of important skin barrier markers, such as loricrin and ZO-1. Addressing the absence of vasculature, we integrate skin organoids into an Organ-on-a-Chip system in cooperation with AG Loskill. This dynamic perfusion system replicates the *in vivo* vascular environment, facilitating studying complex interactions between skin and perfusion. Integration into a chip with precise orientation enhances the viability of organoids within the system, enabling long-term studies of up to 14 days. Co-culture with endothelial cells in the microfluidic channels replicates native skin conditions, as evidenced by cell survival and alignment.

The advancements made in this project have far-reaching implications for diverse scientific and medical applications. From disease modeling to drug testing and beyond, skin organoids' enhanced functionality and reproducibility offer new avenues for research and discovery.

48 - DECIPHERING DRUG RESISTANCE IN ORAL SQUAMOUS CELL CARCINOMA USING PATIENT-DERIVED ORGANOIDS

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Oral squamous cell carcinoma (OSCC) is the most prevalent type of head and neck cancer and originates from different sites in the oral cavity. Half of the patients present to the clinic with advanced metastatic disease that has spread to regional lymph nodes and/or distant organs, such as the lungs. The response to therapy for OSCC is highly variable, and survival rates after first-line treatment remain low compared to other tumour entities. This is due to a lack of understanding of the molecular mechanisms underlying the inter- and intra-patient heterogeneity of OSCC. Therefore, it is crucial to overcome this knowledge gap to develop new and improved therapeutic options for OSCC, especially given its increasing incidence. To address this issue, we collected tissue samples from OSCC patients, including normal oral mucosa and primary, relapsed, and metastatic OSCC. We then established a living biobank of organoids and matched fibroblast lines using this tissue. Patient-derived organoids (PDO) recapitulate the histology of the tumour epithelium of origin and robustly express biomarkers such as CDH1, KRT14, MKI67 and P63. In drug screens, we found PDO-specific responses to inhibitors of key signalling cascades, reflecting inter-patient tumour heterogeneity. Using the patient-matched PDOs and fibroblasts, we are currently characterising the intra-patient drug responses of primary, relapsed, and metastatic OSCC and the influence of the stromal microenvironment. We aim to use our OSCC biobank to define inter- and intra-patient heterogeneity and identify potential new biomarkers and therapeutic targets.

47 - New Approaches to Melanoma Research: Utilising Human Skin Organoids for In Vitro Modelling

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Malignant melanoma is the primary cause of skin cancer-related deaths. Therefore, it is essential to comprehend its pathogenesis and explore new therapeutic approaches. Model systems, such as animal models and human *in vitro* melanoma cell culture systems, have significantly contributed to understanding the complexities of this disease. However, there are still challenges in the translation of findings from animal models to human pathology and in the reproduction of an appropriate microenvironment in human *in vitro* models.

To address these challenges, we have successfully generated skin organoids from human pluripotent stem cells that closely mimic the characteristics of native human skin. These hair-bearing skin organoids provide a physiologically relevant basis for studying melanoma progression. Using this system, we aim to establish a thorough *in vitro* melanoma model. We cultured melanoma cells with skin organoids for 21 days to establish a model that captures the nuances of the tumour microenvironment, allowing the study of melanoma progression and the intricate interactions between tumour and stroma.

To this end, eight different melanoma cell lines were screened for their ability to form tumour spheroids. These cell lines were then co-cultured with skin organoids, resulting in tumour-skin organoids. Immunofluorescence staining was used to analyse tumour markers like HMB45 and assess changes in the microenvironment, particularly the presence of cancer-associated fibroblasts. This approach provides valuable insights into the interaction between melanoma cells and their environment in our model.

In conclusion, this work presents a new method for melanoma research, using human skin organoids to create a physiologically relevant melanoma model. This model overcomes the limitations of existing systems and allows for the investigation of tumour-stroma crosstalk. The research will contribute to the development of more effective melanoma therapies through preclinical drug testing.

46 - Drug effects on human patient-derived small intestinal organoid monolayers

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The small intestine is the site of drug absorption and is thus a highly relevant model for studying drug toxicity, permeability, and influx/efflux. In 3D culture, intestinal organoids form bubble-like structures with the inside (apical side) of the structure corresponding to the lumen (side of absorption) and the outside (basolateral side) corresponding to the blood vessel-facing area. This configuration makes any treatment with compounds that are absorbed on the apical side challenging. If the same organoid line is cultured on a transmembrane insert, however, a polarized monolayer is formed with the apical side on the top and the basolateral side on the bottom, allowing for assessment of drug permeability and barrier integrity.

We assessed the effects of several drugs on monolayer integrity of a patient-derived organoid (PDO) small intestine (duodenum) line. Single cells from duodenum PDO seeded into 24-well inserts produced confluent monolayers at which time trans-epithelial electrical resistance (TEER) was measured daily. The ability to monitor the monolayer culture over a long-time course (at least 3 weeks) permits observation of effects such as cumulative drug toxicity that may be missed with short-term assays. For example, the highly potent chemotherapeutic drug afatinib is poorly tolerated by the gut, resulting in a 96% incidence of diarrheagenic side effects. Treatment of duodenum monolayers with afatinib resulted in cell death and monolayer breakdown at its IC₅₀ value of 0.5 μ M. Treatment at lower concentrations, however, demonstrated a dose-dependent lag in the increase of TEER values over time. In contrast, a hypertension drug, prostacyclin, has a very short half-life in the systemic circulation, making it difficult to ascertain longer-term effects. A TEER time course of duodenum PDO monolayers continuously treated with prostacyclin, however, revealed a drop in TEER values after 11 days compared to the non-treated control, suggestive of a cumulative effect of the drug on membrane integrity.

Patient-derived organoids can provide patient-specific, preclinical, and medically relevant data and provide a predictive model of side effects for therapeutics.

45 - *Galleria mellonella* as a sustainable in vivo model for nanotherapeutics evaluation

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The *Galleria mellonella* insect model (greater wax moth larvae) is an alternative, more ethical, simple and low-cost *in vivo* model that shows a good correlation with mammalian infection models. Besides being a powerful *in vivo* model to study virulence of human pathogenic agents, *G. mellonella* is also being widely used in toxicity studies and to access the efficacy of therapeutic drugs.

In this communication we will present the *G. mellonella* model and its comparison with major invertebrate models. We will also present different applications where we validated this model in the last years, namely studying the cytotoxicity and biocompatibility of inorganic and organic nanoparticles, and bacteriophages.

44 - Plasticity of macrophages from human induced pluripotent stem cells facilitates their use in drug-screening and -safety bioassays

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The field of immunotherapeutics represents a rapidly growing field with novel medicinal products showing highly promising efficacies in several (pre-) clinical studies. Identification and testing of them often relies on animal models, leading to failure in clinical translation. Here, human induced pluripotent stem cells (hiPSCs) have become a widely used alternative to better represent human disease phenotypes allowing a better evaluation of new drugs effectiveness. We therefore developed a GMP-compatible platform, that allows to produce different immune cells from hiPSC at different scales ranging from classical tissue culture plates to intermediate- or even industry-compatible bioreactors. Among immune cells, macrophages are a key cell type that acts as important pro- and anti-inflammatory mediator in the onset and progression of various diseases, including fibrosis, cancer or autoimmunity. Our iPSC-derived macrophages (iPSC-Mac) exhibit convenient phenotypic properties, classical phagocytotic functionality and show a transcriptional profile like blood-derived macrophages, though with a more primitive fingerprint. Given the importance of macrophages in various diseases, iPSC-Mac demonstrate a flexible adaptation into several immunological states *in vivo* and *in vitro*, enabling studies to investigate their impact on numerous disease-relevant processes. Providing specific cytokines, our iPSC-Mac can easily be modulated into pro- or anti-inflammatory phenotypes. In line with their immunogenic plasticity, we developed a highly reproducible platform for (i) testing of novel immune-modulatory drug candidates in potency assays or (ii) safety/pyrogenicity testing in the Monocyte-Activation-Test (MAT). With the development of the MAT, a successful move away from animal testing has been initiated. To consolidate this development, iPSC-Mac can offer a great opportunity by circumventing the current limitations of using artificial cell lines or donor heterogeneity in primary human cells. In this regard, we demonstrated a superior sensitivity of iPSC-Mac towards endotoxins (LPS, MALP-2) and non-endotoxins (Staphylococcus aureus) compared to primary human cells. In summary, we present a scalable and efficient method to generate standardized human macrophages from hiPSC that can be sensitized for different applications, like drug safety, screening, and discovery, but also fundamental biological questions in organ and disease development, altogether helping to replace animal experiments in modern medicine.

43 - Tracking tumor resistance with bioinformatics, clustering, and pseudo-time analysis in 3D Pancreatic cancer tissue models treated with TGF- β and/or gemcitabine

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3D tissue cultures are important to better model diversity of cell behavior in complex environments such as the resistance of PANC1 cell to gemcitabine treatment in tissue niches in the human body. Pancreatic cancer PANC1 cells grown on a decellularized jejunal tissue matrix (SISmuc = Small Intestinal Submucosa with preserved mucosa) respond to treatments with gemcitabine differently depending on tissue niches. TGF- β , induces resistance independently of the tissue niche. Utilizing both 2D and 3D tissue models, this research employs single-cell RNA sequencing to examine the effects of these treatments both alone and in combination. We explored alterations in gene expression across a hypothetical time frame by employing pseudo-time analysis. We reconstruct the developmental trajectory of cells, organizing them along a pseudotime axis. The fundamental assumption is that cells exhibiting comparable gene expression patterns are presumed to be at analogous stages in the developmental process. The study identifies key tumor drivers and pathways through a pipeline integrating spatial-temporal considerations and mathematical objective evaluations, elucidating the mechanisms behind Gemcitabine resistance and the characteristics of invasive cancer cells.

We find proliferation and cytoskeletal markers promoting tumor growth and metastasis, are associated with enhanced tumor motility and differentiation, driving the aggressive and untreatable nature of some cancers. Moreover, marker genes involved in tumor resistance will be shown and are analyzed.

Our analysis supports the strategy of co-treatment with gemcitabine and targeted inhibitors against identified resistance markers in tumor cells. With the support of future clinical studies, this approach should also improve therapeutic outcomes for pancreatic cancer patients.

42 - Matrix-based pancreatic ductal adenocarcinoma (PDAC) models including tissue niches: Replacement of animal-related approaches in drug testing

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PDAC is the most common form of pancreatic cancer with high chemo- and radioresistance which often spreads to bone. One of the main drivers of resistance is the highly desmoplastic tumor microenvironment (TME) defined by a dense envelope of extracellular matrix proteins and stromal cells. Despite the introduction of new models, components of the TME are mostly neglected, leading to high-attrition rates in the clinic.

In order to overcome this limitation, we developed biological and synthetic matrix-based *in-vitro* models which can mimic morphological features of native tumors. We compare the impact of static and dynamic culture conditions in bioreactors and also included mesenchymal cells extracted from patients' material. We were able to replicate features of our previously developed biological matrix on a fully synthetic electrospun matrix. We developed a bone metastasis model including mesenchymal stromal cells differentiated to osteoblasts. Coculture with osteoclasts show in first results a lining of the osteoid-like structures within the generated tissue niche. As an immunotherapeutic approach, we tested efficacy of CAR T cells.

Thus, we demonstrate how a fully synthetic scaffold allows us to produce an animal-free PDAC model which includes stroma components with resemblance to in-vivo conditions offering new options for drug testing.

41 - Hyaluronic acid based bioink platform with multi-functional crosslinkers for the controlled differentiation of MSC

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Synthetic polymers are frequently used as bioink components to ensure a good printability and long-term stability of 3D bioprinted constructs. However, they are often associated with a lack of biocompatibility resulting in compromised cell survival, differentiation, and tissue development. Hyaluronic acid (HA) represents an attractive material for biofabrication approaches since it is a main component of the native extracellular matrix (ECM) providing structural and mechanical support for the embedded cells. Nevertheless, stand-alone bioinks which only consist of modified HA are still rare or lack biological evaluation [1].

Here, we would like to present our versatile bioink platform based on HA derivatives with different crosslinkers for the generation of cartilage as well as customizable bioinks for various biological applications. Previous studies established a bioink utilizing a dual-stage crosslinking mechanism, containing thiolated hyaluronic acid (HA-SH), acrylated (PEG-diacryl) and allylated (PEG-diallyl) polyethylene glycol. Printability is achieved in a Michael Addition, while long-term stability is accomplished by UV-induced thiol-ene click chemistry [2]. Here, we would like to present our newly developed bioink consisting exclusively of reactive HA derivatives. It's also based on a dual-stage crosslinking mechanism, with an initial Michael addition between HA-SH and acrylated HA (HA-A). Secondary crosslinking post-printing is based on a radical polymerization of remaining acrylates providing long-term stability and shape fidelity. Both bioink platforms are variable in polymer content, network density and construct stiffness. All tested bioinks enable chondrogenic differentiation of embedded mesenchymal stromal cells (MSC) allowing to investigate ECM development or the analysis of bioactive substances to counteract inflammatory environments. We intend to further investigate the impact of polymer content, molar ratios, and polymer composition. Additionally, we developed a potential bioink using only HA-A and high molecular HA (hmHA) to generate gradient materials with different network porosity by adjusting the degree of substitution (DS) of the reactive component.

Through further conceivable modifications using peptides or growth factors, the bioink platform presented here is a useful and versatile tool for biofabrication to generate biological constructs and investigating relationships between material properties and biological responses.

[1] Petta D et al., Biofabrication 12:032001, 2020.

[2] Hauptstein J et al., Macromol. Biosci. 22:e2100331, 2022.

40 - Using niche-inspired oral epithelial organoid culture conditions to decipher oral epithelial tissue heterogeneity

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The oral cavity is a multifunctional organ comprising unique tissues with a high degree of structural heterogeneity. Each oral site harbours epithelial stem cells that are influenced by distinct niches. The site-specific molecular mechanisms underlying stem cell fate decisions remain poorly understood. Organoid technology allows stemness modulators to be tested in vitro in a reductionist manner due to the lack of niche cells. We have recently described a protocol for the generation of organoid cultures from mouse tongue epithelium. We have now applied this protocol to other major oral epithelial tissues such as buccal mucosa and hard palate. Using transcriptomics and histology, we found that oral site-specific epithelial differentiation can be maintained in the short term. To develop site-specific culture conditions, we modulated known regulators of epithelial stemness and differentiation such as BMP, Notch, FGF and Wnt signalling in vitro to direct oral site-specific differentiation. Based on our single cell sequencing data, we propose fibroblasts as a driver of oral mucosal site heterogeneity. To understand the oral site-specific interaction and the influence of fibroblasts on OESCs, we performed fibroblast conditioned media experiments on our organoid cultures, revealing fibroblasts as a source of FGF signalling within the oral epithelial stem cell niche. Together with other recent advances, these methods provide new tools for the study and application of oral epithelial tissue heterogeneity, for example to better understand oral tissue regeneration and disease.

39 - Induced vasculogenesis as a strategy for vascular formation in biofabrication

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Vasculature is essential to supply tissues and organs with oxygen and nutrients, as well as for waste removal. Although it is equally crucial in artificially generated tissue-like structures, the *in vitro* generation of functional vascular networks remains a major challenge in the fields of tissue engineering and biofabrication. Current biofabricated models attempt to mimic the triple layered structure of native blood vessels by using adult vascular cells. Nevertheless, the coordinated morphogenetic events during vasculogenesis are key for vessel functionality and stability. We previously demonstrated that iPSC-derived mesodermal progenitor cells (hiMPCs) remain viable and retain their vasculogenic potential after extrusion, making them suitable candidates for extrusion-based 3D bioprinting approaches. Still, their patterning into defined shapes is yet to be established. Our study aims to biofabricate complex hierarchical vasculature based on vasculogenesis by bioprinting hiMPCs. We evaluated the vasculogenic potential of hiMPCs encapsulated in different biomaterials and developed an embedding bioprinting system to print bioinks into tubular structures.

Alginate and gelatin-based formulations were used as biomaterial components in the development of hiMPCs-laden bioinks for 3D bioprinting. Given alginate poor mechanical properties and lack of bioactive moieties, xanthan gum and collagen type I were added for rheological tuning and biological enrichment. Alternatively, gelatin is a popular biomaterial due to its ECM resemblance. GelMA is a chemically modified gelatin molecule that can form covalently crosslinked networks upon light exposure in the presence of a photoinitiator. The biomaterials were combined with hiMPCs and the bioinks were printed into defined tubular constructs using embedding bioprinting. The printed structures remained stable in long-term culture. While the bioinks supported cell viability and differentiation into vascular cell types, the morphogenetic events were ruled by different mechanisms depending on the composition of the matrix. Overall, stem cells showed to be suitable building blocks for the biofabrication of multilayered wall vascular structures and are promising for the *in vitro* production of vasculature.

38 - A modular droplet-based platform for medical, pharmaceutical and biotechnological research

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The challenges to apply microfluidic systems as alternatives to animal models are (i) the generation of *in vivo*-like 3D-cell cultures, (ii) the realization of *in vivo*-like microenvironment, and iii) the robustness and easy handling of the whole system.

Iba developed a droplet-based platform called “pipe based bioreactors (*pbb*)”. Droplets of one μL volume are serially arranged in polytetrafluoroethylen tubes separated by a hydrophobic fluid like perfluorodecalin. Different tumor spheroids, embryonic murine stem cells and primary cells were successfully cultivated. The *pbb*-platform enables all steps, from development of self-organized 3D-cell cultures from single cells, dosing of substances as drugs, medium components or assay reagents to the analysis of cell proliferation or the dose-response, in one closed system. Therefore, one have no problems with evaporation or infections of the performing experimenter. Cross-contamination between each droplet was not detected. The gas permeability of the tubes realize a long-term cultivation under normoxia or hypoxia conditions in a high-throughput manner and allow e.g. the development of therapies for precision medicine. Different microfluidic modules were developed to realize basic functions with high precision: (i) droplet generation, (ii) mixing of cell suspensions and cell culture media in the droplets, (iii) droplet content detection, (iv) active fluid injection into serially arranged droplets, and (v) microscopic and spectroscopic analyses. The analyses of each sample in combination with artificial intelligence algorithms will support a comprehensive assessment of the whole process. The modular character of this platform allows flexible combination of modules for specific medical, pharmaceutical or biotechnological applications. The automation of most processes supports the robustness of the droplet-based microfluidic platform.

37 - Advancing in vitro models for oncological drug development: Integrating 3D-cultures and microphysiological systems

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The complexity and expense of drug discovery processes emphasize the need for innovative *in vitro* models that bridge the gap between traditional cell cultures and animal studies and help to reduce the overall amount of animals used. Our research group addresses this challenge through the development of two distinct yet complementary microfluidic systems, each contributing to the advancement of oncological drug development.

The first system was developed for three-dimensional (3D) cell-based models for cancer research, focusing on the interaction between tumor spheroids and their microenvironment. Within the newly designed cultivation chamber, tumor spheroids were cultivated within a collagen I matrix in the presence of fluid flow to study the invasion of cancer cells from spheroids into the matrix. Here, the morphology of spheroids is influenced by flow and the spheroids in flow conditions are characterized by the absence of a large necrotic core. Additionally, this influence extends to an increase in the size of invasion area, coupled with an increase in expression of genes related to epithelial-mesenchymal transition. These results highlight the importance of fluid flow in cancer research.

Additionally, an integrated pre-clinical microfluidic system was developed to culture organotypic tissues. Central to this system is a novel 3D-polymer chip fabricated from polycarbonate, facilitating the cultivation of precision cut liver and tumor slices. By optimizing perfusion conditions and exploring various medium compositions, we have established a platform for simultaneous efficacy and toxicity testing with increased viability and functionality of the cultured slices. Two organ systems can help to decrease the use of animal models and accelerate drug development, but cannot replicate the complexity of the whole organism. Our future work will focus on integrating further organotypic cultures

In conclusion, our innovative microfluidic systems offer promising solutions to the challenges of drug discovery by providing advanced in vitro models that bridge the gap between traditional cell cultures and animal studies.

36 - Synthetic fibrous scaffold-based 3D small intestinal epithelial *in vitro* models used for bioavailability studies

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Regulatory developments, ethical aspects and differences in drug response between species shifts testing methods more and more from animal studies towards human-based tissue models. Especially in the context of bioavailability of orally administered drugs *in vitro* test systems mimicking the intestinal tract to a high degree are needed. Unfortunately, most current tissue equivalents and even new developments are assembled by animal materials as scaffold material such as collagen or decellularized tissue or based on a simple, semipermeable membrane which is of synthetic nature. Next to the scaffold material, the cell source used for model setup can influence the bioavailability of drugs.

Therefore, we present here a comparison study between different model types representing the intestinal epithelium to demonstrate the relevance of scaffold materials as well as cell sources for bioavailability studies of drugs. In our study we compared Caco-2 cell-based models, organoid-derived cell line-based models and organoid-based models' setup either on a standard synthetic scaffold (PET), a biological scaffold called small intestinal submucosa (SIS) or an electrospun synthetic fibrous 3D scaffold seeded with fibroblasts (StRoMa). Our results demonstrate that organoid-based models as well as organoid-derived cell line-based models' setup either on SIS or StRoMa display organ-specific epithelial barrier integrity investigated by FITC-dextran (4kDa) and transepithelial electrical resistance (TEER) measurements. In addition, a high comparability to the *in vivo* tissue was demonstrated by bioavailability studies with reference substances that are specified by the FDA. In contrast, model's setup on PET demonstrate high TEER values together with low permeability values.

Therefore, our human intestinal tissue models based on StRoMa represents an appropriate *in vitro* test system for bioavailability studies in the context of pre-clinical screenings, as the material is animal-free, standardized and shows a close similarity to the *in vivo* situation.

35 - Semi-automated Cell Isolation for Tumoroid Culture: A Promising Alternative to Animal Testing for Drug Evaluation?

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Tumoroid culture, a novel technique in biomedical research, offers a promising substitute for traditional animal testing in drug evaluation processes. This innovative approach involves growing three-dimensional cell structures derived from patient tumors in a controlled laboratory environment. These tumoroids closely mimic the complex microenvironment and cellular interactions found within tumors *in vivo*, providing a more physiologically relevant model for drug testing compared to 2D cell cultures. Unlike conventional cell lines, tumoroids can retain the genetic heterogeneity and structural features of original tumors, allowing for personalized medicine approaches and tailored drug screening protocols. This enhances the predictive value of drug efficacy and toxicity assessments, potentially filling the gap between preclinical studies and clinical trials. We are currently standardizing our tumoroid culture protocol utilizing patient-derived HNSCC cancer tissue samples. Using the TissueGrinder technology (Fraunhofer IPA) we mechanically dissolve cancer tissue to obtain single tumor cells which we regrow as 3D spheroid culture in specific culturing plates. Once the spheroids have reached a stable phase, they are used for a variety of analyses, especially for toxicity assessment and drug testing. Our goal is to establish a reliable and standardized model for the investigation of onco-modulatory targets on patient derived tumoroids in a cost and time efficient way. Furthermore, we will implement and transfer these protocols towards our in-house microfluidic flow cytometry system CTCelect to further identify and isolate tumor-specific sub populations in the from tissue biopsy isolated cells. In conclusion, tumoroid culture represents a transformative approach to drug testing that offers numerous advantages over traditional animal models, including enhanced physiological relevance, personalized medicine capabilities, and high-throughput screening capacity. As the field continues to advance, tumoroid culture is poised to revolutionize drug discovery and accelerate the translation of promising therapeutics from the laboratory to the clinic, ultimately benefiting patients and advancing cancer research.

34 - Enhanced Optical Evaluation of Bio-Ink Printability

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In tissue engineering, biofabrication techniques aim to create complex 3D structures using cell-laden hydrogel matrices (bioinks) to produce functional tissue models or organs. This approach offers potential reductions in *in vivo* experiments, mitigating significant financial and time investments while addressing ethical considerations associated with animal experimentation. Several biological tests are currently required to assess the suitability of new bioinks. Key considerations when evaluating bioinks include ensuring cell survival during printing, maintaining scaffold shape and resolution for nutrient diffusion, matching mechanical properties to target tissues, and promoting tissue maturation over time.

Rheological tests offer valuable insights into bioink behaviour, such as shear-thinning, elastic recovery, and shear stress. These factors directly impact the printability and exposure of the printed cells. Various printing tests, like Filament Fusion and Collapse Tests, are used to assess the structural stability and material spreading, often with time-dependent considerations. To better understand bioink printing behavior, complementary optical methods, known as A.S.T.R.I.D or “Advanced STRut Investigation Device” and B.E.R.I.T. or “Best Elongation Rheology Investigation Testing”, have been developed alongside traditional shear rheological tests. This enables improved characterization and modeling of bioinks.

This study examines a straightforward imaging setup combined with physical models to better comprehend material behaviour, particularly under extrusion pressure. The research investigates how rheological properties impact printing characteristics and how they can be influenced by polymer concentration and solvent type. Using alginate as a model system, the study evaluates different cell densities to determine the impact of cells on rheological properties, finding that cells do not have a significant effect. Considering these factors, the printability of the bioink primarily hinges on the hydrogel properties. Consequently, valuable insights into the materials' potential can be gained through the development of printable bioinks using analytical tools, eliminating the need for extensive cell printing experiments.

33 - Composite Chitosan Membrane as a replacement of amniotic membrane for the treatment of ocular surface defects

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The ocular surface typically consists of corneal, limbal, and conjunctival epithelial cells. Any defects on these cells may lead to different ocular surface pathologies and even visual loss. Treating ocular surface disorders poses significant challenges, and keratoplasty (corneal transplantation) is often considered as a final option.¹ For decades, amniotic membrane (AM), which is the innermost layer of the placenta, has been used to treat surficial ocular defects because it contains growth factors, cytokines, and other bioactive substances which can help tissue healing and reduce inflammation. In this procedure, the amniotic membrane (AM) is sutured onto the ocular surface with a patch, and it serves as a biological bandage on the damaged area.² However, AM has many advantages like poor availability, unknown composition, opacity, high contamination risk, and fast growth factors depletion. On the other hand, the standardization of the membrane is essential to obtain precise results in clinical use.³ Therefore, one promising strategy to overcome the limitations is using biomaterials with desired ingredients. Upon the broad variety of biomaterials, chitosan was identified to be a promising candidate specifically in the field of ocular surface since it is a FDA approved natural product that is non-toxic, highly transparent, biocompatible, cost-effective, biodegradable, and antimicrobial.^{4,5} For the new composite chitosan membrane, aptamers are combined with chitosan to allow the controlled release of specific growth factors to promote healing over a sufficient period of time. MEW (melt electrowriting) printed polycaprolactone (PCL) fibers are incorporated into chitosan film to provide reinforcement and suturability on eye. Also, fiber-reinforced membranes are produced via casting into SLA-printed molds with micro-sized concentric grooves to facilitate oriented cell growth and migration.

32 - Investigating the role of *Chlamydia trachomatis* infection in cancer development

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Chlamydia trachomatis is an obligate intracellular pathogen, whose genital serovars are the world's leading cause of sexually transmitted diseases. It has been associated with the development of ovarian cancer, but the causality and underlying molecular mechanisms are not yet fully understood. During its biphasic lifecycle, it secretes multiple factors into the host cell to modulate signaling pathways of the host cell to establish and maintain its intracellular niche, acquire nutrients, and evade apoptosis to ensure the completion of their developmental cycle. One of these secreted factors is the nuclear effector (NUE). Upon secretion it is translocated into the host cell nucleus where it associates with chromatin. NUE was also shown to have histone methyltransferase activity towards the host cell histones *in vitro*. Further, *C. trachomatis* promotes proliferation via PI3K- and MEK-ERK pathways and DNA damage in the host cells, while interfering with DNA damage response and downregulating the tumor suppressor p53, all hallmarks of carcinogenesis. During my doctoral studies, I am further investigating the role of *C. trachomatis* infection and the secreted NUE in host DNA damage and cancer development.

31 - Biofabricated gradients for functional tissue models

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One key challenge of the research field of biofabrication is the successful fabrication of functional replacement tissues for humans and disease models to replace animal experiments in pharmaceutical and cancer research. Through the precise deposition of cells and biomaterials with the aid of automated processes such as 3D bioprinting, it becomes possible to mimic the complex, locally varying material properties of native tissue. Most of these tissues contain mechanical and biochemical gradients that, besides the viscoelastic biomaterial properties, are decisive for the fate of cells in the biofabricated construct. To overcome the challenges of extrusion-based bioprinting of gradients, which involves mixing multiple materials with viscoelastic properties and reproducing the complex, multi-material, gradual structures in space and time, suitable fabrication and characterization methods are required. Therefore we developed self-designed printheads with different microfluidic mixing geometries to deposit, in combination with a modified 3D printer, controlled transitions between bioinks, biomaterials, active substances and cells. A suitable quantification protocol enables to determine the quality and the grade of the mixed section. The viability of the cells after extrusion was analyzed for U87 (human glioblastoma cells) and NIH3T3 (mouse fibroblast cells) and compared to commercially available mixers. The results revealed a higher cell viability for the self-designed printheads, while the mixing ratio was similar. Furthermore, we combine standardized testing methods (multi-modal tests in compression, tension, and torsional shear, nanoindentation) with imaging techniques for the characterization of 3D printed gradients. We use nonlinear continuum mechanics modeling and finite element simulations to predict the mechanical response of final printed constructs and to optimize process parameters, the print pattern, and the 3D arrangement in the construct according to the requirements. These approaches enable flexible adaption of the structures to different hierarchies and optimization of bioinks and printheads with regard to printability and shape fidelity of the biofabricated tissue.

30 - Monitoring Neurosphere electrophysiological activity using a novel NeuroMPS with integrated microelectrodes

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In our aging society, the prevalence of neurological diseases is escalating significantly, and human in vitro models are urgently needed to fully capture the complexity of the human brain. The emergence of induced pluripotent stem cells (iPSC) and their differentiation into various cell types have significantly propelled the creation of more authentic human models, such as brain organoids, that accurately mimic human brain features. Yet, the human in vitro models that recapitulate the electrophysiological activity in the brain is still quite limited. To fill this gap, we developed a neuro-microphysiological system (NeuroMPS) integrating iPSC-derived neurospheres and tailored microelectrode arrays, which enables studying the neuronal network activity of the neurospheres in a non-invasive approach.

The neurospheres consist of neurons and glial cells and develop simultaneous network activity after 6 weeks of differentiation. The NeuroMPS is based on two components: i) a custom-designed MEA featuring capped microelectrodes that allow capturing electrophysiological activity from neurites and ii) a tailored glass microwell module.

The novel NeuroMPS enabled the monitoring of the electrophysiological activity of the neurospheres in a time-resolved manner. Additionally, the optically transparent substrate is suitable for various high-resolution microscopy techniques. The platform's sensitivity was tested using multiple compounds (Bicuculine, CNQX, 4-AP) and a neurotoxin (Rotenone). The effects of compound applications were observed from 10 minutes to 48h using electrophysiological and morphological readouts and the effluent analysis. Although changes in electrical activity were detected within 10 min of applying the lowest concentration, morphological and metabolic alterations only appeared hours later and at much higher concentrations. This confirms that electrophysiological assessment of 3D neuronal networks provides a more sensitive approach and possibly better predictability to study the effects of neuroactive and toxic compounds, thereby enhancing the predictability of in vitro methods. The 3D platform discussed here is a truly innovative solution that will aid in developing a new generation of physiologically relevant and high-throughput models for neurological research.

29 - Biofabrication of cellularized and vascularized tissue containers for the transplantation of cells producing therapeutic proteins (project C04, SFB/TRR225)

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The aim of subproject C04 of the SFB/TRR225 is the development of a transplantable tissue container providing *in vivo* secretion of recombinant therapeutic proteins by genetically modified cells, using the rat AV loop model. Recombinant proteins are frequently used as “targeted therapies” However, their usage has several drawbacks, such as high production costs, short half-life, and repeated invasive application. A possible solution is the *in vivo* production of therapeutic proteins by transgenic cells, which are transplanted into the patient's body. As a prototypic example for a biological, we use a recombinant TNFR2-Fc-GpL fusion protein, which corresponds to the clinically approved TNF-blocker Enbrel®. The *Gaussia princeps* luciferase (GpL) domain is used as a highly sensitive reporter. HEK293T cells were lentivirally transfected to stably produce TNFR2-Fc-GpL.

To limit the number of animals used for subsequent experiments, we first tested the producer cells *in vitro* in combination with different bioinks. Alginate-dialdehyde/gelatin (ADA-GEL), as well as recombinant eADF4(C16)-RGD spider silk were identified as suitable hydrogels to support cell viability and secretion of the reporter protein. In order to assess the angiogenic potential, we also performed sprouting assays with endothelial cells.

A hydrogel consisting of 1 % eADF4(C16)-RGD and collagen type I showed the best results *in vitro* and was thus selected for implantation into the rat AV loop model. For this purpose, an arteriovenous loop is formed between the femoral artery and vein. The loop is then placed inside a Teflon chamber filled with the bioink to provide intrinsic vascularization. Five constructs were explanted after four weeks, while the remaining five constructs were explanted after eight weeks.

Blood samples of the rats were taken weekly to measure the levels of the fusion protein, showing detectable levels of TNFR2-Fc-GpL in the serum after two weeks. The further evaluation of the explants is still ongoing. Vascularization and morphology will be analyzed via μ CT, multiphoton microscopy and immunohistochemistry. After suitable combinations of producer cells and bioinks have been identified, the tissue container will be implanted into an arthritis model of the rat, and the therapeutic efficiency will be evaluated.

28 - Visualization of Cas9 proteins in interaction with RNA and DNA by super-resolution microscopy

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Since its discovery, the CRISPR-Cas system has been widely embraced by the gene editing community as a powerful tool to understand and ultimately treat certain types of cancer and genetic diseases. The CRISPR-associated Cas protein can be programmed by a single-guide RNA to bind to specific sequences of the DNA. Depending on the properties of the Cas molecule it can either cause double or single strand breaks, as well as just attach to the DNA without any further enzymatic processes taking place. For a better understanding of the mechanisms of the Cas protein interactions, microscopy methods such as AFM (Atomic force microscopy) or Electron microscopy (EM) have been mainly used. Here we use multicolor super-resolution fluorescence microscopy in combination with Expansion Microscopy (ExM) to visualize the molecular state of the Cas9 molecule bound to the sgRNA and the DNA target at virtually molecular resolution.

27 - Establishment of a hiPSC-derived vascularized *in vitro* model of the endocrine pancreas

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The derivation of pancreatic hormone expressing cells (HECs) from human induced pluripotent stem cells (hiPSCs) presents a routine process described in a variety of publications. However, hiPSC-derived HECs often lack a proper functionality and exhibit an overall immature phenotype compared to their bone-fide counterparts, hindering their use for *in vitro* test systems. Interestingly, transplantation of HECs into a living organism fosters HEC maturation, indicating an essential role of post-transplantation processes for HEC development. One of the possible factors that might trigger HEC maturation is the contact of HECs to newly formed vessels.

To test, whether vascularization can improve the maturation of HECs *in vitro*, we aimed to develop a vascularized *in vitro* model of the endocrine pancreas. To this purpose, we generated HECs as well as arterial endothelial cells (AECs) from hiPSCs according to published protocols. AECs, HECs as well as primary fibroblasts were mixed with a fibrin-based hydrogel and loaded into a microfluidic device, allowing gravity-driven perfusion in a 96-well plate format.

Our data demonstrate the self-assembly of AECs into a vascular network with a laminin and collagen IV containing basal membrane-like structure within 9 days. Connections of the AEC network to the medium channels of the device allowed the perfusion of the vascular bed, proofing the function of the formed network. HECs applied as spheroids together with the AECs could be co-cultured in the microfluidic device. HECs were positive for CPEP, GCG or SST, proving the presence of β -like, α -like and δ -like cells, respectively. AEC networks formed along HEC spheroids, but did not penetrate the already formed spheroids. In an attempt to improve the ingrowth of AECs into the spheroids, pre-endothelialized spheroids consisting of HECs, AECs and fibroblasts were generated. Preliminary data, revealed a positive impact of fibroblasts on the endothelialization.

Summarized, the established microfluidic platform allows the culture of hiPSC-derived HECs in close proximity to a functional, perfusable AEC network. In future, we aim to use the model to study the effects of the vascular interface on pancreatic endocrine development and function and to establish the model for *in vitro* testing.

26 - Automation and Artificial Intelligence in the context of 3R

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In life science laboratories, automated systems can enhanced efficiency, accuracy, and throughput. Nevertheless, automation is mostly limited to work- and time-intensive experimental single process steps rather than entire processes. The **Robotic-enabled biological automation platform** (ReBia) exemplifies the automation of an entire tissue culture workflow for the generation of three-dimensional human tissue models - namely epidermal skin, airway epithelium, and spheroid-based tumor test systems. Besides obvious advantages, e.g. release of capacities, reproducibility and throughput, intelligent process automation in life science contributes to the endeavors of **3R - replacement, reduction, and refinement of animal testing** by increasing availability and quality of alternative testing methods. Artificial Intelligence (AI) techniques further enhance this development. Through integration of AI methods into the ReBia platform, Tissue Engineering processes are monitored to not only increase robustness but to correlate experimental outcomes with process parameters. Moreover, machine learning improves data analysis by automated and non-biased analysis tools, such as the detection of morphological changes in tissue models in response to drug treatments. This lays the groundwork for precise drug screening and evaluation of efficacy. For example, automated AI-assisted analysis of results is applied for the identification of cancer therapy effectiveness, a machine learning model provides a quantitative scoring system. Microscopic image acquisition during automated production and drug administration allows for precise morphological assessments of cultured tumors. A comparative drug screening study demonstrates the full potential of automation and AI in life sciences.

25 - Vascularized metastasis model produced by sacrificial-free direct ink writing

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Human models with high translational capacity are urgently needed for the development of novel anti-cancer drugs. Here, we present a bioprinted cancer model, which allows to study the activity of cytostatic substances [1]. The open design of the model means that any cancer type can be embedded in the hepatic environment simulating liver metastases. The model contains a vascular system which is lined with endothelial cells. It can be connected to a perfusion system of micropumps included in home-made adapters to the multi-well plate. An important step is agar casting to seal the connection between the tubes and the model and to protect the model for long-term cultivation. The model was then used to test the cytostatic activity of anti-cancer drugs on the spheroid embedded in the organ model. After induction of cytochrome P450 enzymes with rifampicin, even a pro-drug (ifosfamide) was converted into its active form and resulted in shrinking of the tumor spheroid. In addition, we aim at replacing animal components in bioprinted organ models. We recently succeeded in producing a fully xeno-free, bioprinted liver model [2]. The first step was to adapt the hepatic cells, which are normally grown in FCS-containing medium to a chemically defined medium. Subsequently, the basement-membrane extract Matrigel in the bioink was replaced by non-animal materials, including collagen from human placenta. Several cell culture methods, such as passaging and cryopreservation were also adjusted to prevent the use of animal components. We believe that xeno-free methods not only contribute to the aim of improving animal welfare, but also produce physiologically more relevant models than conventional cell culture methods making use of animal materials.

[1] Wu, D.; Pang, S.; Berg, J.; Mei, Y.; Ali, A.; Röhrs, V.; Tolksdorf, B.; Hagenbuchner, J.; Ausserlechner, M. J.; Deubzer, H. E.; Gurlo, A.; Kurreck, J. (2024) Bioprinting of Perfusable Vascularized Organ Models for Drug Development via Sacrificial-free Direct Ink Writing, *Adv. Funct. Mater.*, in press.

[2] Ali, A.; Berg, J.; Roehrs, V.; Wu, D.; Hackthal, J.; Braeuning, A.; Woelken, L.; Rauh, C.; Kurreck, J. (2024) Xeno-Free 3D Bioprinted Liver Model for Hepatotoxicity Assessment. *Int. J. Mol. Sci.* 25, 1811.

24 - In-Flow 3D-Bioprinting of Jammed Microgels and Establishment of a New Light-free Cross-linked Hybrid Gel System

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Bioprinting has revolutionized Tissue Engineering and Regenerative Medicine by enabling the fabrication of cell-laden scaffolds with defined spatial organization. It requires bioinks that combine good printability with high cell viability. Recently, jammed microgels have proven to be promising inks as they protect cells during the harmful printing. However, a drawback is their multi-step production since microgels must be created first, then jammed, and finally printed. Here, we introduce an in-flow printing method that combines all these steps to one seamless process. Hereby, cell-loaded microgels are jammed directly into filaments once they leave the microfluidic chip that are printed with an integrated 3D printhead. Our approach has proven to be adapted to various bioinks, including the mostly used alginate gel, ADA-GEL, and an established material that is cross-linked with UV light.

To mitigate the cytotoxicity associated with UV-based cross-linking, we further studied a novel light-free hydrogel system formed via thiol-Michael addition. It combines low molecular weight maleimide-functionalized gelatin with poly-2-oxazoline bearing several thiol groups. The resulting hybrid hydrogels leverage the strengths of both polymer classes, offering enhanced printability and dimensional stability after printing. We delve into the synthesis of the polymers, also investigating the kinetics and variability of the degree of functionalization. Further analysis explores the impact of pH, polymer content, and thiol:maleimide molar ratio on gel formation. The gels exhibit Young's moduli of 5-10 kPa and minimal degradation over 21 days. They show rapid cross-linking in less than 10 sec. First experiments showed that microgels can be created as well.

In the future, the integration of these light-free cross-linking microgels with the described in-flow 3D printing is planned, providing a less cytotoxic and rapid alternative to conventional microgel-based bioinks in biofabrication research.

23 - Monitoring in-vitro wound healing based on a human three-dimensional wounded in-vitro full-thickness skin equivalent using an animal-free derived stromal membrane

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Due to demographic change and the associated increase in chronic wounds new and improved treatments are becoming increasingly important. In order to monitor wound healing processes *in-vitro* according to the 3R principle in the future, there is an urgent need for suitable test systems. In a first step, we developed a healthy human 3D *in-vitro* full-thickness skin equivalent (FTSE) based on an electrospun stromal membrane^[1] that can be wounded. On the other hand, these should also allow the monitoring of wound healing of the dermis and epidermis while testing novel medical products or drug formulations. These wound models may serve as a platform for subsequent research and development studies to mimic chronic wound conditions *in-vitro*.

A FTSE was established by seeding 60,000 human dermal fibroblasts per square centimeter on an electrospun stromal membrane^[1]. After two weeks, 600,000 human epithelial keratinocytes were added to construct an epidermal layer on top of the existing fibroblast-seeded membrane. This systematic approach facilitated the creation of a layered structure that imitates the intricate architecture of human skin. The matured tissue consisting of a dermal and epidermal parts was wounded after 10 days. To demonstrate the wound healing process, a silica gel based open meshed wound pad was applied into the wound defect. The cell migration was analyzed by scanning electron microscopy. Evaluation of wound closure and optimization of the silica gel dressing was performed by optical methods (HE staining, transmitted light microscopy) and quantified by impedance measurements.

The creation of a FTSE for wounded skin, based on an electrospun stromal membrane, is a significant technical advancement that allows for the *in-vitro* monitoring of wound regeneration. To further refine their applicability and efficacy in regenerative medicine research, future work will require the simulation of chronic disease states within these *in-vitro* models.

[1] Weigel T. et al. Fully Synthetic 3D Fibrous Scaffolds for Stromal Tissues—Replacement of Animal-Derived Scaffold Materials Demonstrated by Multilayered Skin. 2022. Advanced Materials vol. 34. DOI: 10.1002/adma.202106780.

22 - Four software tools from network biology reduce animal experiments and make the most of your omics data

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Recent efforts to reduce animal testing in biomedical research have led to the development of alternative methods such as 3D tissue models, or organoids. These approaches offer more ethical and more precise ways to study human cells directly, such as cancer cells (**Cat-E**), or to model drug action (**PROSimat**) in a tissue context, making the most of available omics data (**DataXflow**) and cluster objectively the obtained experimental results (**CFE**).

We support these changes with our new *in silico* tools:

- (i) modelling cancer cell reactions to targeted therapies and other new cancer treatments (e.g. oncolytic virus, immune cells): tool **cancer target explorer CAT-E**.
- (ii) **PROSimat**, the protein network simulation tool explores the cell biology foundations for all involved cellular networks including their dynamics, key pathways, and critical protein interactions for any type of drugs (antibiotics, anticancer, cardiovascular, etc.).
- (iii) Making the most of available omics data is important for fast progress (**DataXflow**)
- (iv) Finally, understanding which different groups and clusters are involved in any of the drug responses is provided by our tool for **characteristic feature extraction (CFE)**, for instance deriving objective clustering in single cell sequencing analysis.

These tools can also be applied to other types of data and experiments, for instance, toxicity experiments, where some animal experiments remain necessary before attempting use in humans, but cell culture data become more and more important for early testing and reducing drug attrition rate. Our goal is to improve therapy and save patient lives. Hence, as far as available, comparison to omics data available from clinical studies by our tools is critical to validate the results of cell culture experiments without the need for animal experiments - and all four tools allow this for their approach. Beyond their usefulness, our tools are distinguished by their user-friendly design, intuitive user interfaces, and streamlined processes which ensure accessibility for persons with varying programming abilities.

Our innovative tools broaden the understanding of the complex ways biological systems work and help a paradigm change towards ethical research techniques and patient-centeredness wherever possible.

21 - In vitro modelling of β -cell plasticity of hiPSC-derived pancreatic endocrine cells

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The question to which extent pancreatic endocrine cells are able to change their cellular state in terms of cellular plasticity is a recurring issue. Recently, bFGF has been shown to induce α - and β -cell dedifferentiation in primary human islets in an attempt to create a diabetes model. Our data show that the effect of bFGF goes beyond dedifferentiation but simultaneously stimulates the emergence of Somatostatin producing δ -like cells, opening up the possibility to study endocrine plasticity *in vitro*.

To establish the model system, we generated human hormone expressing cells (HECs) from human induced pluripotent stem cells (hiPSCs). We stimulated hiPSC-derived HECs with bFGF and analyzed the expression of endocrine cell type associated markers using RT-qPCR and immunofluorescent staining.

bFGF decreased *INS* and *GCG*, and increased *SST* gene expression in a dose- and time-dependent manner. Immunofluorescent staining confirmed an increased SST signal in bFGF treated HECs. In line, bFGF diminished the expression of transcription factors associated with a β -cell (*PDX1*, *NKX6.1*, *MAFA*, *NEUROD1*) and α -cell (*ARX*) state, while elevating δ -cell associated transcription factors (*HHEX*, *EGR1*, *FOS*), indicating that α - and β -dedifferentiation is accompanied by an activation of a δ -cell program. Indeed, bFGF increased the number of cells positive for the δ -cell transcription factor HHEX as well as the progenitor marker SOX9. Interestingly, we found HHEX exclusively in CPEP+ and SST+, but not GCG+ cells, indicating a role of HHEX in a potential β - to δ -cell switch. In order to trace a possible β - to δ -cell conversion, we have begun to engineer a hiPSC triple knockin reporter line for *INS*, *GCG* and *SST* expression using CRISPR/CAS9 DNA cleavage and a donor template-based homology directed repair.

Summarized, our data highlight bFGF as a modulator of pancreatic plasticity by inducing an α - and β -cell dedifferentiation accompanied by an increase of cells with a δ -cell phenotype. In a next step, we aim to address the question whether and how α -/ β -cell dedifferentiation and δ -cell differentiation are connected, which function HHEX plays in the development and plasticity of β - and δ -cells and whether the bFGF induced effects have a relevant clinical role.

20 - 3D co-culture and incorporation of biofabricated fibers for better glomerular cell modeling ex vivo

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Spheroids represent a valuable model for investigating intercellular interactions and communication in a 3D microenvironment more closely resembling *in vivo* conditions. To this end, we conducted a study in which we cultured glomerular cells in both 2D and 3D settings, as well as in mono and co-culture, to analyze cell survival, self-assembly, gene expression, cell-cell interactions, and signaling pathways.

Our findings indicated that the co-culturing of podocytes, glomerular endothelial cells (GEC), and mesangial cells was beneficial for cell survival. Moreover, the expression of podocyte and GEC-specific markers and the extracellular matrix was enhanced by 3D culturing. Furthermore, we demonstrated paracrine signaling within the 3D glomerular co-culture by transport of podocyte-produced VEGF to GEC. Besides, we observed that culturing conditions can affect the expression of housekeeping genes used for qPCR analysis normalization. Indeed, several standard housekeeping genes, including ACTB, GAPDH, UBC, HPRT, or SDHA, showed unacceptable variability in expression depending on the 2D or 3D culture. We identified RPS18 as a stably expressed gene between 2D and 3D glomerular cultures.

Besides the positive effects of 3D cultivation, the expression of hypoxia target genes was also elevated in spheroids compared to 2D. To improve oxygen and nutrition supply, we incorporated submicron fibers into spheroids using a biofabrication approach. These fibers were produced by electrospinning poly-L-lactic acid (PLLA) and subsequent coating with polydopamine (PDA) and gelatin type B. The fibers had an average diameter of 0.5 µm and a length of 60 µm.

The addition of fibers into the cell culture resulted in the formation of larger monoculture spheroids and prolonged cell survival. However, fibers disturbed a specific cell self-arrangement within co-cultured spheroids. RT-qPCR was applied to analyze the further impact of fiber incorporation on the expression of hypoxia target genes and cell-specific markers over time.

In summary, our study suggests that the glomerular 3D co-culture model is a promising platform for studying intercellular communication, disease modeling, and drug screening ex vivo. However, it is crucial to exclude unwanted side effects of artificial agents, such as fibers, before incorporating them into experiments.

19 - Improved culture of primary hepatocytes for the establishment of an *in vitro* liver model

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The successful *in vitro* culture of functional primary hepatocytes is essential for drug toxicity tests and the development of liver disease models. This remains difficult due to the rapid dedifferentiation of primary hepatocytes during culture. There are many approaches to inhibit hepatocyte dedifferentiation, but these oftentimes lack the physiological arrangement of hepatocytes or sufficient hepatocyte function. Here, a three-dimensional (3D) matrix is used to inhibit hepatocyte dedifferentiation during culture.

For this, freshly isolated primary murine hepatocytes were cultured within gelatin-methacryloyl (GelMA) hydrogels.

The 3D GelMA environment significantly improved hepatocyte-like function of the encapsulated cells compared to the standard 2D culture. Importantly, hepatocyte viability was massively enhanced when hydrogels were cultured in gas permeable well plates. Furthermore, cell density and cell-cell contact were improved by culturing hepatocytes in GelMA with an additional melt electrowritten fiber scaffold. However, scaffold addition enhanced the expression of the dedifferentiation marker vimentin, while maintaining hepatocyte-specific gene expression compared to GelMA.

In conclusion, the 3D matrix and enhanced oxygen supply significantly improved hepatocyte viability and hepatocyte-like function. These findings underline the demand for a perfused hepatocyte culture system to further increase oxygen and nutrient supply. This is aimed to be achieved in future by using perfusion chambers with biomimetically structured endothelialized microvascular networks. Furthermore, the 3D environment for hepatocyte culture should be adapted to achieve a more physiological morphology of the hepatocytes while reducing expression of the dedifferentiation marker.

18 - Endothelialized perfusable microvascular networks for biofabrication of standardised in vitro tissue models

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Introduction: Despite the considerable advances in biofabrication, engineering of larger 3D tissue constructs has been restricted by the diffusion limit of oxygen and nutrients. The subproject B02 of the SFB/TRR 225 produces perfusable and fully endothelialized microvascular networks aiming at biofabrication of tissue models with improved oxygen and nutrient transport that could replace animal testing.

Our goal is to adapt the microscopy-compliant vascularized constructs to tissue-specific requirements to enable investigations of e.g. muscle tissue, glomeruli and tumours in physiological-like conditions and disease models.

Methods: Melt Electrowriting (MEW) was used to produce sacrificial structures of thermoresponsive poly(2-oxazoline) (POx), which were then embedded in hydrogels and subsequently removed to create microchannels. Endothelialization was achieved using human umbilical vein endothelial cells and validated using fluorescent staining. Barrier assay and response to stimuli were tested to verify the functionality of the endothelium. Migration of cells in the surrounding hydrogels was investigated

Results: We have successfully created reproducible sacrificial POx scaffolds with biomimetic bifurcating geometries. Using dedicated microscopy-compliant bioreactors developed within the project, POx scaffolds were embedded in a supporting hydrogel and removed by lowering the temperature, which resulted in a formation of bifurcating channel. Upon subsequent endothelial cell seeding, a functional endothelial monolayer was achieved in the channels within 5 days [Ryma et al. Adv Mater 2022]. We have also been able to produce more complex POx scaffolds and a unidirectional flow bioreactor setup to closer mimic physiological conditions. Different hydrogel types have been tested as surrounding matrix that should be compatible with tissue-specific cells and enable cell motility and self-assembly of tissue structures, while supporting the lumen structure stability to maintain perfusability in the bioreactor.

Conclusions and Outlook: The microscopy-compliant vascularised 3D model system developed in B02 project contains functional endothelium and can be adapted to different tissue types to serve as an alternative to animal experiment for e.g. tissue formation, disease modelling or drug screening studies.

In the future, more complex vascular geometries with dedicated bioreactors, advanced cell adhesion and angiogenesis studies, as well as printing of tumour spheroids within the model are planned.

17 - Biofabrication of tissue models using fiber-reinforced 4D scaffolds

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Field of biofabrication is continually expanding due to the increasing life expectancy of humans and the growing need of organ donors. The primary components of most tissues, with few exceptions, consist of ground substances, fibers and cells. Fibers play a crucial role in determining the mechanical properties of tissues, while also serving as guides for cell growth. The most promising technique in biofabrication field is 3D bioprinting allowing direct deposition of various types of materials and cells. Still there are several limitations like low resolution, the challenging printing of hollow tubular structures with low wall thickness due to high shear rates, low mechanical properties and cell alignment. In contrast, 4D biofabrication is a technique where the fourth dimension is introduced by using smart materials, which are capable to undergo shape transformation after the addition of certain stimuli. This additional dimension offers several advantages, such as fabrication of hollow structures, the creation of various shapes, and the preservation of 2D patterns within 3D structure. Based on the design using 4D biofabrication it is possible to fabricate highly complex structures with specific/uniaxial cell alignment that resembles blood vessels, muscle tissues, lungs, etc

In our research, we employed various fiber-forming techniques like electrospinning and melt electrowriting (MEW) in combination with 3D printing to design tubular structures that provide cues for cell alignment. These scaffolds were used for formation of highly aligned tubular tissue models with wide range diameters like skeletal muscle and nerve. Based on materials used and their actuation we were able to achieve superfast shape transformation 10 s. Furthermore, by integrating novel fiber spinning technology in 3D printer, we have successfully developed tissue models of fibroblast with high cell alignment and density. Another approach of us allows to mimic and finely tune mechanical properties of native skin using both soft electrospun meshes and hard MEW structures with negative Poisson's ratios. In summary, fiber-reinforced 4D scaffolds represent a highly promising advancement in the field of biofabrication offering advantages such as high resolution, enhanced mechanical properties, precise cell guidance, and the ability to mimic natural tissues.

16 - Infection of cornea models with different *Neisseria gonorrhoeae* derivatives to study the importance of the Type IV pilus for bacterial adherence and infection outcome.

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Neisseria gonorrhoeae (Ngo) is a human obligate pathogen and is the main cause of *ophthalmia neonatorum*, a type of conjunctivitis in newborns of women infected with Ngo, which untreated can lead to cornea ulceration and to blindness (1). One of the most important virulence factors of Ngo is the Type IV pilus, which are filamentous protein structures that facilitate the adhesion of the bacteria to the target tissue and also play a role in the bacterium-bacterium interaction. In this project, we studied the importance of the Type IV pilus for Ngo adherence, infection and its outcome using cornea tissue models (2). We infected the models with MS11 Ngo derivatives: MS11 F3 (Pili+, RecA+), MS11 N159 (Pili+, RecA-, Adh⁺_{cornea}) and MS11 N191 (Pili+, RecA-, Adh⁻_{cornea}). We observed no significant difference in the tissue integrity between the non-infected and infected models. Measurements of the cell death by LDH method showed that the highest cytotoxicity was present after 72 h of infection in models infected with MS11 N159. The bacterial adherence was also the highest for MS11 N159, which could be confirmed using fluorescence microscopy and SEM. Lastly, to analyze the outcome of the infection, the concentration of different important cytokines for infection was measured. IL-8 was the most secreted cytokine and was the highest in the samples infected with MS11 N159 for 72 h, suggesting a specific response that could be possibly receptor-dependent. These results show the importance of the functional Type IV pilus for the infection of the cornea tissue by Ngo, and its role not only for bacterial adherence but also on the outcome of the infection.

References

1. Tjia, K., van Putten, J., Pels, E., & Zanen, H. (1988). The interaction between *Neisseria gonorrhoeae* and the human cornea in organ culture. *Ophthalmology*, 341-345.
2. Lotz, C., et al. (2016). Alternative methods for the replacement of eye irritation testing. *ALTEX - Alternatives to animal experimentation*. 55-67.

15 - Biomimetic thiol-norbornene functionalized hydrogels for photolithographic bioprinting and tissue fabrication

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Hydrogels are well suited for the development of tissue models to replace animal experiments. A highly versatile and controllable light-inducible biomimetic hydrogel system devoid of animal components was developed. It is based on the thiol-ene chemistry and therefore shows superior cell-compatibility and handling compared to commonly used systems based on the acrylate chemistry. This norbornene-based hydrogel system has a lower risk of cell toxicity since reactive oxygen species are consumed in a step growth crosslinking reaction and are not accumulated as in the acrylate-based system. Other advantages are a faster onset of gel formation.

The cell-compatible photoinitiator Lithium-Phenyl-2,4,6-trimethylbenzoylphosphinat (LAP) was used to yield optimal gel formation by exposure to 405 nm light for two minutes with an intensity of 30 mW/cm². The addition of a small amount of low melting agarose to the pregel solution kept cells dispersed in 3D while positioning in microphysiological systems or bioprinters before illumination. Cell viability was well maintained during this cell culture setup.

The hydrogel system was applied for skin tissue fabrication using human cell lines as well as primary cells. Norbornene-modified dextran or pullulan, combined with the adhesion peptide RGD, photo-crosslinked with hyaluronic acid conjugated to a MMP cleavage site allowed the growth of human fibroblasts inside the gel as well as the formation of a layer of human keratinocytes on top of the gel.

The hydrogel was shaped in a newly developed laser-based bioprinter where structures with a resolution down to 9 µm could be generated. This is, up to date, only reached by two photon photopolymerization. No hydrogel contraction or expansion through the bioprinting procedure was observed. Using this bioprinting system full-thickness skin constructs were generated and remained viable for at least 41 days (Hafa et al., *Adv. Mater.* 2023, 2306258).

In summary, we developed a photo-inducible hydrogel system which is well applicable in bioprinting devices and microphysiological systems to yield highly diversified microarchitectures for complex tissue model fabrications, aiming to replace animal experiments.

14 - Deciphering HSV-1 host shutoff in Air-Liquid Interface models

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Recurrent genital herpes is a lifelong disease caused by two viruses, Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2). These viruses induce host shutoff during their lytic phase through the viral proteins vhs and ICP27. Host expression profiles are thus altered, leading to the suppression of host immune responses and the redirection of cellular resources towards viral gene expression.

However, alterations in major signaling pathways in cell lines typically used in research hinder the study of immune responses against HSV. Moreover, differences in susceptibility and permissivity of different epithelial cell subsets in the urogenital tract as well as cell-cell communication are likely to play a role in the local control of HSV-1. Considering these challenges, alternative models such as Air-Liquid Interface (ALI) models offer a promising approach to study HSV infection with greater physiological relevance. We therefore used a dual-color fluorescent HSV-1 and imaging techniques such as confocal microscopy and electron microscopy to establish HSV-1 infection in ALI models derived from human ectocervix samples.

Our results demonstrate successful HSV-1 infection in ALI models, enabling us to track the progression of the virus over the course of the infection. We are now characterizing the HSV-1 infection of the models, by studying the immune pathways induced in response to the virus (e.g. cytokine expression profiles) and its impact on gene expression. Next, we will use different viruses, including vhs and ICP27 mutants, in combination with single-cell RNA-seq, to study the impact of HSV-1 infection on host gene expression. This should allow us to gain insight into the impact of HSV-1 host shutoff on cell-specific as well as paracrine immune mechanisms.

13 - 3D mucosal infection models as validation tools for vaccine development

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The use of 3D infection models as validation tool for vaccine testing could provide an additional step between 2D cell cultures and animal experiments. By mimicking important features of the native tissue architecture in a defined laboratory environment, these models offer a reproducible and relatively complex system to understand host-pathogen interactions. These in vitro 3D models provide a valuable tool to assess human specific infections, thus enabling a more precise assessment of the complex host-pathogen interactions compared to flat cultures. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are causative agents of the most common sexually transmitted infections. There are however no effective vaccines available. Although they can be treated with antibiotics, the rapid increase in resistance to established treatments for *N. gonorrhoeae* is a cause for concern, thus highlighting the importance of vaccine development for these pathogens. This study aims to establish immunocompetent in vitro 3D urovaginal mucosa models to assess the interaction of these two pathogens with the host mucosa. The models are developed by co-culturing primary cervical fibroblasts and epithelial cells on a compressed collagen scaffold. The compressed collagen method provides a homogenous meshwork of collagen and cells with minor shrinkage of the hydrogel. These models show high in vivo - in vitro correlation, with a stratified squamous epithelium and possess barrier properties. They also express markers typical of the ex vivo cervical mucosa. The responses of the urovaginal mucosa model to *C. trachomatis*, *N. gonorrhoeae* and *HSV-2* will be investigated. The complexity of the models will be increased with the integration of immune cells such as neutrophils, macrophages and t-cells. The integration of these models into a microfluidic system could provide further complexity for the tested Tissue-Pathogen-Immune cell interactions.

12 - Human *in vitro* test systems of inflammatory bowel diseases for evaluating PROTAC efficacy

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Crohn's Disease (CD) is characterized by a multi-factorial pathophysiology, including genetic perturbations in the regulation of innate immune mechanisms and epithelial barrier integrity. So far, animals treated with dextran sulfate sodium (DSS) to induce chronic inflammation in the intestine are one of the most widely used models for CD to investigate pathophysiological mechanisms or perform efficacy tests of therapeutic substances. However, such models lack organism specific genetic backgrounds. Therefore, organoid cultures derived from intestinal crypts isolated from CD patient biopsies recapitulate the human-specific background of genetic susceptibilities to the pathology. Such platforms can be used for accurate drug screening of targets involved in the development of chronic inflammation such as the soluble epoxide hydrolase (sEH). Here we present an *in vitro* test system based on generated human CD enteroids, as an alternative to mouse models.

Histological analysis revealed a confluent epithelial layer in CD and healthy donor-derived Transwell®-like models, while mucus secretion was found to be increased in CD donor lines. On the contrary, TEER was found to be decreased in CD-derived epithelial models compared to the control (enteroids without CD pathology), accompanied by reduced expression levels of tight junction genes. In the context of treatment strategies, we tested different proteolysis targeting chimeras (PROTACS) used for the degradation of sEH, which were applied on both model types. In general, sEH expression is similar in both models but effects on healthy or diseased tissue models are different. While healthy models showed for up to 6 h of treatment a reduction in sEH protein expression, the diseased models demonstrated a reduction in sEH protein expression for up to 24 h. In addition, healthy models even showed an increase in sEH protein expression after 6 h of treatment. Taken together, we observed a partial degradation of sEH in our models as expected, which even occurs preferred in models with a disease background.

Therefore, our human intestinal tissue model represents an appropriate *in vitro* test system to further investigate underlining mechanisms of CD pathophysiology and defines our model as model used in the context of drug testing of potential therapeutics for CD.

11 - Development of microparticles for mechanical stress sensing in bioprinting

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A precise understanding of the mechanical stress effecting cells during the bioprinting procedure is essential for targeted controlling and development of biofabrication processes. To reach this aim, we develop mechanosensitive sensor particles mimicking the mechanical behavior of living cells and combine mechanical measurements of these particles with computer simulations.

Microparticles consisting of a polyoxazoline and thiol-modified hyaluronic acid were produced via droplet microfluidics. Measurement of the particles' Young's Modulus was conducted via Atomic Force Microscopy and moduli in the range of cells were determined. The deformation of the particles in microchannels was recorded and a flow behavior comparable to cells could be observed.

Based on our experimental results we can develop simulations that predict the flow behavior of cells during the bioprinting process.

10 - 3D printing and characterization of complex anatomical structures using two hydrogel system: obtaining shape fidelity

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3D printing has emerged as the premier method for creating tissue implants and organ models due to its ability to accurately replicate intricate geometries. However, 3D bioprinting faces unique challenges, especially regarding the viscosity of bioink and the complexity of biological structures. Key challenges include achieving shape fidelity, dense vascularization, and native-like cell density. Mechanical strength and post-print conditioning are also critical hurdles. Despite exploring various bioink materials, achieving consistent success and reproducibility remains a challenge.

Our focus is on achieving shape fidelity, and we have demonstrated a method for bioink preparation and 3D printing using chitosan, dissolved in an alkaline solvent, enabling crosslinking with hot water. The bioink exhibits shear-thinning properties, which are crucial for 3D bioprinting. Printing occurs within gelatin support, preventing structure collapse, which is then melted away post-printing, allowing chitosan to crosslink and ensuring structural integrity. Water-based crosslinking is non-toxic, rapid, and cost-effective. This approach was used to successfully create prototypes of complex structures such as human tri-leaflet heart valves, sections of coronary arterial trees, scaled-down kidney structures, and human ears, all with precise dimensional accuracy and mechanical properties suitable for human tissue. The mechanical strength and suturability depend on the crosslinking temperature of the printed structures and fall in the range of human tissue.

The degradation study demonstrates the stability of these structures under various conditions. This study highlights the potential for creating intricate structures for organ model development and advancements in tissue engineering and biomedical applications.

9 - Mechanoresponsiveness and Viability Changes of hMSC-TERT Printed with Pluronic F127® and POx/POzi

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In three-dimensional (3D) bioprinting, the integration of living cells into hydrogel scaffolds represents a complex interplay of physiochemical properties such as viscosity, temperature, and concentration. These parameters influence the functionality of the printed constructs and the biological effects on the embedded cells. This study investigates the short-term effects of 3D printing telomerase-immortalized human mesenchymal stromal cells (hMSC-TERT) incubated for 24 hours in the bioinert hydrogels, Pluronic® F127, and a synthesized poly(2-methyl-2-oxazoline)-block-poly(2-n-propyl-2-oxazin) (POx/POzi).

The hydrogels were prepared by dissolving autoclaved polymers in sterile Eagle's Minimum Essential Medium (MEM). The medium was supplemented with 10 % (v) heat-inactivated fetal bovine serum (FBS), 50 µg/mL gentamicin sulfate, and 100 nM sodium selenite. The polymer concentration was 27.8 % (w/v). Mixing with the cell suspension achieved a final concentration of 25 % (w/v) and an hMSC-TERT concentration of 5×10^6 cells/mL. These bioinks were printed using a 22 gauge nozzle and a 30 gauge needle into plastic petri dishes and incubated for 24 hours in a humidification chamber. The scaffolds were dissolved by adding cold phosphate-buffered saline (PBS) to extract the cells for further analysis.

A key finding of this study is the reduction in cell viability by about 50 % within 24 hours after printing. Although cell viability after printing with POx/POzi was higher under the chosen printing conditions, a comparable reduction in viability was observed after incubation. Interestingly, the expression levels of the mechanoresponsive genes FOS and PTGS2 remained essentially unchanged, suggesting that the shear stress from 3D bioprinting with these hydrogels does not significantly influence these specific genetic pathways.

This study showed that bioinert POx/POzi hydrogel is better suited for the 3D printing and incubation of hMSC-TERT than Pluronic F127®. Since no response to mechanical stimuli was observed in hMSC-TERT, no undesirable, premature differentiation of these cells is expected. These results indicate the potential of using bioinert hydrogels as a sacrificial matrix for 3D bioprinting of hMSC-TERT, suitable for up to four hours.

8 - The Glomerular Filtration Barrier: Fabrication and Testing of a 3D in-vitro Model

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The kidney is a very dynamic organ that operates the key function of filtering waste from the blood in 2 million glomeruli through the filtration barrier: a triple layer of highly specialized cells, podocytes, the basement membrane, and glomerular endothelial cells (GEC). Failures in this organ originate many kidney diseases. It is, therefore, interesting to study in more details its mechanisms. The challenge lies in replicating a functional in vitro model that addresses the needs of these cells and can be versatile. So far, only a few studies have focused on the artificial membrane. We hypothesise using a biomimetic membrane to support the co-culture of glomerular cells, tested in a custom-built bioreactor.

Aligned nanofibers of Poly-L-Lactic Acid were fabricated through electrospinning, and then functionalized with polydopamine-assisted gelatin immobilization. The surface characterization of the membrane was analyzed using Scanning Electron Microscopy and Raman spectroscopy and micro-BCA assay. Human GECs and podocytes were seeded on each side of the membrane in static condition. Later, we 3D printed a millifluidic bioreactor, assembled the membranes in it, and cultured cells on either side of the membrane. Flow simulations were run to optimise the structure and then perfused media separately on either channel. Furthermore, albumin was used as a model protein to evaluate the membrane's function.

The membrane presented an average thickness of 10 µm. Raman spectroscopy shows the typical peaks of the amide bond of gelatin in addition to the PLLA peaks. Cells present an elongated morphology, spread, attach, and form a tight monolayer. Thereafter, we analyze the high viability of cells under flow. The SEM images revealed that both cell types remained attached on each side after 5-days of flow. Further, we observed the high viability of cells during their culture time. The Actin staining revealed that cell morphology was significantly affected under dynamic culture conditions and cell-typical structures are present.

Further studies foresee the optimization of the tissue maturation to replicate diseased tissue models.

7 - Increasing sensitivity and sustainability in early toxicity screening - Serum-free culture of HepG2 cells

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In pharmaceutical drug development, the use of *in vitro* cell, tissue and organ culture is an important tool for preclinical and nonclinical testing of drug candidates for efficacy, DMPK and safety. With the increasing complexity of available cell culture models, much progress has been made to approximate the human *in vivo* situation, thereby reducing the need for animal experiments. However, human *in vitro* cell culture systems often require animal components for supplementation (Weihe, 1985).

FBS is an important ingredient in cell culture media and the current standard when maintaining and proliferating cells *in vitro*. However, the use of FBS is being controversially discussed for several reasons, including ethical concerns, political and societal pressure, as well as scientific problems. Nevertheless, scientists hesitate to change the paradigm without solid data de-risking the switch within their assays.

This study focused on one of the major work horses in early toxicity testing, the HepG2 cell line. Out of ten different tested media, only two showed a long-term cultivation capacity comparable to our standard media containing FBS and were selected for further testing.

Due to their use in drug toxicity testing, cellular functionality was assessed by using different early toxicology assays. For these experiments, compounds described by the MIP-DILI consortium were used alongside internal assay specific control substances (Dragovic et al., 2016). Throughout the different toxicology assays, serum-free cultivated cells displayed a similar or slightly increased drug response in the assays focusing on viability/cell death, while the oxidative stress response was generally lower in serum-free media. Additionally, cells were compared on the protein level after treatment with test compounds using different protein markers. Cells cultivated under serum-free conditions showed stronger drug responses when assessing markers related to cell death/apoptosis and stress response. In addition, depending on the compound a stronger or similar autophagy response compared to cells cultivated in standard conditions was observed. In contrast, the oxidative stress response was again lower in both serum-free media.

Taken together, the obtained results demonstrate that HepG2 can be cultured and used under serum-free conditions for early drug screening in *in vitro* toxicology.

6 - Development of primary cell-based infection models to study pathogenesis of *Campylobacter jejuni*

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Campylobacter jejuni is currently the leading cause of bacterial foodborne gastroenteritis in humans. However, only a few *C. jejuni* virulence-determining factors involved in host cell interactions *in-vivo* have been identified, in part due to the lack of appropriate infection models that accurately reflect the human host environment. So far, infection studies have mainly employed animal or *in-vitro* cell culture models based on immortalised cell lines (e.g., Caco-2 cells), which are limited in their ability to model the complexity of intact three-dimensional (3D) tissue in the human host. To add physical characteristics of host intestinal tissue, like the structure of crypts and villi, we previously developed a 3D infection model based on tissue-engineering that incorporates a biological scaffold, the small intestinal submucosa, seeded with Caco-2 cells. This 3D infection model allowed for monitoring pathogenesis related phenotypes of *C. jejuni* mutant strains analogous to phenotypes previously observed in animal models, which could not be reflected in 2D monolayers. Nevertheless, the use of immortalized cell lines in an infection model limits the resemblance of specific host tissue characteristic, such as cell type diversity. These shortcomings hinder the identification of host cell types targeted by *C. jejuni*, as well as the determination of bacterial survival and replication within host cells. Therefore, we are now adapting primary cell-based *in-vitro* infection models of the small intestine by employing human intestinal organoids for *C. jejuni* infections. So far, we have successfully set up 3D and 2D monolayer cultures of human jejunum and ileum organoids in our lab. First infection experiments of organoid-derived monolayers demonstrate that independent of the multiplicity of infection (MOI) and patient origin of organoids, *C. jejuni* can infect primary jejunum cells and in several of these, clusters of multiple bacterial cells can be observed. Using these humanized infection models, we aim to get a deeper understanding of *C. jejuni* pathogenesis, including host target cell identification as well as epithelial and pathogen factors mediating infections.

5 - Translation and Reproducibility: Standardized HUMAN Biomaterials

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Background: Despite the ongoing developments in new biomaterials over the last decades, scientific research, the starting point for many medical applications, is still performed with human cells embedded in animal-derived materials. Animal-derived materials are not only regularly associated with the sacrifice of many animals, but the cultivation of human cells in combination with animal-origin products also does not reflect an ideal human cell environment. An alternative source of biomaterials is human placenta tissue which is globally available in large quantities.

Objective: By using human placenta tissue, a so-called medical waste, THT Biomaterials reuses clinical material that would otherwise be discarded after birth to develop new human placenta-derived biomaterials.

Methods: Human placenta extracellular proteins are isolated using enzymatic or non-enzymatic strategies allowing the obtention of large proteins quantities through a sustainable process. THT human placenta-derived biomaterials have been successfully used in cutting-edge life sciences applications such as 3D cell culture (stem cells, organoids), organ-on-a-chips, and 3D bioprinting replacing popular materials like mice tumor-based hydrogels and fetal calf serum (3Rs).

Results: The human placenta-derived biomaterials have demonstrated a vast potential not only to replace animal materials in science but also to boost the translation of in vitro results into clinical applications. In prospect, THT Biomaterials based on a circular economy also aims to integrate these human biomaterials into clinical applications since they have a recognized potential as medical products.

Conclusion: THT solution minimizes the lifecycle environmental impact of new products and can therefore contribute to a more sustainable future in light of the Sustainable Development Goals (SDGs) from the United Nations.

4 - Uncovering host-pathogen interaction dynamics in human intestinal epithelial cells

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Salmonella enterica serovar Typhimurium (henceforth *Salmonella*) is a common cause of gastroenteritis in humans. *Salmonella* bacteria infect the intestine and actively invade intestinal epithelial cells (IECs), where they either remain in the *Salmonella*-containing vacuole (SCV) or escape into the cytosol. The determinants of these different lifestyles remain poorly understood. Research on those two distinct populations of *Salmonella* inside epithelial cells has mostly been done on immortalized cancer cell lines, which are known to lack components of the *in vivo* tissue that might be crucial to infection with *Salmonella*. The growing field of organoid research provides new models, such as organoid-derived monolayers, that bridge the practicability of cell lines with a higher level of complexity, by providing different cell types of a certain tissue, in a 2D monolayer structure. Here we characterize a human small intestinal epithelial organoid-derived monolayer model for infection with *Salmonella*, using single-cell RNA sequencing (scRNA-seq). We furthermore infect the organoid-derived monolayer model with *Salmonella* and investigate the existence of vacuolar and cytosolic *Salmonella* respectively, using a staining for the SCV surface marker LAMP-1. To study the dynamics of the infection, we have implemented a high-throughput method, combining single-cell RNA-seq and RNA metabolic labeling. Altogether, we expect our multi-dimensional single-cell analysis to reveal the host factors that underlie the fate decision of bacterial divergent lifestyles.

3 - TOWARD PERFECTION: NAIVE HUMAN PLURIPOTENT STEM CELLS IN DISEASE MODELING AND CRISPR TECHNOLOGIES

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Human PSCs emerged as a key tool for biomedical research, due to their inherent features. However, primed hPSCs show skewed differentiation, a defective genomic methylation pattern, sensitivity to single-cell dissociation, and poor amenability to genome editing. Thus, the establishment of a stable human-naïve culture has gained priority in the stem cell community. Naïve hPSCs correspond to the pre-implantation epiblast, offer a unique window into early embryogenesis studies, exhibit plasticity, and unbiased differentiation potential into extraembryonic and embryonic tissues, including the special capability to generate human primordial germ cells (PGCs). Originally, in 2013, the first successful trial was conducted to generate transgene-free naïve-like cells by supplementing growth factors with small chemical inhibitors that suppress specific protein kinases involved in differentiation, cell adhesion, and survival. Mainly, the JAK/STAT3 pathway, along with key kinases MEK, GSK, SRC, PKC, WNT, and BRAF, are the essential targets of naïve hPSCs deriving protocols. The two most effective protocols for generating naïve-like stem cells are t2iLGö and 4iLA/5iLA(F). Surprisingly, however, naïve hPSCs exhibit certain hallmarks of mammalian naïve pluripotency, the generated cultures show limited cellular proliferation and stop proliferating at ~20 passages without continuously expressing exogenous factors, where chromosome anomalies and massive loss of methylation are highly to occur, especially within 10 passages. Therefore, conditions to sustain human naïve pluripotency are still suboptimal and need more investigation. In this work, we developed condition to generate naïve-like hPSCs, where 5iLA/F served as the base medium. The generated cells showed naïve pluripotency features from the dome-shaped colony, positive to naïve specific markers, robust upregulation of the essential naïve-specific genes, to the potential to generate derivatives of all three germ layers, and reactivation of *XIST*. We investigated enhancing the efficiency of Crispr-cas12a in the derived cells. The robust increase in the fraction cleaved in the generated cells, implies enhanced genome editing capability in the naïve cells, which represents promising perspectives for systematic investigation of pathophysiological mechanisms or drug screening for potential therapeutics.

2 - *Neisseria meningitidis* disrupts P-glycoprotein but not BCRP functional activity in induced pluripotent stem cell-derived brain-like endothelial cells

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The blood-brain- and meningeal blood-CSF barrier (mBCSFB) are formed by brain endothelial cells (BECs), protecting the brain from harmful substances. BECs express efflux transporters, P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP), which actively pump endogenous and exogenous substances from the brain to the blood. Despite the crucial role of efflux transporters in the brain, there is still a considerable gap in our understanding of their activity in response to environmental factors such as pathogens.

Neisseria meningitidis (*Nm*), a commensal bacterium of the respiratory tract, can cause meningitis through systemic spread and transmigration across mBCSFB. Little is known about the impact of *Nm* on efflux transporters' activity in brain endothelial cells (BECs). This study aims to decipher the impact of *Nm* on P-gp and BCRP activity in BECs.

Induced pluripotent stem cells (iPSCs) were differentiated into iBECs as previously described. The impact of *Nm* on P-gp and BCRP was analyzed by RT-qPCR and immunoblotting. P-gp and BCRP activity was assessed by measuring intracellular accumulation of Rhodamine 123 (R123) and Chlorin e6 (Ce6), respectively.

The uptake of R123 and Ce6 by P-gp and BCRP under exposure to their inhibitors, PSC833 and Ko143, respectively confirmed their activity in iBECs. Following *Nm* infection, a significant decrease in P-gp activity despite the alteration in expression and protein level was observed. The addition of PSC833 during infection exhibited less increase in R123 accumulation compared to the inhibitor, proposing a competitive interaction between *Nm* and PSC833. In contrast, the BCRP activity remained unaffected. Our data suggested an essential role for the *Nm* capsule in P-gp dysfunction since non-capsulated *Nm* did not impact the R123 accumulation. Moreover, we observed that live *Nm* is required for P-gp inhibition.

The decline in P-gp activity during *Nm* infection suggests a dynamic response to *Nm* at the level of activity. Consistent with earlier research, unaltered BCRP activity suggests a potential compensatory relationship with P-gp. Our data identified a crucial role for the *Nm* capsule in P-gp inhibition, presenting a potential therapeutic target for drug delivery. Our observation emphasizes the requirement of live *Nm* for P-gp inhibition, highlighting the host-pathogen interaction.

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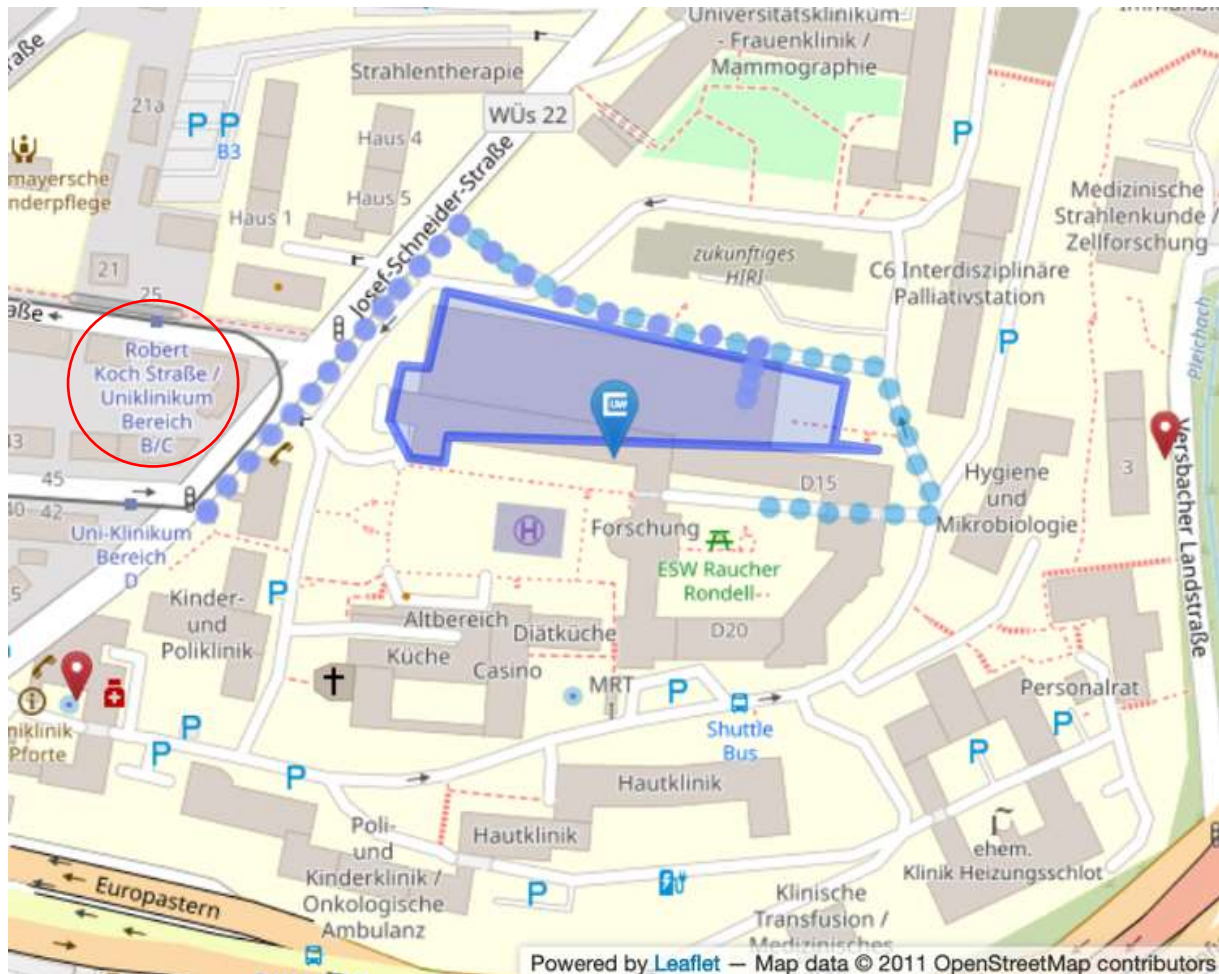
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Luisa-Marie	Pfeifer	Merck Healthcare KGaA	Early & Investigative Toxicology	Germany
Frederic	Pipp	Merck KGaA	Corporate Animal Affairs	Germany
Christina	Popp	Fraunhofer Institute for Silicate Research IS	Fraunhofer Translational Center Regenerative Ther	Germany
Vivien	Priebe	Universitätsklinikum Würzburg	Department for Functional Materials in Medicine an	Germany
Jörn	Probst	Fraunhofer ISC	TLZ-RT	Germany
Cynthia	Sharma	University of Würzburg	Chair of Molecular Infection Biology II	Germany
Peter	Prusseit	Deutsche Forschungsgemeinschaft (DFG)	Law department	Germany
Alexander	Radüchel	University Hospital of Würzburg	Department for Functional Materials	Germany
Anna	Rederer	University Hospital Erlangen	Department of Nephrology, TRC	Germany
Amelie	Reigl	Fraunhofer ISC TLZ-RT	Skin in vitro Testsystems	Germany
Alice	Rein	University Hospital	FMZ	Germany
Silke	Riegger	3R-Center Tübingen	University of Tübingen	Germany
Jonas	Röder	Friedrich-Alexander-Universität Erlangen-Nü	Biomaterials	Germany
Julia	Roosz	NMI	MicroOrganoLab	Germany
Claire	Rousseau	University of Würzburg	Virologie	Germany
Matthias	Ryma	University Clinic Würzburg	Department for Functional Materials in Medicine an	Germany
Sahar	Salehi-Müller	University of Bayreuth	Biomaterials	Germany
Evelin	Sandor	Uniklinikum Erlangen	Department of Plastic and Hand Surgery	Germany
Natascha	Schäfer	Universitätsklinikum Würzburg	Institut für klinische Neurobiologie	Germany
Martha	Schamel	Tutogen Medical GmbH	Technical Operations	Germany
Michael	Schell	Cenibra	-	Germany
Maximilian	Schinke	Fraunhofer ITEM	Division of Cardiovascular Research	Germany
Luisa	Schmitt	Universitätsklinikum Würzburg	Lehrstuhl für Tissue Engineering und regenerative M	Germany
Regine	Schneider-Stock	University Hospital Erlangen	Experimental Tumor Pathology	Germany
Nina	Schukraft	Universitätsklinikum Würzburg	Institut für Klinische Neurobiologie	Germany
Julian	Schwebler	University Hospital Würzburg	Department of Ophthalmology	Germany
Fabian	Seidl	NEROW	EFRE	Germany
Anna	Seubert	University Hospital Würzburg	MSNZ	Germany
Ioanna	Skandali	THORLABS GmbH	Marketing & Communications	Germany
Rosie	Smith	Novogene GmbH	Marketing	Germany
Sabrina	Stecher	SFB/TRR225	Chirurgie II	Germany
Maria	Steinke	Fraunhofer ISC	TLZ-RT	Germany
Linda	Stelz	JMU Würzburg/Biocenter	Biotechnology and Biophysics	Germany
Valeska	Stephan	SKTF/UMR	SKTF	Germany
Janis	Stiefel	Fraunhofer IMM	Diagnostics	Germany
Jennifer	Stotzem	Deutsche Forschungsgemeinschaft	Zoologie	Germany
Lili	Szabo	University Hospital Würzburg	Mildred Scheel Early Career Center (MSNZ Würzbur	Germany
Katinka	Theis	Uniklinik Würzburg	Department for Functional Materials FMZ	Germany
Esra	Türker	Universitatklinikum Würzburg	Institute of Clinical Neurobiology	Germany
Moritz	Väthróder	Universitätsklinikum Würzburg	Nuclear medicine	Germany
Brigitte	Vollmar	University Medical Center Rostock	Institute for Experimental Surgery	Germany
Sylvia	Wagner	Fraunhofer Institute for Biomedical Enginee	Bioprocessing & Bioanalytics	Germany
Tobias	Weigel	Fraunhofer ISC	Translational Center for Regenerative Therapies	Germany
Jeanette	Weigelt	University of Würzburg	Department of Functional Materials for Medicine ar	Germany
Daniela	werkmann	Merck KGaA	Site Management	Germany
Franziska	Werner	Universität Würzburg	Physiologisches Institut	Germany
Lisa	Wiesmüller	Ulm University	Department of Obstetrics and Gynecology	Germany
Jana	Wilken	Initiative Tierversuche verstehen	not applicable	Germany
Svenja	Wingert	University of Tübingen	Department for Microphysiological Systems, Institu	Germany
Philipp	Wörsdörfer	University of Würzburg	Institute of Anatomy and Cell Biology	Germany
Maximiliane	Wußmann	Fraunhofer ISC	Translational Center for Regenerative Therapies	Germany
Daniela	Zdzieblo	Fraunhofer Institute for Silicate Research	Translational Center Regenerative Therapies	Germany
Janina	Zeqiraj	VDI/VDE Innovation + Technik GmbH	Projekträgerschaft Lebenswissenschaften	Germany
Saskia	Zöphel	Fraunhofer ISC	TLZ-RT	Germany

HOW TO FIND US

Public transport

You can take the tram lines 1 and 5 from the main train station to the Würzburg **"Uni-Klinikum / Bereich D" stop**. Or you can take a cab (phone 0931 19410) from the main station with the destination **Josef-Schneider-Straße 2, house D15, Rudolf-Virchow-Zentrum**.

Follow the footpath, marked purple on the map, barrier-free to the main entrance (foyer) of house D15.



By car

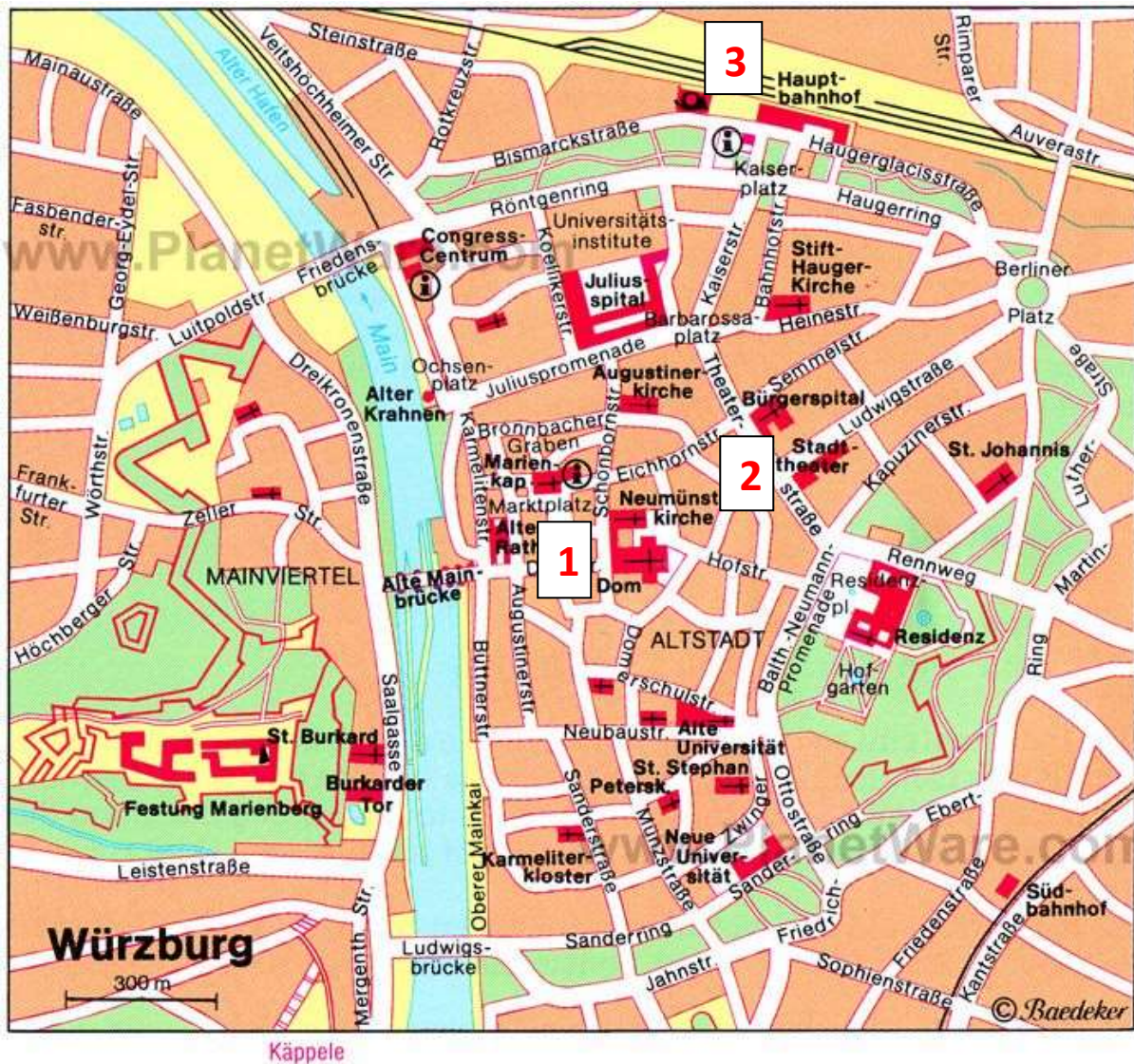
Due to construction work near building D15 parking spaces in the UKW area C-D (near building D15) are very limited. Additional parking is available in the UKW area A-B (near ZIM/ZOM). The shuttle bus service of the University Hospital can be used free of charge. The bus runs every 10-15 minutes from Monday to Friday between 5:45 and 19:15.

Shuttelbus Tour:



Entrance to RVZ main entry (Building D15)

CITY MAP OF WÜRZBURG



Source: Pinterest

1. Wednesday, 5th June, 7-10pm, Get together of Young Investigators: MAM-MAM Burger restaurant

Blasiusgasse 3, Würzburg (from RVZ use Tram line 1 or 5, exit at 'Dom' station)

2. Thursday, 6th June, 7.30-11pm, Conference Dinner: Bürgerspital

Theater Strasse 19, Würzburg

3. Würzburg main station/Würzburg HBF

4. G-Hotel

Schweinfurter Str. 3, Würzburg