PROGRAM

& ABSTRACT BOOK

VENUE:

Rudolf Virchow Center

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

3D Infect GRK 2157 Research Training Group www.uni-wuerzburg.de/grk2157

TRR 225 Biofab

DFG Deutsche Forschungsgemeinschaft

This symposium is kindly sponsored by:

Biomol GmbH Hamburg

https://www.biomol.com

I&L Biosystems

https://www.il-biosystems.com

GeSiM GmbH

https://gesim.de/

CONTENT

Editorial Deadline: 17.11.2022

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:
https://event.fourwaves.com/bmi/pages

REGISTRATION HOURS

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

23.11.22: 1.30 – 3 pm
24.11.22: 8 – 12 am
25.11.22: 8.30 – 9 am

PRESENTATION UPLOAD

Speakers should upload their presentations during the registration hours at the registration desk. 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. 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

- Sina Bartfeld, Institute for Molecular Infection Biology, University of Würzburg / Medical Biotechnology, Technical University Berlin
- Jürgen Groll, Department for Functional Materials in Medicine and Dentistry, University of Würzburg
- Tomasz Jüngst, Department for Functional Materials in Medicine and Dentistry, University of Würzburg
- Vera Kozjak-Pavlovic, Department of Microbiology, University of Würzburg
- Marco Metzger, Fraunhofer Translational Center Regenerative Therapies TLC-RT, Würzburg, Germany
- Jennifer Ritzer, Department for Functional Materials in Medicine and Dentistry, University of Würzburg
- Thomas Rudel, Department of Microbiology, University of Würzburg
- Jörg Teßmar, Department for Functional Materials in Medicine and Dentistry, University of Würzburg

Local Organizing Committee

- Landing Page/Poster: Nathaly Cabezas, Marco Metzger, Amelie Reigl, Jennifer Ritzer, Meike Scheuring
- Sponsoring with Online-Registration and Preliminary Program: Zan Lamberger, Mindaugas Pauzuolis, Xuen Ng, Saskia Roth
- Evening program: Kirilo Krjukov, Spyridon Damigos
- Catering: Meike Scheuring, Jennifer Ritzer
- Speaker invitation and organization: Eva Rühl, Anna Reinert, Saskia Roth, Ravisha Rawal, Amelie Reigl, Jonas Röder

WELCOME TO WÜRZBURG

Welcome to the Biofabrication meets Infection Symposium 2022,

On behalf of the scientific and local organization committees, we would like to welcome you to our joint international symposium “Biofabrication meets Infection”.

Biofabrication concerns the fabrication of stratified three dimensionally organized cell-material constructs, with the aim to generate biologically functional tissue models. The SFB/TRR 225 consortium is focused on this field of research. The GRK2157 on the other hand deals with the application of 3D tissue models for infection research. Both consortia have joined forces to organize this international symposium which will focus on topics related to biofabrication and complex tissue models and their application to infection research based on this unique local clustering of expertise in these fields.

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 biofabrication, tissue model development and infection research to identify synergies and boost new research ideas and cooperations.

We want to highlight that the symposium has been organized by PhD students from 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 25 lectures and 55 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.

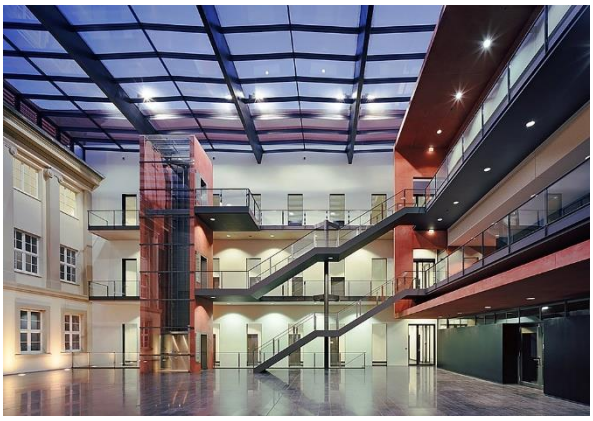
We are looking forward to a conference with exciting science, stimulating discussions and exchange and multiple opportunities to initiate collaborations!

Jürgen Groll and *Thomas Rudel*

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 required):

Welcome Reception: November 23, 2022 – 6:00 pm **(only TRR225 and GRK2157 members)**
The welcome reception will take place at the event venue ***Rudolf Virchow Center (RVZ).*** The RVZ is a popular venue for different types of conferences and events.

Guided Tour through Würzburg city: Thursday, November 24, 2022 – 6:15 pm

Experience the history and scenery of Würzburg on an extraordinary tour through one of Germany's most beautiful and historic cities. On the road with the **Night-watchman of Würzburg**, the tour is a balanced mix of humorous entertainment and historical as well as cultural information, narrated with either original Franconian dialect or in English.

The guided tour will start at the Dom St. Kilian (Cathedral), Domstraße 40, 97070 Würzburg and ends at the *Bürgerspital Weinstuben* at 7:15 pm (place of our Gala Dinner). From RVZ 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 73-75.

Symposium Dinner: Thursday, November 24, 2022 – 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 *Bürgerzimmer*, 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! Please find more information about the restaurant here:

https://www.buergerspital-weinstuben.de/
Address: Theater Strasse 19, 97070 Würzburg

SCIENTIFIC PROGRAM

Wednesday, November 23, 2022 **(internal meeting of TRR225 and GRK2157 members)**

2:00 pm	Arrival of TRR225 and GRK2157 members and attachment of posters
3:00 – 4:00 pm	**Jürgen Groll and Thomas Rudel** Welcome and Introduction (RVZ lecture Hall)
4:00 – 5:30 pm	**Internal poster session** Internal poster session with SFB TRR225 and GRK2157 members Part 1: SFB TRR225 4:00 pm – 4:45 pm Part 2: GRK2157 4:45 pm – 5:30 pm
5:30 – 6:00 pm	**Internal meeting with SFB TRR225 and GRK2157 members** PhD students of TRR225 and GRK2157
6:00 – 8:30 pm	***Welcome reception / Get together***

Thursday, November 24, 2022

8:00 am	Arrival of external participants and attachment of posters
9:00 – 9:30 am	**Jürgen Groll and Thomas Rudel** Welcome and Introduction (RVZ lecture Hall)
	Session I: Biofabricated tissue models
9:30 – 10:00 am	**Jos Malda**, University Medical Center Utrecht, Netherlands Converged Biofabrication technologies for 3D implants and models
10:00 – 10:30 am	**Daniela Duarte Campos**, University Heidelberg, Germany Bioprinting for tissue and organ engineering (digital)
10:30 –11:00 am	**Heunsgoo Shin**, Hanyang University Seoul, South Korea Engineering multi-cellular spheroids using bioinspired materials for biofabrication of 3D tissue
11:00 – 11:30 am	***Coffee Break / Poster Session***
	Session II: Cellular guidance structures
11:30 – 12:00 am	**Jochen Salber**, Ruhr Universität Bochum, Germany From single-cell control to coordinated vascular-nerve bundle extrusion to complex tissues and organs - The cooperativity of molecular gradients and guidance structures

12:00 – 12:30 pm	**Laura DeLaporte**, RTWH Aachen, Germany Injectable synthetic colloidal building blocks to assemble into structured 3D tissue engineering constructs
12:30 – 12:45 pm	**Marie Piantino**, Osaka University, Japan Development of a three-dimensional blood-brain barrier network with opening capillary structures for drug transport screening assays (selected talk #3)
12:45 – 1:45 pm	***Lunch Break / Poster Session***
	Session III: Vascularization
1:45 – 2:15 pm	**Debby Gawlitta**, University Medical Center Utrecht, Netherlands Designing the vascular tree in complex tissue models
2:15 – 2:45 pm	**Michiya Matsusaki**, Osaka University, Japan Vascularized 3D Tissue Models for Infection Assays of Cancer Cells and Bacteria (selected talk #3, 11, 13, 25)
2:45 – 3:00 pm	**Matthias Ryma**, University Würzburg, Germany Sacrificial templating via thermoresponsive polymers for simple, reproducible, and accurate in vitro vascularization (selected talk #4, 49, 53)
3:00 – 3:30 pm	***Coffee Break / Poster Session***
	Session IV: Organoids and 3D Infection models
3:30 – 4:00 pm	**Andrew Thorely**, Genentech, USA Use of primary human lung cells to model epithelial-macrophage innate immune responses (digital)
4:00 – 4:30 pm	**Daniel J. Kelly**, Trinity College Dublin, Ireland Biofabrication and bioprinting for bone and joint regeneration
4:30 – 5:00 pm	**Matthias Lutolf**, EPFL Lausanne, Switzerland Engineering organoids (digital)
5:00 – 5:30 pm	**Francesco Boccellato**, University of Oxford, UK Stem-cell driven models to understand epithelial response to infections and cancer initiation
6:15 – 7:15 pm	**Guided tour with Night-watchmen** Meeting point: *Dom St. Kilian*, Domstraße 40, Würzburg
7:30 – 10:00 pm	***Symposium Dinner*** Restaurant *Bürgerspital Weinstuben*, Theater Strasse 19, Würzburg

Friday, November 25, 2022

	Session I: Biofunctionalization and cell tracking
9:00 – 9:30 am	**Ana Paula Pego,** University of Porto, Portugal Engineering the nervous system with the help of biomaterials
9:30 – 10:00 am	**Viola Vogel,** ETH Zürich, Switzerland Mechanobiology of platelets
10:00 – 10:30 am	**Ali Doryab,** Helmholtz-Zentrum München, Germany A Bioinspired "Breathing" In Vitro Mini-Lung Fibrosis Model
10:30 – 10:45 am	**Niklas Pallmann,** University Würzburg, Germany Airway tissue models based on a novel synthetic scaffold to replace animal experiments (selected talk #24, 30)
10:45 – 11:00 am	**Ivana Jeremic,** University Erlangen-Nürnberg, Germany Quantitative imaging and analysis of biofabricate quality and maturation (selected talk #35)
11:00 – 11:15 am	**David Böhringer,** University Erlangen-Nürnberg, Germany Traction forces of migrating immune cells" (selected talk #5, 47)
11:15 – 11:30 am	***Coffee Break***
	Session II: Advanced human infection models
11:30 – 12:00 am	**Eileen Gentleman**, Kings College London, UK Modular hydrogels for organoid-based disease modelling (digital)
12:00 – 12:15 pm	**Leo Endres**, University Würzburg, Germany Modeling Neisseria meningitidis interaction with the meningeal blood-CSF barrier using iPSC and multi-cell culture approaches (selected talk #16)
12:15 – 12:30 pm	**Thomas Däullary**, University Würzburg, Germany Salmonella Typhimurium infection in a primary in vitro model of the human small intestine reveals the induction of OLFM4 expression as host cell response" (selected talk #21, 32, 55)
12:30 – 1:15 pm	***Lunch Break***
	Session III: Virulence factors in infection

1:15 – 1:45 pm **Mikael Sellin**, Uppsala University, Sweden
Pathogen Attack on the Intestinal Epithelium – Insights from Enteroid Imaging

1:45 – 2:15 pm **Steve Boulant**, University of Florida, USA
Understanding enteric virus-host interactions at the single cell level using intestinal organoids (digital)

2:15 – 2:30 pm **Mindaugas Pauzuolis**, University Würzburg, Germany
Helicobacter pylori shows tropism to gastric differentiated pit cells dependent on urea chemotaxis (selected talk #6)

2:30 – 3:00 pm ***Awards and closing remarks***

Snacks and Departure

POSTER ABSTRACTS

56 - Modelling how soluble VE-cadherin disrupts endothelial barrier&function via VE- PTP/RhoA signalling

Mahshid Danesh[1], Juna-Lisa Knop[2], Sven Flemming[2], Natalie Burkard[2], Matthias Hiermaier[3], Mughda Srivastava[4], Nicolas Schlegel[2], Thomas Dandekar[1]

[1]University of Würzburg, Department of Bioinformatics, Biocenter, Würzburg, Germany University of Würzburg, Department of Bioinformatics, Biocenter, Würzburg, Germany, [2]University Hospital Würzburg, Department of general, visceral, vascular and paediatric surgery, Würzburg, Germany, [3]University of Munich, Institute of Anatomy and Cell Biology; Munich, Germany, [4]University Hospital Würzburg, Core Unit Systems Medicine, Würzburg

Increased levels of soluble vascular endothelial VE-cadherin fragments (sVE-cadherin) have previously been linked with inflammation-induced loss of endothelial barrier function. Here we tested whether sVE-cadherin is critically involved in the onset of endothelial barrier dysfunction independent of pro-inflammatory stimuli. Recent studies have shown that the 5th domain of VE-cadherin protein binds to the 17th domain of VE-PTP (vascular endothelial protein tyrosine phosphatase) protein is important in endothelial adherens junction integrity. To study this interaction, we modeled this binding in silico. At first, we separated these two defined domains from other parts of the proteins. Active amino acids were selected and molecular docking was performed. After selecting the best complex, it was used to do a molecular dynamics simulation (MD) using GROMACS 2021.5 and AMBER99SB + ILDN force field. The RMSD parameter was calculated to evaluate the stability of the complex. To investigate the amino acids involved in the interaction, the final structure of the MD was used as input for the pdbsum generation. In the VE-PTP and Cadherin interfaces,10 and 11amino acids were recognized respectively. Also, 3 salt bridges, 6 hydrogen interactions, and 59 non-bonded contacts were formed between two proteins. Van der Waals and electrostatic energies between the two chains were calculated to be -202.3 and -421 kg/mol, respectively. Before starting the experiments, we checked the Homology modeling of EC1-5 of VE-cadherin structure in rats and humans which was 86%. Using Chimera, the RMSD between these two structures was 0.306 Å which is less than 2Å and considered very close. Therefore, this justified using of the human sVE-cadherin $^{EC1-5}$ construct for the in vivo experiments in rats. Then in the laboratory, recombinant human sVE-cadherin $^{EC1-5}$ showed dose-dependent induced loss of endothelial barrier function in vitro and in vivo. While induction of endothelial cell death was not observed, sVE-cadherin $^{EC1-5}$ interfered with homophilic VE-cadherin interaction. In addition, sVE-cadherin $^{EC1-5}$ perturbed VE-PTP/ VE-cadherin interaction as revealed by molecular modeling and loss of VE-cadherin/VE-PTP at cell borders. Downstream effects involve VE-PTP-dependent RhoA activation which was attenuated by AKB9778. Rho-kinase inhibitor Y27632 blocked sVE-cadherin $^{EC1-5}$ induced loss of endothelial barrier function.

55 - Establishment of a primary human hypoxic colonoid-derived tissue model

Gohar Mädler[1], Sabrina Hermann[2], Christina Fey[2], Thomas Däullary[2], Daniela Zdzieblo[2], Marco Metzger[2], Alexander Westermann[1]

[1]1Helmholtz-Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Josef-Schneider-Str. 2 / D15, 97080 Würzburg, Germany, [2]Department of Tissue Engineering and Regenerative Medicine (TERM), University Hospital Würzburg, 97080 Würzburg, Germany

The large intestine plays a crucial role in nutrient absorption, water transport, and maintenance of the microbiota that—together with the immune system of the host—provides defense against pathogen invasion. However, experimental model systems to functionally study host-microbiota interactions are limited. Two-dimensional monocultures of immortalized cell lines are oversimplified surrogates of the three-dimensional and dynamic structure of the mammalian gut, while mouse models require intensive maintenance and are only to some degree experimentally amenable. Here, we established a primary hypoxic tissue model of the human large intestine. Specifically, crypts isolated from human colon biopsies were propagated as colonoids in Matrigel drops. In parallel, porcine colon tissue was decellularized, with crypts retaining their natural 3D structure, and used as a biological scaffold to seed the human colon stem cells. We evaluated diverse parameters such as the cell seeding density, centrifugation vs air-liquid interface for optimal crypt colonization, and static vs dynamic culturing. The optimized model can be cultured in a hypoxic milieu and mimics several aspects of the *in-vivo* niche, including three-dimensional crypt structures of the gut epithelium and a mucus layer. We will next apply single-cell RNA sequencing to profile the cell type composition of the tissue model and test how colonization with representative microbiota members affects the mucus layer and underlying epithelium. In summary, we expect our advanced human colon tissue model to fill the gap between simple cell culture and whole-animal models, and to become an invaluable tool in mechanistic gut microbiota research.

54 - Investigating the role of Chlamydia trachomatis infection in cancer development using organoids

Paul Köhling[1], Bhupesh Prusty[2], Nadine Vollmuth[1], Thomas Rudel[1]

[1]Chair of Microbiology, Biocenter, University of Würzburg, [2]Institute for Virology and Immunology, University of Würzburg

Chlamydia trachomatis is an obligate intracellular pathogen, who's 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, inter alia, modulate signaling pathways of the host cell 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 host DNA damage and proliferation, while interfering with DNA damage response and downregulating the tumor suppressor p53, all hallmarks of carcinogenesis. During my doctoral studies, I want to further investigate the role of *C. trachomatis* infection and the secreted NUE in host DNA damage and cancer development by infecting organoids with different genetic predispositions in order to drive the organoids into tumor formation.

53 - Development of a perfusable vascularized in vitro skin model for infection studies with Trypanosoma Brucei

Amelie Reigl[1, 2], Laura Hauf[3], Matthias Ryma[4], Jürgen Groll[4], Philipp Wörsdörfer[5], Süleyman Ergün[5], Marco Metzger[1, 2, 6], Markus Engstler[5], Florian Groeber-Becker[1, 2, 6], Dieter Groneberg[1, 6, 7]

[1]Fraunhofer Project Center for Stem Cell Process Engineering, Würzburg, Germany, [2]Institute of Tissue Engineering and Regenerative Medicine, Würzburg, Germany, [3]Department of Cell and Developmental Biology (Zoology I), University of Würzburg, Germany, [4]Department of Functional Materials in Medicine and Dentistry at the Institute of Functional Materials and Biofabrication and Bavarian Polymer InstituteJulius-Maximilians-Universität Würzburg, Germany , [5]Institute of Anatomy and Cell Biology, Julius-Maximilians University Würzburg, Germany, [6]Translational Center for Regenerative Therapies, Fraunhofer-Institute for Silicate Research ISC, Würzburg, Germany, [7]Institute of Physiology, Julius-Maximilians University Würzburg, Germany

To investigate infectious diseases such as sleeping sickness in humans, which is caused by the African parasite Trypanosome Brucei there are suitable skin models necessary. The skin is unique with its complex architecture. Due to this complexcity the production of an *in vitro* skin with hair follicle and skin layers is currently still challenging. Existing simple models lack both complexcity and in addition a perfusable vascularization. Combination of skin organoids with a perfusable vasculaized hydrogel could be used to study interaction of T.Brucei and the skin.

We identified in the hiPSC derived skin organoids 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). Skin organoids at day 140 were stung by flies infected with Trypanosoma brucei. We saw at the injection site infiltration of Trypanosoma brucei expressing the fluorophore tdTomato into the dermal layer, without infiltration into the epidermal part. After 7 days post infection, the parasites distributed throughout the complete organoid and accumulate in specific areas of the dermal layer without infiltration of epidermal parts. In parallel, the development of a vascularized perfusable hydrogel is realised by using a Scaffold of PcycloPrOx via Melt Electro Writing and a hydrogel composed of GelMA and ColMA in a bioreactor. Vascularization will be realised with iPSC derived vascular organoids. We could identify a vascular network (VE-Cadherin), with endothelial cells (CD31), pericytes (NO-GC, PDGFRß) and smooth muscle cells (SMMHC). This complex skin model is intended to further investigate the mechanism of invasion of the parasite into the human blood stream.

52 - Infection of Upper Respiratory Tract Air Liquid Interface Models Reveals Basal Cells as Important Site of SARS-CoV-2 Replication

Morris Baumgardt[1], Benedikt Obermayer[2], Anita Balázs[3], Anna Löwa[1], Emanuel Wyler[4], Luiz Gustavo Teixeira Alves[4], Katharina Hellwig[1], Dieter Beule[2], Marcus Mall[3], Markus Landthaler[4], Christian Drosten[5], Stefan Hippenstiel[1], Andreas C. Hocke[1], Katja Hönzke[1]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Department of Infectious Diseases and Respiratory Medicine, Charitéplatz 1, 10117 Berlin, Germany., [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Core Unit Bioinformatics, Charitéplatz 1, 10117 Berlin, Germany. , [3]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany., [4]Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC) and IRI Life Sciences, Institute for Biology, Humboldt Universität zu Berlin, Berlin, Germany., [5]Institute of Virology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany.

Background: The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) causes the coronavirus disease 2019 (COVID-19) and predominantly infects cells of the upper respiratory tract (URT). The human URT comprises cells of the nasal and bronchial airway epithelium consisting e.g. of secretory, basal and ciliated cells. SARS-CoV-2 tropism at the cellular level is not fully defined, whereas ciliated cells have been highlighted as a major infected cell cluster in the current literature. The question of SARS-CoV-2 induced genes and their influence also remains open.

Methods: We performed SARS-CoV-2 infections with wild type and B.1.1.7 variant of nasal and bronchial air liquid interface (ALI) models generated from primary human material. Camostat mesylate, as SARS-CoV-2 cell entry inhibitor, was applied. Replication kinetic analysis, spectral microscopy and single-cell RNA sequencing (scRNA-seq) were used to elaborate distinctions in the different conditions.

Results: SARS-CoV-2 wild type virus and B.1.1.7 variant showed a similar replication in both model types which was effectively inhibited by Camostat mesylate. Interestingly, analysis of scRNA-seq data and immunohistochemistry revealed basal cells as fraction with the highest viral load, correlating with higher replication in donors with high numbers of basal cells. As co-incidence, female donors present the highest number of basal cells. Genes associated with a high viral load include e.g. *TMPRSS2*, *HOXB2* or *MUC13*.

Conclusions: In contrast to other findings, basal cells were found to play an important role in infection. In addition to *TMPRSS2*, *HOXB* and *MUC* genes also seem to be involved in higher viral loads. In order to statistically robustly highlight the sex effect, more donors are needed. Also the influence of HPV associated *HOXB* and inflammatory *MUC* genes could be highlighted in this way.

51 - SFB/TRR 225 Project C01: Recombinant spider-silk-based hydrogels for cardiac tissue engineering

Tilman U. Esser[1], Xuen J. Ng[2], Vanessa Trossmann[2], Felix B. Engel[1], Thomas Scheibel[2, 3, 4, 5, 6]

[1]Nephropathologische Abteilung, Uniklinikum Erlangen, [2]Lehrstuhl Biomaterialien, Fakultät für Ingenieurwissenschaften Universität Bayreuth, [3]Bayreuther Zentrum für Kolloide und Grenzflächen (BZKGG), [4]Bayreuther Zentrum für Molekulare Biowissenschaften (BZMB), [5]Bayreuther Materialzentrum, [6]Bayrisches Polymerinstitut (BPI)

Biofabrication allows for the generation of complex tissue models for drug testing and disease modeling in vitro, and even transplantation in vivo. Such models are for example valuable for cardiovascular research, where novel curative therapies need to be developed to combat heart disease. While the field of biofabrication has been rapidly developing in the last decade, the development of novel materials with exciting properties and processing capacities, that allow high cell viability and functionality and at the same time processability, is still an ongoing endeavor.[1] Recombinant spider silk proteins have already been shown to exhibit remarkable properties for biofabrication. While they are biocompatible, hypoallergenic and non-inflammatory they can be processed into various forms, including shear thinning hydrogels, which are solely cross-linked by physical interactions, and can further be tailored by molecular engineering.[2, 3] Films made of positively charged, or RGD-tagged variants of recombinant spider silk allowed the culture of neonatal and hiPSC-derived cardiomyocytes.[4, 5, 6] Further, hydrogels of recombinant spider silk proteins showed the capacity to promote neovascularization in the arteriovenous loop model in rats.[7] In addition, silk-based composite hydrogels enable the generation of contractile cardiac tissues using hiPSC-derived cardiomyocytes. To further progress towards the fabrication of complex and hierarchical structured tissue models, silk-based composite and hybrid inks are developed with other polymers and pre-structuring components such as fiber fragments to suit 3D printing technologies.

References

[1] T. Jungst et al., Chemical reviews 2016, 116(3), 1496-1539.
[2] E. Doblhofer et al., Applied microbiology and biotechnology 2015, 99(22), 9361-9380.
[3] S. Salehi et al., Molecules 2020, 25(3), 737.
[4] J. Petzold et al., Advanced Functional Materials 2017, 27(36), 1701427.
[5] J. Kramer et al., Scientific reports 2020, 10(1), 1-12.
[6] T. U. Esser et al. Materials Today Bio 2021, 11, 100114.[7] D. Steiner et al., Biofabrication 2021, 13(4), 045003.

50 - A human adipose tissue model for Trypanosoma brucei infection

Laura Hauf[1], Carina Blum[2], Torsten Blunk[3], Markus Engstler[1]

[1]Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Würzburg, Germany, [2]Lehrstuhl für Funktionswerkstoffe der Medizin und der Zahnheilkunde, Institut für Funktionsmaterialien und Biofabrikation, Universitätsklinikum Würzburg und KeyLab Polymers for Medicine des bayerischen Polymer Instituts (BPI), Würzburg, Germany, [3]Klinik und Poliklinik für Unfall-, Hand-, Plastische und Wiederstellungschirurgie, Universitätsklinikum Würzburg, Würzburg, Germany

Despite awareness of *Trypanosoma brucei* colonizing multiple host tissues, biological mechanisms underlying parasite life within extravascular spaces remain unresolved. One tissue that is vital to our understanding of this parasite is the skin. At the site of transmission, the interface between host, parasite, and the tsetse fly vector is created.

To investigate African trypanosomes within host skin, advanced primary human skin equivalents were developed, comprising an epidermal and dermal layer. These human skin equivalents were utilized to characterize parasite differentiation and persistence following natural infection vs. syringe transmission.

Taking yet another step forward, we are aiming to extend our model by incorporating the hypodermis as the most interior skin layer. Based on a previously described adipose tissue construct, we are establishing an infection system to monitor *T. brucei* inside the fat.

For the preparation of fat-like tissue layers, spheroids are generated from human adipose-derived stromal cells (ASC) and seeded into fibrous scaffolds produced by melt electrowriting (MEW). Subsequently, ASCs within the tissue sheets are differentiated into the adipogenic lineage.

So far, the artificial adipose tissue model was successfully co-cultured with bloodstream-form cells as well as infected with vector-borne metacyclic forms of *T. brucei*. With this, we now have the tools to combine the fat tissue component and human skin equivalents in a sandwich approach and address complex questions regarding metabolism and tissue tropism of the parasites.

49 - SFB/TRR225 Project B02: Endothelialized perfusable microvascular networks for biofabrication of standardised in vitro tissue models

Robin Dai[1], Sven Heilig[2], Hatice Genc[1], Matthias Ryma[2], Iwona Cicha[1], Jürgen Groll[2]

[1]Section of Experimental Oncology and Nanomedicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91054 Erlangen, Germany, [2]Department of Functional Materials in Medicine and Dentistry, University Hospital Würzburg, 97070 Würzburg, Germany

INTRODUCTION Despite the large advances that tissue engineering has made over the past few decades, fabrication of functional tissue models has been restricted by the diffusion limit of oxygen and nutrients. To overcome these limitations the subproject B02 from the SFB/TRR 225 aims to biofabricate perfusable and fully endothelialized microvascular networks.

MAIN RESULTS/PRELIMINARY WORK In the first funding phase, reproducible thermoresponsive sacrificial poly(2-oxazoline) (POx) constructs with biomimetic geometries were successfully created by Melt Electrowriting (MEW). The constructs were subsequently embedded in a supporting hydrogel using dedicated microscopy-compliant bioreactors developed within the project. To manufacture perfusable microchannel networks, the embedded POx scaffolds were removed by lowering the temperature, which resulted in a formation of bifurcating channel. Upon endothelial cell seeding, channel endothelialisation was achieved within 5 days and resulted in a functional cell monolayer responding to stimuli.

METHODS AND WORK PACKAGES During the second funding phase, project B02 will continue to improve the design and fabrication of network structures to enable fabrication of more complex structures. Furthermore, our goal is to adapt the basic vascularized tissue model to tissue-specific requirements of e.g. muscle tissue, glomeruli and tumours. Furthermore, different bioreactors will be developed to support multi-fluid cell culture and complex three-dimensional structures.

To further characterise the model, cell spheroids of different cell types will be placed in the gel to evaluate their interactions with the endothelial layer, including signal transduction, migration and neovascularisation. Moreover, the endothelialized lumen will be analysed regarding the structure and functionality. This includes modelling of the blood flow in the endothelialized channels, testing of the endothelial barrier function using fluorescently labelled dextrans, cell response to inflammatory stimuli, as well as the evaluation of interactions between endothelial cells and circulating human platelets and inflammatory cells.

INTEGRATION INTO DISCIPLINARY TOPICS B02 aims to provide a microscopy-compliant vascularised model system adaptable to the needs of the subprojects B03, C01, C04 and C06. Reporter cells and imaging techniques required for the planned studies of the endothelial cell layer influence on viability and function on hydrogel-embedded cells will be provided by Z03 and Z02, respectively.

48 - Adipose Tissue Model Generated from MEW Scaffolds and 3D Multicellular Spheroids – Long-term Characterization of Adipogenesis and ECM Development

Tamara Weidemeier[1], Laura Hauf[2], Carina Blum[3], Petra Bauer-Kreisel[1], Markus Engstler[2], Torsten Blunk[1]

[1]Department of Trauma, Hand, Plastic and Reconstructive Surgery, University of Würzburg, Würzburg, Germany, [2]Department of Cell and Developmental Biology, Julius-Maximilians University, Würzburg, Germany, [3]Lehrstuhl für Funktionswerkstoffe der Medizin und der Zahnheilkunde, Institut für Funktionsmaterialien und Biofabrikation, Universitätsklinikum Würzburg und KeyLab Polymers for Medicine des bayerischen Polymer Instituts (BPI), Würzburg, Germany

Fat tissue serves as main energy storage depot and represents the largest endocrine organ. More recently, adipose tissue has been increasingly reported as a target of early infection and as a reservoir in which many organisms persist during infection, from virus, to bacteria and parasites [1]. For in vitro infection experiments, 3D adipose tissue models similar to native fat appear desirable. Such models may better represent the natural environment, as precursor cells show a superior differentiation potential, compared to conventional 2D culture, and display more native-like cell-cell and cell-matrix interactions. Based on previous work [2], here we established long-term cultures of 3D sheet-like adipose constructs generated using melt-electrowritten (MEW) scaffolds and 3D multicellular spheroids.

Box-structured MEW scaffolds were seeded with multicellular spheroids made from adipose-derived stromal cells (ASC). Fibronectin coating of MEW scaffolds distinctly improved the seeding rate of ASC spheroids. Adipogenesis was induced with induction cocktail for 7 days, followed by 2-9 weeks of culture in maintenance medium. Adipogenic differentiation was detected by the expression of adipogenic marker genes and intracellular triglyceride accumulation, which was further enhanced by the addition of ascorbic acid. The formation of an adipose tissue-like extracellular matrix was demonstrated by markedly increased laminin and collagen IV content. A positive influence of ascorbic acid on extracellular matrix formation was verified by quantification of the collagen content. The secretion of leptin and adiponectin also demonstrated native-like behavior as an endocrine organ. First results of the 10-week study showed long-term stability and viability of the 3D model. In addition, strongly enlarged triglyceride vacuoles were observed. In summary, we present a stable, viable and functional adipose tissue model that may be suitable for infection experiments. The sheet-like constructs are planned to be further investigated as subcutaneous fat layer in 3D skin equivalents, in order to elucidate infection with African trypanosomes such as *T. brucei*.

[1] Tanowitz HB, Scherer PE, Mota MM, Figueiredo LM. *Trends in Parasitology* 33, 276-284 (**2017**).

[2] McMaster R, Hoefner C, Hrynevich A, Blum C, Wiesner M, Wittmann K, Dargaville TR, Bauer-Kreisel P, Groll J, Dalton P D, Blunk T. *Advanced Healthcare Materials* 8, 1801326 (**2019**).

47 - SFB/TRR 225 Project C02: Biofabrication of 3D models for functional investigations of stromal parameters affecting the behaviour of breast cancer cells

Sabrina Stecher[1], Thabang Matseke[2], Hannes Horder[1], David Böhringer[2], Franziska Dusi[1], Petra Bauer-Kreisel[1], Ben Fabry[2], Torsten Blunk[1]

[1]Department of Trauma, Hand, Plastic and Reconstructive Surgery, University of Würzburg, 97080 Würzburg, Germany, [2]Department of Physics, Friedrich-Alexander University Erlangen-Nürnberg, 91052 Erlangen, Germany

The reciprocal communication between tumour cells and the surrounding microenvironment plays an important role in cancer development and progression. In breast cancer, adipose tissue as a major part of the tumour microenvironment undergoes structural and metabolic alterations, driving migration/invasion of neighbouring tumour cells. Using 3D printing, we aim to reconstruct this tumour microenvironment in vitro to study how structural heterogeneity and dynamic changes of the stroma influence local invasion and migration of breast cancer cells.Therefore, we developed two 3D bioprinted co-culture models using human adipose-derived stromal cells (ASC), adipose spheroids, and MDA-MB-231 breast cancer cells. In the first model, quantification of triglyceride content, immunohistochemical staining, and adipogenic marker gene expression revealed that 3D bioprinting of ASC spheroids does not impair adipogenesis.Co-culture with MDA-MB-231 cells results in triglyceride reduction in adipose microtissues and profibrotic ECM remodelling (increased collagen I, VI, and fibronectin expression), recapitulating pathophysiological events observed in vivo. With the second two-compartment co-culture model, 3D migration of tumour cells in dependence of stromal cell-secreted proteins was studied. Live cell imaging measurements of MDA-MB-231 cell migration indicate increased persistence, invasion distance, and motile fraction triggered by proteins secreted by printed ASC/adipocyte spheroids.The described biofabricated models allowed us to investigate key aspects of the bidirectional breast cancer-adipose tissue crosstalk, i.e. cancer cell-induced reduction of lipid content and ECM remodelling, and stromal cell-induced stimulation of cancer cell migration. This will provide the basis to further elucidate the complex interaction of breast cancer cells and the surrounding stroma in a defined, native-like environment.

References:
Fischer L, Nosratlo M, Hast K, Karakaya E, Strohlein N, Esser T, Gerum R, Richter S, Engel F, Detsch R, Fabry B, Thievessen I, Biofabrication 14 (2022).
Horder H, Guaza Lasheras M, Grummel N, Nadernezhad A, Herbig J, Ergun S, Tessmar J, Groll J, Fabry B, Bauer-Kreisel P, Blunk T, Cells 10 (2021).
McMaster R, Hoefner C, Hrynevich A, Blum C, Wiesner M, Wittmann K, Dargaville TR, Bauer-Kreisel P, Groll J, Dalton PD, Blunk T, Adv Healthc Mater 8 (2019).

46 - SFB/TRR 225 Project C02: Interleukin-2 stimulated natural killer cells fail to respond to chemoattractants

Thabang Matseke[1], Tina Czerwinski[1], David Boehringer[1], Christoph Mark[1], Caroline Bosch-Voskens[2], Ben Fabry[1]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, [2]Universitätsklinikum Erlangen

Immune cell therapy against solid tumors poses numerous challenges, including poor tumor homing of ex-vivo expanded autologous natural killer (NK) cells. These cells are highly motile in dense 3-D collagen gels (1). However, they migrate without clear directional preference towards tumor cells (2), patient-derived cancer-associated fibroblast spheroids, or gradients of Macrophage Inflammatory Protein-1 Alpha (MIP-1α) (a chemokine that stimulates migration of native NK cells). We hypothesize that the absence of directed migration is attributable to the continuous (over-)stimulation of expanded NK cells by interleukin-2 (IL-2) administration, which is necessary to differentiate the cells and to induce motility. To test this idea, we measure the stiffness (an indicator of cell activation) of NK-like NK92 cells in response to increasing concentrations of the chemokine MIP-1α (0, 0.1, 1, 10, 100, or 1000 ng/ml). After 15 min of incubation in a 2% alginate solution containing MIP-1α, the cells are pressed (3 bar) through a capillary (200 µm), which exposes the cells to shear stress and causes measurable cell elongation, depending on cell stiffness. We find that cell stiffness increases only slightly in response to very high MIP-1α concentrations (from 575 Pa (control) to 685 Pa (1000 ng/ml MIP-1α)). This finding supports our hypothesis and may explain why IL-2 (over-)stimulated ex-vivo expanded NK cells show impaired tumor homing in-vivo.

(1) Mark C, Czerwinski T, Roessner S, Mainka A, Hörsch F, Heublein L, Winterl A, Sanokowski S, Richter S, Bauer N, Angelini TE, Schuler G, Fabry B, Voskens CJ. Cryopreservation impairs 3-D migration and cytotoxicity of natural killer cells. Nat Commun. 2020;11:5224. doi: 10.1038/s41467-020-19094-0.

(2) Metzner C, Hörsch F, Mark C, Czerwinski T, Winterl A, Voskens C, Fabry B. Detecting long-range interactions between migrating cells. Sci Rep. 2021;11:15031. doi: 10.1038/s41598-021-94458-0.

45 - SFB/TRR 225 Project A02: Hyaluronic acid based bioink platform with multi-funcitonal crosslinkers for the controlled differentiation of MSC

Paula Büttner [1], Jeanette Weigelt[2], Torsten Blunk [1], Jörg Teßmar[2]

[1]Departement of Trauma, Hand, Plastic and Reconstructive Surgery, University Hospital Würzburg , [2]Departement for Functional Materials for Medicine and Dentistry, University Hospital Würzburg

Current 3D bioprinting frequently involves the application of concentrated polymeric bioinks, which ensure sufficient fabrication stability, but often lack the suitable properties for cell survival and differentiation. Another challenge is to find a bioink for an appropriate tissue development, which leads to a good distribution of the formed extracellular matrix (ECM) in the construct ideally. Hyaluronic acid (HA) appears to be an attractive material for biofabrication approaches, as it is a main compound of the extracellular matrix (ECM) providing structural and mechanical support for the embedded cells as well as functioning as signaling molecule.

Previous studies established a newly developed bioink platform utilizing a dual-stage crosslinking mechanism, containing thiolated hyaluronic acid (HA-SH), acrylated (PEG-diacryl) and allylated (PEG-diallyl) polyethylene glycol. Printability is achieved by the initial crosslinking step between the HA-SH and the PEG-diacryl in a Micheal-Addition at neutral pH. Long-term stability is accomplished by UV-induced thiol-ene click chemistry.

By using high molecular hyaluronic acid components like HA-SH larger than 200 kDa, it was possible to lower the polymer contents to 2 % in the hydrogel formulations, which allowed adjustment of the biofabrication window and the overall mechanical stability of the bioink. The formation of a coherent ECM in the chondrogenically differentiated hydrogels associated with distinctly improved tissue stiffness was enhanced by the lower polymer content. In a second approach the stepwise crosslinked bioink was further modified using a thiolated growth factor (TGF-beta1) to enhance differentiation of the incorporated MSC.

We intend to expand the material platform using specific variations of the physicochemical properties and to optimize the biological functionalization of the HA-based bioink. Hence, we aim to optimize the bioink using newly synthesized gelatin-based polymers and pentenoyl-functionalized HA (HA-PA) to reduce the polymer content of the gel. The newly developed gels will be evaluated regarding MSC differentiation as well as the production and homogenous distribution of the ECM.

Hauptstein, J. et al., Advanced healthcare materials, 9(15), e2000737, 2020.
Hauptstein J. et al., Macromol. Biosci. 22:e2100331, 2022.
Hauptstein J. et al., Int. J. Mol. Sci. 23:924, 2022.

44 - SFB/TRR 225 Project B05: Membrane engineering as a tool to control the behaviour of mesenchymal stem cells in biofabrication processes

Marcel Groß[1], Kirilo Krjukov[2], Stephan Altmann[2], Jürgen Mut[1], Jürgen Seibel[1], Regina Ebert[2]

[1]Institute of Organic Chemistry Julius-Maximilians University of Würzburg, [2]Department of Musculoskeletal Tissue Regeneration Julius-Maximilians University of Würzburg

Biofabrication is an interdisciplinary scientific research area which combines tissue engineering, material science, and 3D printing. Biofabrication aims to create biomimetic materials which display hierarchical arrangement within a scaffold and imitate biological properties closer to native tissue than classical tissue engineering scaffolds. One challenge constantly faced during the biofabrication process is choosing between cell viability and structure fidelity. In the 3D printing process, higher structure fidelity is often achieved using firmer bioinks and nozzles with a smaller diameter. The latter can decrease cell viability due to the growing shear stress with increasing nozzle gauge. Our goal is to increase the cell viability of various cell types during the 3D printing process by modifying the cell membrane with two different methods.

The first method is called glycoengineering, and it modifies a cell's glycocalyx by incorporating various modified sugars via metabolic processes. The second method involves a synthesized molecule with cholesterol "anchors", which can directly be integrated into a cell's lipid bilayer due to the highly lipophilic nature of the cholesterol molecules. According to our preliminary research, two tetraacylated mannose monosaccharides displayed the most promising results, which were quantified using copper-catalyzed click chemistry. Fluorescent dyes, with chemically complementary groups used in this reaction, showed the highest fluorescence and, therefore, the most effective integration of mannose into the glycocalyx compared to glucose and galactose. The downside of glycoengineering is a long 72 h incubation time, which may be reduced by using the fast-acting cholesterol "anchors." They integrate into a cell's membrane within minutes. However, they also dissociate from the membrane within 15-30 minutes.

We aim to identify which method of membrane engineering is better suitable for increasing cell viability within the 3D printing process. We will compare glycoengineering to the membrane "anchors" regarding viability, cell adhesion, cell-cell interaction, and cell-bioink interaction. Furthermore, we will investigate if and to what extent those membrane modifications change the stiffness of the cells and if increasing or decreasing the stiffness shows better results for the criteria mentioned above.

43 - Manufacturing and assessment of a multi-well osteoblast-osteoclast 3D in vitro model.

Alexander Sieberath[1]

[1]University Hospital Knappschaftskrankenhaus Bochum

Understanding how bone remodelling is regulated is key for the effective prevention and treatment of osteoporosis. Consequently, research has focused on the development of new in vitro models using cell lines or primary cells to gain more relevant preclinical research results. The generation of new osteoblasts and osteoclasts co-culture models is necessary due to the major role of both cell types during osteoporosis disease progression.

Co-culture models of osteoblasts and osteoclasts offer an alternative to animal testing and are considered to have the potential to improve the drug development processes for bone related diseases in the future. Here, we report the development of a 3D in vitro model of the bone remodelling process containing osteoblast- and osteoclast-like cells.

A collagen-supplemented ten times simulated body fluid like solution (10x SBF collagen) was used to deposit a calcium phosphate collagen composite coating on a SLA printed cell culture insert. The designed cell culture insert consisted of 88 microwells with a central pin in trabecula bone scale located in the centre of each well.

Human bone marrow derived osteoblast- (OB) and osteoclast-like cells (OC) were co-cultured on coated SLA printed geometries and coated tissue culture pastic. Our results show that the net remodelling activity of osteoblasts and osteoclasts could be evaluted by fluorescent staining techniques in a quick and effective manner.

The developed model has the potential to be used in osteoporosis drug discovery and disease modelling applications.

42 - Bioorthogonal Click-Labelling of SARS-CoV2 Spike on Pseudotyped Vesicular Stomatitis Virus for SMLM and Live Cell Imaging

Marvin Jungblut[1], Simone Backes[2], Marcel Streit[3], Matthew Lycas[4], Markus Sauer[1], Gerti Beliu[3]

[1]Department of Biotechnology and Biophysiks, Julius-Maximilians-Universität Würzburg, [2]Department of Virology and Immunobiology, Julius-Maximilians University Würzburg, [3]Rudolf Virchow Center, Center for Integrative and Translational Bioimaging; Julius-Maximilians University Würzburg, [4]EPFL, Institute of the Physics of Biological Systems

The last years demonstrated impressively that viral infections have to be investigated at high spatial and temporal resolution to enable the development of new refined therapeutics. Microscopic investigations of viral infections so far have been limited by the small size of viral particles that cannot be resolved by conventional fluorescence microscopy. Super-resolution microscopy with a resolution of few nanometers now offers the capability of more detailed studies of the early stages of infection. However, a major challenge in the application of super-resolution microscopy is fluorescence labelling. Standard immunolabelling often blocks the interaction of the viral particle with the host cell. Therefore, we used genetic code expansion (GCE) and bioorthogonal click labelling with tetrazine-dyes for site-specific labelling of Spike proteins, without affecting their function during infection. We investigated viral infection by pseudotyping vesicular stomatitis virus (VSV) with a clickable variant of the SARS-CoV-2 Spike protein. We performed infection studies on Vero cells expressing angiotensin-converting enzyme 2 (ACE2) alongside *direct* stochastic optical reconstruction microscopy (*d*STORM) measurements of immobilized VSVs. Here we found that the size and shape of these particles is consistent with structural data from previous studies. In addition, click labelling offers advantages in labeling efficiency compared to immunostaining and allows for quantification of Spike proteins present on the virion. Further, we could image the infection of live cells - from docking to the subsequent expression of GFP in the host cells induced by VSVs - using lattice light-sheet microscopy. Our method of GCE and bioorthogonal labelling of viruses will improve future microscopic investigations of viral infections.

41 - Development of bioresins for volumetric printing for vascular applications

Csaba Gergely[1], Michael Bartolf-Kopp[1], Tomasz Jüngst[1], Lisa Galaba[1]

[1]Department for Functional Materials in Medicine and Dentistry University of Würzburg

Volumetric printing (VP) is a novel cell-friendly 3d printing method capable of in situ encapsulation of cells into CAD designed models in a one-step process within seconds, leading to fast manufacturing of usable sample sizes of complex hydrogel scaffolds. With this method, not restricted to a layer-by-layer structure, large objects can be created which were not able to be printed with conventional biofabrication methods, for example multi material objects ore intricate tubular branching constructs. These tubular models can be post-seeded with endothelial cells and cultivated under physiological flow to further investigate artificial small diameter blood vessel models. Especially models for coronary arteries are in need for medical training and therapeutic replacement purposes. For that even 1:1 copies of 3d scanned tissue can be used as for example CT scans of blood vessel systems of the coronary tree. To prevent cell- sedimentation enhance cell distribution and provide a beneficial physiological environment for cell maturation, special properties for the resin have to be established. First and foremost, bioresins, which are a bioink formulation used for vat and vial based biofabrication techniques, a cytocompatible and photocrosslinkable hydrogel precursor, with methacrylated Gelatin (GelMA) as prototypical representative, has to be chosen. GelMA has thermal gelling behavior and good optical transparency, its properties can be tuned via the formulation of the precursor solution. This results in different mechanical stiffness, printing resolution and biological behavior to mimic the extracellular matrix (ECM) for the various cell types in volumetric bioprinting. But these properties can only be optimized up to a certain point. Research into alternatives or new polymeric options for photosensitive fabrication needs to be conducted to push the limits of printability and biocompatibility. Further, certain limitations of this printing method have to be addressed like the limited scaffold size and convergence with other biofabrication and tissue engineering methods.

By combining chemistry and biofabrication with tight feedback loops to biology and biomechanics we are aiming for advanced in-vitro vasculature models. We apply a variety of biofabrication methods like for example VP and MEW to model small vascular grafts with optimized mechanical properties and cultivate in bioreactors.

40 - SFB/TRR 225 Project C05: Ultraweak hydrogels for molecular and biological functional analyses of cell-matrix and cell-cell 3D networks in neuronal cell culture systems

Mateo Andrade Mier[1], Carmen Villmann[1], Reiner Strick[2], Anna Reinert [2], Esra Türker[3]

[1]Universitätsklinikum Würzburg (SFB/TRR225), [2]Universitätsklinikum Erlangen (SFB/TRR225), [3]Universitätsklinikum Würzburg

Hydrogel matrices mimicking native brain extracellular matrix (ECM) environment have been shown to be capable of promoting neural network formation. The matrices used need to recapitulate the chemical composition as well as mechanical properties of the brain. Matrigel (mouse sarcoma extracted ECM) and hyaluronic acid-based hydrogels (in coculture with astrocytes) have proven to be suitable candidates for neuronal development. Since brain is one of the softest tissues, both hydrogels are used ultra-weak with elastic moduli below 100 Pa. To offer appropriate mechanical support for cell growth and handling in the lab, melt-electro written polycaprolactone (PCL) frames can be used to reinforce said hydrogels. In both, synapse formation and dendrite outgrowth 3D composites outperform 2D *in vitro* controls. Additionally, the neural networks formed in 3D are functional.

Simulating brain disease states, besides neurons, glial cells and glioblastoma cells can also be successfully cultured in similar 3D constructs. Each of these cell types corresponds to different physiological and pathological states of the brain. Using glial cells and glioblastoma cells, we were able to determine that the composition of the hydrogel has an impact on cellular behavior. Furthermore, the design of the PCL frames, that initially served as a support structure, has a profound influence on cell morphological development and cellular migration aspects.

The platform of ultraweak hydrogels reinforced by carefully designed MEW printed PCL frames will further allow the development of in vitro composites to study glioblastoma. Tumor cells need to interact with native CNS cells and the brain ECM to promote their progression. In this process astrocytes and neurons play an important role. The development of co-cultures of two or three cell types will help further unravel interactions between glioma and native cells and glioma cells and matrix in terms of functional, phenotypical, and mechanical changes.

39 - Analysis of innate immune response of gastrointestinal epithelium to EPEC infection using human 3D organoids derived 2D monolayers as a model

Mastura Neyazi[1], Carmen Aguilar[1], Nicolas Schlegel[2], Kirsten Glaser[3], Sina Bartfeld[1, 4, 5]

[1]Research Centre for Infectious Diseases, Institute for Molecular Infection Biology, Julius Maximilian Universität Würzburg, Würzburg, Germany, [2]Department of General, Visceral, Vascular and Paediatric Surgery, University Hospital of Würzburg, Würzburg, Germany, [3]Division of Neonatology, Department of Women's and Children's Health, University of Leipzig Medical Center, Leipzig, Germany, [4]Medical Biotechnology, Institute for Biotechnology, Technische Universität Berlin, Berlin, Germany, [5]Si-M/'Der Simulierte Mensch', a Science Framework of Technische Universität Berlin and Charité-Universitätsmedizin Berlin, Berlin, Germany

Introduction: The innate immune response of the host cell activated by pattern recognition receptors (PRRs) and microbe associated molecular patterns (MAMPs) initiates NF-kB response that results in inflammatory cytokine production upon microbial exposure. Profiling of human intestinal organoids grown from the different segments of the gastrointestinal tract show segment specific gene expression. This can be utilised to study gastrointestinal infection using patient derived primary cells. Enteropathogenic E. coli (EPEC) is human specific, gram-negative bacteria that causes diarrhoea in infants and children in developing countries leading to severe illness and even death. The key component of EPEC pathogenicity is type III secretion system (T3SS) that inject effector proteins into the host cell to block the NF-kB pathway and prevent inflammatory response.

Material/methods: Organoids are generated *in vitro* from resection material from human Jejunum. Here we establish 2D cultures from 3D organoids. Barrier function is measured using transepithelial electric resistance (TEER). Using different media composition, we analyse the differentiation of the cells in 2D using qRT-PCR and immunofluorescence. We infect the primary monolayers with EPEC. Attachment of bacteria is determined by microscopy and FACS.

Results: Within 7-8 days, organoid-derived cells form a tight monolayer. The monolayers are stable for at least 10 days. Using differention media, cultures can be directed either towards adsorptive lineages, enterocytes, or secretory cells (goblet cells marked by MUC2 and enteroendocrine cells marked by CHGA). EPEC can attach and infect Jejunum monolayer cells and form characteristic actin-rearrangements ("pedestals") on the apical side of the cells, one of the hallmarks of EPEC infection. With FACS analysis we detect about 50% of cells EPEC+ in 2D monolayers compared to 75% in HeLa cells.

Discussion: Primary human jejunum monolayers grown from 3D jejunum organoids show characteristics similar to that of physiological conditions namely, tight junction formation and upregulation of various differentiated cell. This provides an easily achievable tool to better understand the interaction of pathogens with the host cells, which is otherwise difficult to investigate in human setting. This therefore provides a platform to identify the importance of both EPEC and host factors for selective infection in the human gastrointestinal tract.

38 - SFB/TRR 225 Project B04: 3D Bioprinting of Vascular Structures using Vascular Wall-Resident Stem Cells and Human iPSC-derived Multipotent Progenitor Cells

Nathaly Chicaiza Cabezas[1], Leyla Dogan[2], Ruben Scheuring[1], Philipp Wörsdörfer[2], Jürgen Groll[1], Süleyman Ergün[2]

[1]Department for Functional Materials in Medicine and Dentistry, University Hospital Würzburg, [2]Institute of Anatomy and Cell Biology, University of Würzburg

Vascular networks are essential structural components that supply tissues with nutrients and oxygen. Proper vascularization is crucial in artificially generated structures; however, the in vitro generation of functional hierarchical vascular networks remains one of the major challenges of tissue engineering. Our study aims to biofabricate complex and hierarchical vascular systems using vascular wall-resident stem cells (VW-SCs) and iPSC-derived mesodermal progenitor cells (hiMPCs). Both cell types have the capacity to deliver all cell types present in the vascular wall, namely endothelial cells, smooth muscle cells, perycites, and fibroblasts. The biofabrication of such complex structures requires an appropriate matrix material that is printable, supports cell attachment, viability, and proliferation, and promotes morphogenetic processes. We supplemented an alginate-based bioink with extracellular matrix (ECM) components such as col I and laminin to promote cell adhesion and differentiation. Additionally, we used xanthan gum as a thickener/sacrificial material to improve printability and enhance microporosity.

Bioinks composed of stem cells and alginate-based hydrogels supplemented with ECM components were extruded into molds to evaluate cellular behavior after extrusion. Similar parameters were then used to print the bioinks into defined tubular structures using in-gel bioprinting. Cellular viability and morphogenetic capacity were evaluated using a viability assay and immunohistochemistry. The preliminary results showed an overall cell viability of 90% and early start of cell migration and elongation within the hydrogel mixture supplemented with ECM components and sacrificial materials. Overtime, cells entered differentiation and morphogenesis, resulting in the formation of cell cords. It was therefore suggested that cell proliferation, interaction, and differentiation depend on the morphology and chemistry of the matrix. We previously demonstrated that hiMPCs encapsulated in alginate/col I hydrogels survive extrusion and retain their capacity to form complex vascular structures that display a multilayered and hierarchical network, in a process that mimics the embryonic steps of vessel formation by vasculogenesis. Further analysis of the printed cells into defined tubular structures is under study. In conclusion, the combination of VW-SCs/hiMPCs and a proper hydrogel mixture could result in the generation of hierarchical vascular structures composed of small and large vessels with multilayered wall displaying intima, media and adventitia-like structures.

37 - SFB/TRR225 Project A08: Vascular supply for 3D tissue based on shape-changing polymers and recombinant spider silk

Saskia Roth[1], Christina Heinritz[2], Leonid Ionov[1], Thomas Scheibel[2, 3, 4, 5, 6]

[1]Professur für Biofabrikation, Fakultät für Ingenieurwissenschaften, Universität Bayreuth, [2]Lehrstuhl Biomaterialien, Fakultät für Ingenieurwissenschaften, Universität Bayreuth, [3]Bayreuther Zentrum für Kolloide und Grenzflächen (BZKG), [4]Bayreuther Zentrum für Molekulare Biowissenschaften (BZMB), [5]Bayreuther Materialzentrum (BayMAT), [6]Bayerisches Polymerinstitut (BPI)

Tissue engineering is a steadily evolving method of replacing damaged or lost tissue/organs [1, 2] in case the self-healing capabilities of the human body reach their limits. A major problem in the development of biological 3D scaffolds is the adequate supply of oxygen and nutrients due to the lack of a functioning vascular network.

The project goal is to explore possibilities for fabrication of a supply structure for oxygen and nutrients in engineered tissue scaffolds. At the core are tubes populated by unidirectionally aligned smooth muscle and endothelial cells, which mimic the hierarchical organization of natural blood vessels. For this endeavor, 3D bioprinting and fiber spinning processes will be combined with a 4D biofabrication approach, which allows the generation of complex structures with high precision. First, a flat layered construct is fabricated and populated with cells, followed by controlled shape-transformation into a tubular structure [3]. The chosen surface topography will control cell adhesion and directional growth [3, 4]. Next to synthetic biopolymers, engineered recombinant spider silk will be one structural component for cell adhesion and alignement. Silk materials are biocompatible, hypoallergenic, non-inflammatory, biodegradable and possess extraordinary mechanical properties, in particular high elasticity and strength [5]. Furthermore, they can be easily modified for addressing a specific cellular interaction and, since a modified silk variant has previously been shown to enhance vascularization of hydrogel scaffolds in AV-loop experiments [6], is a promising candidate for its application in engineering of complex vascularized tissues.

References:

[1] F.J. O'Brien, Biomaterials & scaffolds for tissue engineering, Materials Today **2011**, 14(3) 88-95.
[2] R. Langer, J.P. Vacanti, Tissue engineering, Science **1993**, 260(5110) 920.
[3] G. Costante *et al*., ACS Applied Materials and Interfaces **2021**, 13(11) 12767-12776.
[4] J. Uribe-Gomez *et al*., ACS Applied Bio Materials **2021**, 4(7) 5585-5597.
[5] E. Doblhofer, Applied microbiology and biotechnology **2015**, 99 (22) 9361-9380.
[6] D. Steiner *et al.,* Biofabrication **2021**, 13 045003

36 - SFB/TRR225 Project C03: Analysis of tumor dormancy and progression in biofabricated vascularized 3D models

Evelin Sandor[1], Tannaz Karimi[1], Carolin Eckert[2], Andreas Prof. Dr. Arkudas[3], Anja Prof. Dr. Bosserhoff[2], Rafael Schmid[1], Sonja Schmidt[2], Annika PD Dr. Kengelbach-Weigand[3]

[1]Laboratory for Tissue Engineering and Regenerative Medicine, Department of Plastic and Hand Surgery, University Hospital of Erlangen, Friedrich-Alexander University Erlangen-Nürnberg (FAU), 91054 Erlangen, Germany, [2]FAU Erlangen-Nürnberg, Department for Biochemistry and Molecular Medicine, [3]University Hospital of Erlangen, Department of Plastic and Hand Surgery

The melanoma is a highly malignant tumor of the melanocytes. Critical issues are the early metastasis from small primary tumors and possible resistance against therapies as well as tumor dormancy. The phenomenon of tumor dormancy, where tumor cells remain in the body for years to decades without any tumor progression, is barely understood yet. The aim of the project is the biofabrication of in vitro and in vivo tumor models in the vascularized system of the arteriovenous loop (AV Loop) of the rat. Selected melanoma cell lines (isolated from primary tumor and metastases) will be printed and analyzed in different inks, also in co-culture models with ADSC (Human Adipose-Derived Stem Cells) and MSC (Human Mesenchymal Stem/Stromal Cells). The cells will be printed as single cell suspensions and tumor spheroid in established dormancy and progression models. We found different materials to mimic tumor dormancy as well as progression. HAA / Cellulose-Alg and AlgHyaGel will be used as dormancy model and progression model, respectively. The focus will now be on investigating why and how a tumor cell transitions from dormancy to proliferation. Based on our previous experience, we will use biofabrication to reproduce more complex tumors with different areas, stiffness and cell types and analyze the behavior of the tumor cells. Survival and proliferation can be analyzed by fluorescence microscopy using reporter cell lines. The RNA can be isolated from the bioinks and later used for RNA sequencing. The AV loop model is a great option to observe among others the cell behavior, the tumor growing properties, the cell-cell and cell-matrix interactions and additional the (tumor) anginogenesis effects. In the long term, the models established here will help to clarify basic scientific questions about tumor development and progression and will serve as a standard model for testing anti-cancer therapies.

35 – Selected Talk and Poster: SFB/TRR225 Project Z02: Quantitative imaging and analysis of biofabricate quality and maturation

Julian Bauer[1], Ivana Jeremic[2], Joachim Schenk[3], Matthias Weiss[2], Oliver Friedrich[1], Katrin Heinze[3]

[1]Institute of Medical Biotechnology, Friedrich-Alexander-University Erlangen-Nürnberg, [2]Institute of Experimental Physics I, University of Bayreuth, [3]Institute of Molecular Microscopy, Rudolf-Virchow-Zentrum, Julius-Maximilians-University Würzburg

The process of biofabrication, or more specifically the development and production of functional, structurally integral three-dimensional tissue models, requires continuous, non-invasive quality control regarding production quality and subsequent construct maturation. The subproject Z02 provides this quality control, by applying state-of-the-art light microscopy techniques such as Multiphoton (MPM), label-free Second-Harmonic generation (SHG) and Selective Plane Illumination Microscopy (SPIM), while constantly developing new imaging approaches and software-based image analysis tools. This ongoing development process aims to simultaneously optimize imaging parameters like temporal and spatial resolution and to expedite data acquisition, processing and analysis for every investigated sample. In addition to the service network character of the project, every involved research group additionally incorporates an individual work package focused on the development of novel advanced imaging systems and bioreactor solutions, to further facilitate sample handling and optical analysis. Firstly, Würzburg is focussing on expanding the device portfolio with a far-infrared Lightsheet Fluorescence Microscopy (LSFM) application, which is customized for three-dimensional analysis of fixed and optically cleared samples. Bayreuth, on the other hand, is concentrating on the establishment of a SPIM imaging system specifically tailored for live cell imaging inside of biofabricates, with a strong emphasis on the characterization of cell migration. Lastly, to establish a common reproducible foundation for sample handling and cultivation during imaging at every research site of the joint project, Erlangen is enhancing and validating a modular micro bioreactor environment, which will allow for real-time imaging during construct maturation, due to integrated flexible thin-layer windows.

34 - M-CSF treatment enhances tissue resident alveolar macrophage function to protect from pulmonary Aspergillus fumigatus infection

Dalia Sheta[1, 2], Zeinab Mokhtari[3], Marlene Strobel[4], Yidong Yu[1], Jorge Amich[5], Nora Trinks[2, 6], Sebastian Reinhard[2, 6], Sina Thusek[1, 2], Hermann Einsele[1], Katrin G. Heinze[7], Ulrich Terpitz[6], Andreas Beilhack[1, 2]

[1]Department of Medicine II, University Hospital, Würzburg, Germany, [2]Graduate School of Life Sciences, Würzburg, Germany, [3]GSK plc., [4]Biotest AG, [5]Manchester Fungal Infection Group (MFIG), School of Biological Sciences, University of Manchester, Manchester, United Kingdom, [6] Biocenter, University of Würzburg, Germany, [7]Rudolf-Virchow-Center, University of Würzburg, Germany

Patients undergoing hematopoietic cell transplantation (HCT) are at high risk of life-threatening invasive aspergillosis. Therefore, we investigated first-line host defense mechanisms to protect from pulmonary *A. fumigatus* infection and asked whether cytokines, including M-CSF and IL-34, affect alveolar macrophages (AMs) function to improve protection from invasive aspergillosis after allogeneic allo-HCT.

To study the impact of these cytokines on AMs phenotype, migration and phagocytic function, we employed super-resolution, dynamic laser-scanning-microscopy and functional in vitro assays. To study the outcome of exogenous cytokine stimulation on the pulmonary immune response in vivo we employed mouse models of allogeneic allo-HCT and invasive *A. fumigatus* infection. To this end, we monitored the survival of the mice and investigated local host-pathogen interaction using flow cytometry and 3D light sheet fluorescence microscopy.

Allo-HCT recipients survived *A. fumigatus* infection when infected 6 days but not 4 days after HCT. AMs showed the highest frequency, proliferation and phagocytic activity in the lung, when compared to neutrophils and monocytes, suggesting that tissue-resident AMs were responsible for protecting mice from *A. fumigatus* infection after allo-HCT. This observation was confirmed by selective AM depletion, which rendered mice vulnerable to uncontainable infection. Subsequently, we investigated whether cytokines could boost AMs to achieve earlier protection. In vitro, M-CSF but not IL-34, increased AMs migration speed (0.52 vs. 0.3 µm/min, respectively) and versatility (diffusion co-efficient of 0.78 vs. 0.6 μm^2/min, respectively). Next, we treated HCT-recipients with M-CSF and subsequently infected them intratracheally with *A. fumigatus* 4 days after allo-HCT. M-CSF boosted myelopoiesis by 2-fold and, importantly, locally expanded tissue-resident AMs by 1.5-fold. Additionally, M-CSF improved AM killing capacity and protected 90% of allo-HCT recipients from *A. fumigatus* infection on day 4 after allo-HCT. Additionally, M-CSF fostered lung tissue integrity and reduced serum levels of pro-inflammatory cytokines. Finally, M-CSF treatment did not protect from lethal aspergillosis upon local depletion of AMs in allo-HCT recipients supporting the importance of a functional pool of tissue-resident AMs to protect from pulmonary infections.

Conclusively, these data support that M-CSF holds great potential for clinical application by fostering tissue-resident AMs responsiveness to protect from early *A. fumigatus* infections in allo-HCT recipients.

33 - SFB/TRR225 Project B09: Biofabricated gradients for functional tissue models

Jessica Faber[1], Florian Hofmann[2], Silvia Budday[1], Tomasz Jüngst[2]

[1]Friedrich-Alexander-University Erlangen-Nürnberg, Institute of Applied Mechanics, [2]University of Würzburg, Department for Functional Materials in Medicine and Dentistry

Gradients in native tissues are decisive for the fate of cells and therefore need to bereproduced for the successful fabrication of functional tissue analogues [1, 2]. As a result, it isa key challenge to mimic the complex, viscoelastic properties of soft tissues in space and timeby suitable biomaterials and to create tissue models with locally varying material properties.This becomes possible with the aid of automated processes, such as 3D bioprinting, wherecells and biomaterials can be precisely deposited.The viscoelastic properties of biomaterials can lead to challenges in printing gradients, wheremixing of multiple materials is necessary. To face these difficulties, a suitable platformtechnology needs to be developed that is capable of biofabricating defined and reproduciblegradients in space and time.For this purpose, we developed printheads with self-designed microfluidic mixing geometriesto mix biomaterial inks of different viscosities and to print controlled transitions of materials,active substances, and cells. Furthermore, we use suitable testing techniques with welldesigned,standardized testing protocols to reliably quantify the mechanical properties ofgradients within biofabricated constructs across scales. To this end, we combine mechanicaltesting using a rheometer to perform dynamic, quasi-static and stress relaxation experimentsunder multiple loading modes, e.g., compression, tension, and torsional shear, withindentation measurements and imaging. In addition, we use appropriate approaches forconstitutive modeling based on continuum mechanics to quantify the complex materialproperties and to systematically optimize process parameters, the print pattern, and the 3Darrangement within biofabricated constructs. Corresponding computational models based onthe finite element method (FEM) help to predict the biomaterial behavior under therespective loading and boundary conditions and serve as a valuable tool to optimize thefabrication process of functional tissue models.

[1] Koser DE; Thompson AJ; Foster SK; Dwivedy A; Pillai EK; Sheridan GK; Svoboda H; Viana M;Costa LD; Guck J; Holt CE; Franze K. Nat Neurosci 2016, 19 (12), 1592.[2] Li C; Ouyang L; Armstrong JPK; Stevens MM. Trends Biotechnol 2021, 39 (2), 150.

32 – Selected talk (no poster): Salmonella Typhimurium infection in a primary in vitro model of the human small intestine reveals the induction of OLFM4 expression as host cell response

Thomas Däullary[1, 2], Fabian Imdahl[3], Oliver Dietrich[3], Laura Hepp[2], Christina Fey[2], Tobias Krammer[3], Alexander J. Westermann[3, 4], Jörg Vogel[3, 4], Marco Metzger[2, 5, 6], Emmanuel Saliba[3, 4], Daniela Zdzieblo[2, 5, 6]

[1]University Würzburg, Chair of Microbiology, [2]University Clinic Würzburg, Chair of Tissue Engineering and Regenerative Medicine, [3]Helmholtz-Institut for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany, [4]Institute for Molecular Infection Biology (IMIB), Unjversity of Würzburg, Würzburg, Germany, [5]Fraunhofer Institute for Silicate Research (ISC), Translational Center Regenerative Therapies (TLC-RT), Würzburg, Germany, [6]Fraunhofer Institute for Silicate Research, Project Center for Stem Cell Process Engineering, Würzburg, Germany

Salmonella, including the non-typhoidal *Salmonella enterica* serotype Typhimurium (STm), is a foodborne pathogen causing moderate to severe infections in the intestinal tissue and is considered 1 of 4 key causes of diarrheal diseases worldwide. Although fundamental insights into STm infection biology have been gained in recent decades using classical cancer cell lines and animal studies, these systems are reaching the limits of their informative value with respect to human pathogenesis. Modern infection research therefore requires alternative approaches such as tissue engineering-inspired model systems that more accurately resemble the human physiology. We therefore developed a primary human tissue model for infection research that mimics biological and physical properties of the human intestinal epithelium. It consists of a natural biological scaffold reflecting the extracellular matrix combined with primary intestinal organoids. With confocal and electron microscopy as well as single cell transcriptomic analysis, we demonstrated that our model exhibits characteristics similar to native tissue, including (ultra-)structural features, appropriate barrier functions, and a cellular profile similar in composition to the native one. We established an infection protocol that enables the direct interaction of STm with the apical epithelial surface and the further observation of the intracellular *Salmonella* life progression. By spatiotemporal analysis, we show that the key steps of the STm invasion of the epithelium are recapitulated, which includes bacterial adhesion, F-actin remodelling and ruffling, bacterial invasion, F-actin cytoskeleton restoration. Further, we observed apical-basolateral intracellular migration and the development of heterogonous *Salmonella* phenotypes, including non-dividing and filamentous *Salmonella*. With this infection model, we further investigated the transcriptomic epithelial host cell response via single cell RNA-sequencing and observed among others changes in expression of the stem cell marker Olfactomedin-4 (OLFM4). Via HCR-FISH and protein expression analysis, we could demonstrate for the first time that OLFM4 expression is a NOTCH mediated epithelial response towards Salmonella infection, suggesting a previously unknown cellular mechanism in the human pathogenesis of *Salmonella* Typhimurium infection.

31 - Heterogeneity in defense between cervical squamous and columnar epithelia against Chlamydia infection at single-cell resolution

Pon Ganish Prakash[1]

[1]Doctoral Researcher in Chumduri lab

Chlamydia trachomatis (CT), an obligate intracellular, Gram-negative bacterium that causes sexually transmitted infections, is linked to cervical cancer development, especially in patients with human papillomavirus (HPV) coinfection. The human cervix hosts two distinct types of epithelial cells: the ectocervix (outer part composed of stratified squamous cells) and the endocervix (inner part composed of glandular columnar cells). The transition zone (TZ), where these two types of epithelia meet, is highly predisposed to infections and cancer development. Cervical cancer is the fourth most common type in women. It occurs in two different histological forms: squamous cell carcinoma (SCC), often arising from squamous cells at the TZ, and adenocarcinoma (ADC), developing from glandular columnar cells. Cervical cancers in patients are usually SCC (9/10 times). This indicates that CT can easily infect squamous cells, whereas the columnar cells fight back in protecting the host to a certain extent. Here, our goal is to study the innate immune discrepancies and capture discrete defense mechanisms equipped by ecto and endocervix to protect the host against CT. Hence, we used human patient-derived ectocervix and endocervix *in vitro* 3D organoid models and employed a single-cell RNA sequencing technique to study CT infection at a higher resolution. Our analysis identified transcriptionally distinct cellular subtypes with concrete information on lineage development in both epithelia. In addition, we deconvoluted the innate immune differences between ecto and endocervix pre-and post-CT infection. This highlighted that the early/stem cells of endocervix (Co1A and Co1B) have superiority over ectocervix, naturally to safeguard the host cells against pathogens and clearing out microbes substantiated by stronger gene expression levels of Mucins, Pattern Recognition Receptors (PRRs) and Anti-Microbial Peptides (AMPs). Strikingly, we identified a unique, remodeled subpopulation of columnar cells upon CT infection with exclusive gene expression profiles correlating to a robust interferon-gamma response, Il-6 Jak/stat3 signaling, and cytokine signaling. Physiologically, we observed that ectocervical organoids with CT infection gets disrupted easily over time; however, endocervical organoids remained intact. This underlines the fact that infection in columnar epithelia transcriptionally renovates them with high defensive capabilities with elevated immune response mediated mechanism via IFNg signaling to prevent reinfection.

30 - Establishment of human Coronavirus NL63 in Cell Lines and Human Respiratory ALI Models

Kristina Meierhans[1], Kshitija Gadekar [1], Rinu Sivarajan [2], Niklas Pallmann[2], Maria Steinke[2], Maria Steinke [3], Christine Krempl[1]

[1]1. Institute for Virology and Immunobiology, Julius-Maximilians-University Würzburg, Würzburg, Germany, [2]2. Chair of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Würzburg, Germany, [3]3. Translational Center Regenerative Therapies, Fraunhofer Institute for Silicate Research ISC, Würzburg, Germany

Human Coronavirus (HCoV) NL-63 that was isolated in 2004 has been reported to use the transmembrane protein angiotensin converting enzyme 2 (ACE2) as receptor for attaching to target cells, as does the severe acute respiratory syndrome (SARS) CoV and SARS-CoV-2. However, they differ greatly in their pathogenicity as HCoV-NL63 predominantly causes mild respiratory disease. For the purpose of comparing the patho-mechanisms of these viruses, in particular with respect of age- and gender-dependent mechanisms in human respiratory 3D models, we established the infection with HCoV-NL63 in our lab.

Four different epithelial cell lines namely LLC-MK-2, CaCo-2, Calu-3 and Vero E6 were chosen for this study. Susceptibility of virus to these cells were checked using immunofluorescence staining of the viral Nucleocapsid protein. These cell lines were also used for the assessment of virus propagation by understanding its growth kinetics. Along with this, commercially sourced 3D Respiratory Airway tissue models (MUCILAIR™) were infected with NL-63 to check the susceptibility of the virus at the air liquid interface.

Characterization of the virus titers by growth curves showed that the maximum virus titers were achieved in LLC-MK-2, Vero E6 and CaCo-2 cells from 3 to 7 days post infection. Surprisingly, in Calu-3 cells, i.e., a human pulmonary cell line known to be susceptible for SARS-CoV-2, virus proliferation found to be abortive. Initial characterization in terms of receptor utilization of HCo -NL63 confirmed the essential role of ACE2 for the virus infection in A549 cells. Additionally, confocal microscopy screening detected the virus in airway epithelial cells using N-Protein immunostaining. It has been observed that this N-protein and subsequently the virus is co-localised in the ciliated cells particularly on the cilia and at its base. According to this data, ciliated cells could be considered as one of the primary targets of the virus particles. Initial observations showed that the number of ciliated cells was reduced when the microscopic images were compared from Day 1 and Day 7 post infection, which highlights the importance of ciliary cells in the infection.

29 - On the nonlinear mechanical properties of alginate-gelatin hydrogels and bioprinted constructs with varying mesostructures

Anahita Ahmadi Soufivand[1], Jessica Faber[1], Jan Hinrichsen[1], Silvia Budday[1]

[1]Institute of Applied Mechanics, Department of Mechanical Engineering, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany

3D printing is a promising technology to produce various structures layer by layer. It is feasible to fabricate complicated geometries which are impossible with conventional manufacturing methods [1]. 3D bioprinting applies this technique in the biomedical field. In microextrusion bioprinting, the bioink is extruded from the nozzle through pressure. During the extrusion process, the viscous bioink is exposed to the shear stresses that may affect the material properties [2]. Moreover, geometrical parameters controlled through the print pattern can change the mechanical properties of the final printed construct [1, 2].

In this study, we examine the complex mechanical properties of 2 % (w/v) alginate - 5% (w/v) gelatin hydrogel samples. We investigate the effects of the extrusion process and geometrical parameters, i.e., different mesostructures, on mechanical properties of bioprinted constructs. We perform cyclic compression-tension and stress relaxation tests on the molded and printed samples [3] and evaluate the time-independent properties of the hydrogel using the Ogden model and an inverse parameter identification scheme using the finite element method (FEM).

Our results show that the molded and printed samples exhibit a significantly different mechanical response in both compression and tension with higher stresses for molded samples. This is also represented in the material parameters of the Ogden model identified through the inverse FE scheme, where we obtain μ=11.7 kPa and α= 2 for molded and μ=6.5 kPa and α= 5.6 for printed samples. In addition, the different mesostructures lead to a distinct mechanical response during cyclic and stress relaxation tests, demonstrating that geometrical parameters can be used to tune the mechanical properties of the final printed construct. We may conclude that the extrusion process and structural parameters significantly influence the nonlinear mechanical properties of alginate-gelatin hydrogels, which has important implications for the advancement of functional tissue replacements fabricated through 3D bioprinting in the future.

28 - SFB/TRR225 Project C04: Biofabrication of cellularized and by the AV loop vascularized tissue container for the transplantation of cells producing therapeutic proteins

Raymund Horch[1], Harald Wajant[2], Isabell Lang[2], Yvonne Kulicke[1]

[1]Plastisch- und Handchirurgische Klinik, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), [2]Abt. Molekulare Innere Medizin, Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg

The aim of project C04 of the SFB-TRR225 is the development of a transplantable tissue container providing *in vivo* secretion of therapeutic proteins by genetically modified cells.

Recombinant proteins are frequently used as “targeted therapies” for the treatment of cancer and autoimmune diseases. However, their usage has several drawbacks, such as high production costs, short half-life and difficult delivery. A possible solution is the *in vivo* production of therapeutic proteins by transgenic cells, which are transplanted into the patient’s body.

During the first funding period, different producer cell lines were developed. These cells stably express an Fc-TNFR2 fusion protein, which corresponds to the clinically approved TNF-blocker Enbrel®. The *Gaussia princeps* luciferase (GpL) domain is used as a reporter. Furthermore, the vascularization of different hydrogels in the rat arteriovenous (AV) loop model was investigated.

In the second funding period, new producer cell lines will be generated by lentiviral transfection, and clones with high recombinant protein expression will be selected. Moreover, the release of “danger” signals from producing cells and their immunostimulating potential will be analyzed.

The producer cells will then be incorporated into different bioinks and implanted in the rat AV loop model. For this purpose, the femoral artery and vein are anastomosed with an interpositional vein graft from the other side, and the loop is placed inside a tissue chamber filled with bioink to provide intrinsic vascularization. Angiogenesis will be further optimized by using different 3D print designs of the bioink. After explantation, the vascularization and morphology will be analyzed by micro-computed tomography (µCT), multiphoton microscopy and immunohistochemistry. After suitable combinations of producer cells and bioinks have been identified, the cellularized vascularized tissue container will be implanted in the pristan-induced arthritis (PIA) model of the rat, and the therapeutic efficiency will be evaluated.

27 - SFB/TRR225 Project B07: Mechanically defined sensor particles for bioprinting

Richard Kellnberger[1], Steffen Trippmacher[1], Katinka Theis[1]

[1]Coauthor

For targeted controlling and development of biofabrication processes a precise understanding of the mechanical stress effecting cells during the printing procedure is essential. We develop microgels functioning as mechanosensitive sensor particles that mimic the mechanical behavior of living cells. These sensor particles allow deeper insights into the mechanical processes occurring in the course of bioprinting – enabling the design of new and reliable printing technologies as well as bioinks.

We develop two kinds of sensor particles: *fracture sensors* and *deformation sensors*. *Fracture sensors* are hydrogel particles containing dye-filled liposomes which break when a threshold force is exceeded, resulting in an easily detectable fluorescence signal. Cell-mimicking *deformation sensors* can be used for time-resolved and in-situ monitoring of deformation in microchannels. A direct comparison to living cells will allow the distinction between passive mechanical (sensors) and active biological (cells) effects occurring during the bioprinting process.

The sensor particles are characterized by static and dynamic AFM indentation experiments to obtain information about their mechanical properties. Experiments in microchannels will correlate the mechanical properties with the deformation of particles during the printing process.

Furthermore, we develop simulations to model the mechanical stress on the cells or sensor particles. Using data gathered from experiments with sensor particles and cells, the intrinsic mechanical properties of the cells can be determined and the simulations can be used to optimize the printing process.

26 - 3D mucosal infection models as validation tools for vaccine development

Helene Mehling[1], David Kessie[1], Florian Groeber-Becker[1], Thomas Rudel[1]

[11]Chair of Microbiology, Biocenter, University of Würzburg

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.

25 - Engineering Biomacromolecule-fueled Transient Volume Transition of a Hydrogel

Masahiko Nakamoto[1], Michiya Matsusaki[1]

[1]Division of Applied Chemistry, Graduate School of Engineering, Osaka University

The metabolic cycle, comprising nutrient intake from and waste excretion to the external environment combined with internal cascade anabolic and catabolic pathways, is essential for life forms operating under out-of-equilibrium conditions. Recently, artificial materials that perform work under out-of-equilibrium conditions have attracted significant attention. We report for the first time a metabolic cycle-inspired hydrogel (MC gel) which exhibits the biomacromolecule-fueled transient volume phase transition. This hydrogel has the affinity and digestive capacity for a fuel α-poly-L-lysine (PL) by incorporating acrylic acid and trypsin. The MC gel underwent the transient volume change in response to PL. This study forms the basis of astrategy for engineering hydrogels that afford the dynamic regulation of biological processes via the target biomacromolecule-fueled TVPT.

24 – Selected Talk and Poster: Airway tissue models based on a novel synthetic scaffold to replace animal experiments

Niklas Pallmann[1], Heike Oberwinkler[1], Tobias Weigel[1], Rinu Sivarajan[1], Stephan Hackenberg[2], Agmal Scherzad[3], Jochen Bodem[4], Maria Steinke[1]

[1]Department for Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, 97070 Würzburg, Germany, [2]Department of Oto-Rhino-Laryngology – Head and Neck Surgery, Rheinisch-Westfälische Technische Hochschule Aachen (RWTH) Aachen University Hospital, Aachen, Germany, [3]Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, University Hospital Würzburg, Würzburg, Germany, [4]Institute of Virology und Immunobiology, Julius-Maximilians-Universität Würzburg, Versbacher Str. 7, 97078 Würzburg, Germany

Introduction and study aim: To build complex tissue models in vitro, several research groups apply biological scaffolds that are based on decellularized animal organs or isolated extracellular matrix proteins. To obtain such biological scaffolds, animal experiments are needed. To reduce animal components in human airway tissue models, which often come with biological variation, and to replace animal experiments, we aimed to use a fully synthetic 3D fibrous scaffold to build the stromal compartment of our airway tissue models. We further aimed to find out if experimental data obtained from novel fiber-based vs. our current biological airway tissue models were of comparable quality.

Methods: Biological and fiber-based scaffolds were obtained from porcine small intestinal submucosa (SIS) and from electrospun polyamide 6, respectively. Both scaffolds were seeded with human primary airway fibroblasts and epithelial cells. High speed video microscopy was used to monitor mucociliary differentiation of the tissue models. Mucosal barrier properties were assessed using the fluorescein isothiocyanate-dextran assay. Further, we used histological and immunohistochemical staining to characterize the morphology of the developed models. We comparatively analyzed all the experimental data obtained from SIS- and fiber-based tissue models.

Results: Both scaffolds support the migration and even distribution of primary human airway fibroblasts and allow adherence and differentiation of primary human nasal epithelial cells. The fibroblasts migrated in the synthetic scaffold and synthesized an extracellular matrix, which morphologically resembled the biological scaffold. We observed differences in the barrier formation between unloaded scaffolds and fibroblast-loaded scaffolds. Seeding of epithelial cells further improved barrier properties. Comparing fiber-based with SIS-based airway tissue models, we did not observe any significant differences.

Discussion: Our preliminary results indicate similar outcomes in airway tissue models based on biological and fiber-based scaffolds. However, due to donor variance, we require multiple repetitions to finally make a valid decision concerning the replacement of the biological matrix. This work has the potential to significantly contribute to reduce and replace animal experiments and to improve our models by decreasing the variability in the scaffold composition and thickness.

23 - Design Evolution and Attachment of a Melt Electrowritten Cardiac Patch to Regenerate Ischemic Heart Disease

Johannes Braig[1], Carolin Kropmaier [1], Tomasz Jüngst[1], Jürgen Groll[1]

[1]Department of Functional Materials in Medicine and Dentistry, Institute of Functional Materials and Biofabrication and Bavarian Polymer Institute, University of Würzburg, Würzburg, Germany

Introduction: Ischemic heart disease is the worldwide leading cause of death for men and women. Due to the limited heart transplantations, mechanical ventricular assist devices present the current standard to treat damaged hearts, although it is not a long-lasting solution. A biological ventricular assist device (BioVAD) aims for a regenerative alternative and is composed of two scaffold zones manufactured via Melt Electrowriting (MEW). The inner regenerative part reinforces a hydrogel cultured with human induced pluripotent stem cells to create cardiac tissue that integrates into the patient's heart to provide mechanical support and a lifelong solution. An outer support area attaches the BioVAD to the heart and supports tissue integration by preventing micromovement without negatively influencing the heart contractions. The present work outlines the BioVAD design evolution of the support area based on a modular concept and investigates the attachment with glues and suturing to cardiac tissue in custom build mechanical testing set-ups.

Materials and methods: Custom build MEW devices are used to manufacture the BioVAD scaffolds. The regenerative zone is manufactured with a hexagonal pore pattern according to our collaboration partner. The outer support zone is tailored from a variety of design elements such as sinusoidal and straight fibers.

Results and discussion: The outer support area has a complex function with opposing requirements. On the one hand, it needs to transmit forces generated by the cells in the regenerative zone to the heart and on the other side, it needs to show high elasticity not to interfere with the natural movement. This was achieved with a scaffold design composed of different zones with characteristic mechanics; one providing a stiff force transmission zone and the others creating elastic areas using a sinusoidal fiber pattern that pull the BioVAD to the cardiac surface to ensure integration.

Conclusions: Scaffold manufacturing via melt electrowriting was proven to generate scaffolds with characteristic mechanical properties that meet the biomechanical requirements of the heart. The modular concept allowed to tailor a full BioVAD which was examined in FDM created mechanical testing set-ups. Further equipment of porcine cardiac tissue enabled to characterize the attachment with glues or suturing.

22 - Synthetic fibrous scaffold for 3D small intestinal epithelial in vitro models

Christina Fey[1], Sabrina Herrmann[2], Tobias Weigel[1], Jörn Probst[1], Marco Metzger[1, 2], Daniela Zdzieblo[2, 3]

[1]Fraunhofer Institute for Silicate Research (ISC), Translational Center Regenerative Therapies (TLC-RT), Würzburg, Germany, [2]University Hospital Würzburg, Department of Tissue Engineering and Regenerative Medicine (TERM), Würzburg, Germany, [3]Fraunhofer Institute for Silicate Research (ISC), Project Center for Stem Cell Process Engineering, Würzburg, Germany

Extracellular matrices (ECMs) of animal origin such as Matrigel® or decellularized biological scaffolds are still used as major building blocks for Tissue Engineering-inspired cell culture applications. In addition to ethical concerns in the context of their production, biological ECMs are also known for batch-to-batch variations and high production costs. Synthetic scaffolds are therefore of great interest for Tissue Engineering in general. Modern synthetic scaffold materials should be bioactive and should represent a biomimetic structure similar to the *in vivo* situation, as this massively impacts on cell and therefore organ/tissue function *in vitro*. In this context, we present here a flexible, highly porous fibrous scaffold that is seeded with primary enteroid-derived intestinal epithelial cells. Thereby, the human small intestine is modeled as an *in vitro* test system compliant with the 3R principle *i.e.*, to reduce, replace or refine animal-based research. modeling the human small intestine in an *in vitro* test system that is compliant with the 3R principle.

A conventional electrospinning process (polyamide) was modified by defined combination with particular porogens (NaCl porogen particles), resulting in a highly porous scaffold. For the setup of small intestinal *in vitro* test systems, the 3D scaffolds were seeded with small intestinal stromal tissue cells (hiF) for 2 weeks. Fibroblasts were able to rearrange the fibrous structure and led to the synthesis of ECM, as shown by histological analysis for collagen I. Subsequently, primary enteroids were seeded as single cells on the respective scaffolds, forming a confluent epithelial cell layer after a proliferation phase of 3 days and a differentiation phase of 5 days. Epithelial integrity was investigated by FITC-dextran (4kDa) as well as transepithelial electrical resistance (TEER) measurements. In addition, a high comparability to the *in vivo* tissue was demonstrated by histological and gene expression analyses for the respective cell types found in the native tissue such as enterocytes (fatty acid binding protein 2; FABP2), goblet cells (Mucin-2, Muc2) or paneth cells (Lysozyme; Lyz).

Therefore, our human intestinal tissue model based on synthetic fibrous scaffolds represents an appropriate *in vitro* test system used in the context of drug testing *e.g.*, efficacy, toxicity and transport.

21 - Immune-competent human intestinal tissue 3D model of S. Typhimurium infection

Spyridon Damigos[1], Thomas Däullary[1, 2], Alexander Westermann[3], Marco Metzger[1, 4], Daniela Zdzieblo[1, 5]

[1]Department of Tissue Engineering and Regenerative Medicine, University Hospital of Wuerzburg, [2]Department of Microbiology, University of Wuerzburg, [3]Helmholtz Institute for RNA-based Infection Research, Helmholtz Centre for Infection Research (HZI), University of Würzburg, [4]Translational Center for Regenerative Therapies (TLC-RT), Fraunhofer Institute for Silicate Research (ISC), Würzburg , [5]Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Silicate Research (ISC), Würzburg

Mucosal tissues including the intestinal mucosal interphase, are constituting a barrier within the inner organs and the external environment and have a protective function against pathogen infections. Assuming the intestinal epithelial barrier as a borderline of the host-environmental phases, the native tissue including the luminal micro-organisms comprise interactive immune components. In this context, the sub-epithelial compartment's stromal cells and phagocytes orchestrate the transition to the adaptive immune response and the migration of antigens to the gut associated lymphoid tissues (GALT).

Salmonella enterica serovar Typhimurium (STm) is an invasive enteric pathogen to the human causing non-typhoidal gastroenteritis. So far, it is assumed that STm infects intestinal epithelial cells and utilizes sub-epithelial phagocytes as "vehicles" to further migrate to the GALT or mesenteric lymph nodes. Human-based models that recapitulate the primary mucosal interphase transition of the pathogen's virulence cycle are lacking. Here we present a novel primary human intestinal tissue co-culture infection model, based on primary intestinal epithelial cells, seeded on porcine organ specific decellularized submucosa [1], with embedded monocyte-derived macrophages and intestinal stromal cells. Interestingly, we show the complexity of the *S*.Typhimurium infection method on a 3D tissue model, compared to conventional 2D infection of intestinal epithelial monolayers or phagocytes and stromal cells. In conclusion, we show an up-to date 3D intestinal tissue infection model to representative the native situation.

References:

1. Schweinlin, M., Wilhelm, S., Schwedhelm, I. et al. (2016). *Tissue Engineering Part C Methods 22*, 873-883. doi: 10.1089/ten.TEC.2016.0101

20 - A 3D cell culture system for bioengineering human neuromuscular junctions

Bita Massih[1], Patrick Lüningschrör[1]

[1]Institute of Clinical Neurobiology, University Hospital Würzburg, Germany

The signals that coordinate and control animal movement are transmitted from motoneurons to their target muscle cells at neuromuscular junctions (NMJs). Human NMJs display unique structural and physiological features, which make them vulnerable to pathological processes. NMJs are an early target in the pathology of motoneuron diseases. Synaptic dysfunction and synapse elimination precede motoneuron (MN) loss suggesting that the NMJ is the starting point of the pathophysiological cascade leading to motoneuron death. Therefore, the study of human MNs in health and disease requires cell culture systems that enable the connection to their target muscle cells for NMJ formation.

Here, we present a human neuromuscular co-culture system consisting of induced pluripotent stem cell (iPSC)-derived MNs and 3D skeletal muscle tissue derived from myoblasts. We used self-microfabricated silicone dishes combined with Velcro hooks to support the formation of 3D muscle tissue in a defined extracellular matrix, which enhances NMJ function and maturity. Using a combination of immunohistochemistry, calcium imaging, and pharmacological stimulations, we characterized and confirmed the function of the 3D muscle tissue and the 3D neuromuscular co-cultures.

In summary, we present a human 3D neuromuscular cell culture system that recapitulates aspects of human physiology in a controlled *in vitro* setting.

19 - SFB/TRR225 Project B03: Printing of biofabricates and customized bioreactors for skeletal muscle tissue (B03)

Johann Schorzmann[1], Lys Sprenger[2], Hsuan-Heng Lu[3], Sahar Salehi[2], Aldo Boccaccini[3], Frank Döpper[1]

[1]Chair of Manufacturing and Remanufacturing Technology - University of Bayreuth, [2]Chair for Biomaterials – University of Bayreuth, [3]Chair of Biomaterials - Friedrich-Alexander University Erlangen-Nuremberg

The increasing demand for regenerative skeletal muscle tissue (SMT) in medicine has intensified efforts to generate this tissue in vitro in order to approximate the properties of native SMT as closely as possible. However, the complex interactions between motor neurons, myoblasts, extracellular matrix (ECM) and its anisotropic structure, as well as electrical and bio-mechanical stimulation, make biofabrication a major challenge. The lack of neuronal connectivity of myotubes leads to low development of functional SMG and increased degradation, which can be compensated by external electrical stimulation. Electrical stimulation can enhance myoblast differentiation and accelerate the rate and speed of myotube formation. In addition, the mechanical properties and electrical conductivity (EC) of the substrates lead to an increase in C2C12 myogenesis and better myotube development. However, the reason for the increased myogenesis on EC substrates is not yet fully understood.

The sub-project B03 will develop two different composite bio-inks with organic and inorganic fillers for the formation of SMG by using biofabrication and bioreactor technologies. Biofabrication is used to flexibly fabricate constructs to replicate the hierarchical and anisotropic structures of the SMG. Printable composite ink with inorganic bioactive fillers (BIF) will be developed, which on the one hand lead to the post-cross-linking of the bio-ink and thus to defined mechanical properties and pore sizes. On the other hand, the released ions should biochemically stimulate the printed cells myogenically. The second anisotropic composite ink (ribbon composite ink) contains embedded, highly cell-loaded EC ribbons as cell transporters. Both BIF and ribbon composite inks will be optimised for SMG biofabrication and transferred to printing bioreactor technology. At the cellular level, C2C12 cells and skeletal muscle reporter cells will be used in this TP. The bioreactor system will be designed for the growth and maturation of SMG. Dynamic perfusion and external stimulation (biochemical and electrical stimulation) will be applied in the 3D printed bioreactor to improve cell development. The development of bioreactor technology with additive manufacturing of bioreactors with higher functionality, which can be adapted depending on the tissue, and it is researching the functionality of electrical stimulation and possibilities of monitoring tissue models are focused.

18 - Studying Chlamydia trachomatis infection in human fallopian tube polarized epithelial cell culture model

Pargev Hovhannisyan[1], Cindrilla Chumduri[1], Thomas Rudel[1]

[11]Chair of Microbiology, Biocenter, University of Würzburg *Chlamydia trachomatis* is a Gram-negative human-specific pathogenic bacterium, which causes most frequent sexually transmitted bacterial infections worldwide. In women, infection often localizes to the cervix, but when left untreated, it may spread to the upper genital tract and cause serious complications. In our study, we are using human fallopian tube adult stem cell-derived organoids for the development of polarized epithelial monolayers to study the *C. trachomatis* infection in a setting, which better recapitulates the architecture and infection environment of the human fallopian tube tissue. Organoids allow having a stable and expandable source of primary epithelial cells from a single donor. We have been able to generate an intact monolayer of polarized epithelial cells based on cell culture inserts in the air-liquid interface setting. The cells proliferate and polarize in a time-dependent manner and express markers of polarization and differentiation after maturation. Infection studies showed that the model has a higher resistance to the infection in comparison with the conventionally used cell line models. In order to have a global view of the infection process, we performed dual RNA-sequencing of the infected models. The analysis reveals a strong regulation of gene networks involved in innate immunity and metabolic pathways.

17 - Establishing long-term live-cell imaging of human airway organoids in a microfluidic system

Mara Fischer[1], Anna Löwa[1], Zeynep Demir[1], Maren Mieth[1], Katharina Hellwig[1], Doris Frey[1], Stefan Hippenstiel[1], Andreas Hocke[1]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Department of Infectious Diseases and Respiratory Medicine, Charitéplatz 1, 10117 Berlin, Germany

Background: Pneumonia is one of the most common lethal infectious diseases worldwide. To find innovative treatments, models that best reflect the patient's (patho)physiological situation are urgently needed. Adult stem cell-derived organoids show great potential for translational research, as they reflect 3-dimensional tissue-like differentiated epithelial cell types allowing the study of long-term effects for testing therapeutic approaches. However, many assays provide only static read-outs at single time points, limiting the ability to dynamically capture host-pathogen interactions. Functional molecular live-cell imaging (LCI) can remedy this situation and allows observation in microfluidic systems.

Methods: Human airway organoids grown in a dome-shaped extracellular matrix (ECM) were detached and placed on a commercially available microfluidic slide with grafted ECM coating suitable for microscopy. After attachment, organoids were monitored by confocal LCI imaging comparing static and dynamic conditions using a commercially available microfluidic system (ibidi).

Results: Dynamic cultivation, compared to static cultivation, ensured that respiratory organoid deposits in the medium were removed and organoid viability was stable over time. We demonstrated that at flow rates up to 2.5 ml/min, corresponding to a shear stress of 1.5 dynes/cm², most of the partially embedded organoids remained at their initial location. However, to keep the shear stress as low as possible, a threshold of up to 0.11 dyn/cm^2 (equivalent to 0.18 ml/min) was used, which still ensured media supply to the organoids. By creating a humid microenvironment, evaporation of the organoid media from the gas-permeable tubes traversing the ambient air was avoided. In addition, titration of the medium ensured that the pH of the medium remained stable at ambient CO_2 level in the microscope cage.

Conclusion: In summary, we have succeeded in establishing an efficient system for monitoring 3D organoids over several days in a confocal microscope system, which could now be applied for live monitoring of host-pathogen interaction.

16 - Selected talk and poster: Modeling Neisseria meningitidis interaction with the meningeal blood-CSF barrier using iPSC and multi-cell culture approaches

Leo Endres[1], Marvin Jungblut[2], Mustafa Divyapicigil[3], Markus Sauer[2], Christian Stigloher[4], Myron Christodoulides[5], Brandon Kim[3], Alexandra Schubert-Unkmeir[1]

[1]Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany, [2]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany, [3]Department of Biological Sciences, University of Alabama, Tuscaloosa AL, USA, [4]Imaging Core Facility, Biocenter, University of Würzburg, Würzburg, Germany, [5]Molecular Microbiology, School of Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, UK

Introduction: *Neisseria meningitidis* (*Nm,* meningococcus) is a human-specific bacterium that can cause meningitis by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB) and infecting the leptomeninges. Previous research on this host-pathogen interaction has mostly been done on immortalized brain endothelial cells (BECs) alone, which do not retain certain physiological barrier properties *in vitro*, and has not yet included leptomeningeal cells (LMC) to model the physiological microenvironment. Here, we report on the development and application of BEC-LMC co-culture models to examine Nm interaction at the mBCSFB in a more physiologically relevant context.

Methods: We used BEC-like cells derived from induced pluripotent stem cells (iBECs) or hCMEC/D3 cells in co-culture with meningioma derived LMCs. Transmission electron microscopy, confocal and super-resolution microscopy techniques were applied for model characterization and detection of interacting bacteria. Tightness and integrity of the cell barriers was analyzed via transendothelial electrical resistance (TEER) and sodium fluorescein permeability. We used Gentamicin protection and transmigration assays to determine bacterial interaction and penetration of the barrier models and examined changes in host gene expression in response to infection via qPCR and ELISA.

Results: We observed characteristic expression of BEC markers including tight junction proteins in iBEC, which remained distinct from LMCs in co-culture. LMC co-culture improved barrier tightness and stability of iBEC layers for seven days. BEC response to infection was generally not altered by LMC co-culture. Interestingly, we discovered bacteria already traversing in small numbers 6 h post-challenge, when barrier integrity was still high, suggesting a transcellular route. By 30 h, deterioration of the barrier properties has been detected, including loss of TEER and reduced expression of cell-junction components. Finally, we found proinflammatory activation of BECs upon Nm infection.

Discussion: Our work highlights the usefulness of advanced in vitro models of the human mBCSFB for infection studies that more closely mimic the meningeal microenvironment. Further advancement of the model can be achieved by adding other relevant cell types such as immune cells or introducing more physiological parameters such as shear stress.

15 - The bacterial factors necessary for the survival of Neisseria gonorrhoeae in neutrophils

Ravisha Rawal[1], Thomas Rudel[1], Vera Kozjak-Pavlovic[1]

[11]Chair of Microbiology, Biocenter, University of Würzburg The facultative intracellular and human-specific pathogenic bacterium *Neisseria gonorrhoeae* is the causative agent of the sexually transmitted infectious disease (STI) Gonorrhea. Infection with *N. gonorrhoeae* results in a potent neutrophil (polymorphonuclear leukocyte, PMN)-driven inflammatory response. Despite a robust immune response, viable Gc are recovered from neutrophil-rich patient exudates.

Understanding how the bacteria can survive within PMNs inside the human body and identifying specific factors that facilitate that survival is essential to combating persistent gonococcal infection. In the previous work, we have used the *N. gonorrhoeae* transposon library screen to identify factors that might play a role in the interaction of gonococci with as well as their survival upon exposure to PMNs. The list of identified factors included proteins important for the pilus assembly, as well as candidates involved in the lactate metabolism. Characterizing those factors and their influence on neisserial survival within neutrophils will provide an insight into the mechanism of interaction of gonococci and PMNs.

Further on, to perform our studies under close to natural conditions we plan to use complex 3D tissue models of epithelial, fibroblast and endothelial cells, to which neutrophils are added upon infection. For this, we have already performed initial experiments with a perfusion-based Bioreactor system and observed neutrophil transmigration to the site of infection. However, the difficulties we encountered in the recovery of neutrophils from these models, point to the necessary modifications of this approach.

14 - Transcriptional and metabolic dynamics of primary human lung cells acutely infected by RSV

Kevin Berg[1], Sibylle Haid[2], Ehsan Vafadarnejad[3], Antoine-Emmanuel Saliba[3], Thomas Pietschmann[2], Florian Erhard[1]

[1]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany, [2]Twincore-Centre for Experimental and Clinical Infection research, Dept. Experimental Virology, Hannover, Germany, [3]HIRI- Helmholtz Institute for RNA-based Infection Research, Dept. single cell genomics, Würzburg, Germany

Acute infection with Respiratory Syncytial Virus (RSV) is the leading cause of severe lower respiratory tract infection in children. The consequences of infection among children is highly variable and the principles that control severe courses are not well defined.

Here we analyze RSV infection-induced transcriptional changes of well-differentiated human airway epithelial cells in an air-liquid interface system from six donors over a timeframe of 7 days at single cell resolution. We characterized host and viral transcriptomes in the major cell types that were clustered according to the human lung atlas (Travaglini et al, Nature 2020). Only few RSV infected cells produced IFN-I/III but strong induction of interferon stimulated genes (ISGs) was observed in bystander as well as infected cells. Consistent with well-described viral immune evasion mechanisms, ISG expression, however, was negatively correlated with viral load. Marker gene analyses revealed that ciliated cells, the main target of RSV, dedifferentiate into a basal cell-like state by downregulating cilia-related transcription and thereby likely leading to a loss of their clearing function.

Further investigation showed that only a minor fraction of all cells was infected at any given time and that viral gene expression levels had the same pattern across all infected cells indicating constant turnover of de-novo infection and cell death. Pseudo-bulk ranking and grouping of cells by their viral load revealed several waves of specific transcriptional programs. These transcriptional changes encompass interferon responses in cells with low viral load but move on to rearrangements in cilium organization as well as microtubule formation and are dominated by apoptosis-related gene expression patterns like protein refolding and other cellular stresses in cells with highest viral load.

In summary, our analyses revealed cell type-dependent and time-structured responses of the human lung cell epithelium and highlights key cellular pathways perturbed by an RSV infection.

13 - Quantitative analysis of Granular Support Bath Materials Residues in Printed biological structures for Embedded 3D Printing

Jinfeng Zeng[1], Nozomi Kasahara[1], Zhengtian Xie[1], Fiona Louis[2], Donghee Kang[1], Michiya Matsusaki[1]

[1]Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Japan, [2]Joint Research Laboratory (TOPPAN) for Advanced Cell Regulatory Chemistry, Graduate School of Engineering, Osaka University

Biomedical application of three-dimensional (3D) printing demands complex hydrogel-based constructs laden with living cells. Embedded 3D printing facilitates the fabrication of such biological structures using soft protein and polysaccharide hydrogels that are challenging or impossible to create by traditional fabrication approaches. Although this targeting strategy is appealing, the residues of support materials in printed objects have been overlooked. This investigation began with the findings that residual support materials altered the surface and mechanical properties of fibrin gel sheets, impeding cell adhesion and spreading. Here, we quantified the residues in programmed structures and analyzed in detail the effects of support material properties on the attachment of bath pieces. Fluorescent granular baths were prepared to visualize the location of support materials. Cross-sectional images showed that most of the microgel particles distributed over the whole filament surface, while a small amount diffused into the center. Our results demonstrated that baths with smaller particle size and lower viscosity spread faster and deeper in extruded inks. The deeply embedded gel particles undoubtedly increased the difficulty of cleaning. Under mild post-processing, the residual amount of support materials depends mainly on the percentage of liquefaction of the granular gel bath. By quantitative analysis, detectable residues were still present, even with easily removable baths. This study draws attention to the impacts of support materials on programmed 3D biological structures, and encourages the development of new strategies to diminish the residues or take advantage of the residual support materials to improve the performance of printed objects.

12 - Impact of Neisseria meningitidis infection on the efflux transporter P-glycoprotein in brain endothelial cells

Fatemeh Nosratabadi[1], Leo Enders[1], Brandon J. Kim[2], Alexandra Schubert-Unkmeir[1]

[1]Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany, [2]Department of Biological Sciences, University of Alabama, Tuscaloosa AL, USA

Introduction: *Neisseria meningitidis* (*Nm*) is a commensal bacterium that colonizes the human nasopharynx and occasionally crosses the nasopharyngeal barrier, followed by systemic spread and transmigration of the meningeal blood-cerebrospinal fluid barrier (mBCSFB) causing meningitis. The mBCSFB is composed of specialized brain endothelial cells (BECs) that tightly regulate the blood-to-brain traffic of compounds due to unique properties including the presence of complex tight junctions and special efflux transporters such as P-glycoprotein (P-gp). Dysregulation of P-gp was recently observed for *Streptococcus agalactiae* in human induced pluripotent stem cell-derived BECs (iPSC-BECs). Here, we investigate the effect of *Nm* infection on P-gp using human *in vitro* BEC models.

Methods: hiPSCs were differentiated into iPSC-BECs according to recently described protocols. The human BEC line, hCMEC/D3 was used in parallel. Immunofluorescence staining of adherence and tight junction markers and TEER measurements were performed to verify the quality of the iPSC-BECs. The impact of *Nm* on P-gp was analyzed by RT-qPCR and immunoblotting. Rhodamine 123 (R123) accumulation assays together with pharmacological inhibition of P-gp using Cyclosporin A (CsA) and valspodar (PSC833) was performed to assess P-gp activity in our cellular models with or without *Nm* infection. Inhibition of other transporters, breast cancer resistance protein (BCRP) and multidrug resistance protein (MRP) family, was done to investigate if R123 efflux is specific to P-gp in our models.

Results: P-gp inhibition using CsA or PSC833 lead to a significant increase R123 accumulation, confirming activity of P-gp in our models. Interestingly, second generation inhibitor PSC833 increased R123 accumulation more than CsA (> 400 %). Both compounds affected P-gp activity more substantially than the BCRP and MRP inhibitors, Ko143 and MK571 respectively, demonstrating their specificity. We observed significant downregulation of P-gp expression after 24h of *Nm* infection. Furthermore, we found elevated accumulation of R123 in BECs infected with *Nm*, indicating reduction in P-gp function.

Discussion: We were able to confirm P-gp activity in iPSC-derived BECs and hCMEC/D3s and our data suggest that *Nm* infection disrupts P-gp function in BECs. Future aims include studying the role of different *Nm* virulence factors on P-gp dysfunction during infection and evaluating other efflux transporters.

11 - Fabrication of cell scaffold capable of sustained oxygen supply by hydroxyapatite formation on calcium peroxide

Daisuke Tomioka[1], Michiya Matsusaki[1]

[1]Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Japan

In tissue engineering field, in vitro construction of thick three-dimensional (3D) tissues is still a major challenge because limited diffusion of oxygen inside tissues causes cell necrosis. To address the oxygen shortage in 3D tissues, oxygen releasing materials have recently attracted much attention. Calcium peroxide (CaO_2) is one of the most common oxygen sources for oxygen releasing materials because it generates oxygen, hydrogen peroxide (H_2O_2) and calcium hydroxide ($Ca(OH)_2$) by the reaction with water. However, the initial burst release of oxygen from CaO_2 is big issue due to the rapid reaction with water [1].

Herein, we have recently achieved the sustained oxygen release by suppressing the reaction between CaO_2 and water through the formation of hydroxyapatite (HAp) on the surface of CaO_2 using phosphate buffer (PB). It is known that the reaction between $Ca(OH)_2$ and phosphoric acid provides HAp which has lower water solubility. Thus, $Ca(OH)_2$ produced by immersing CaO_2 in PB is expected to react with phosphoric acid in PB to form HAp on CaO_2

For the application of HAp-CaO_2 as an oxygen releasing material, we fabricated oxygen releasable gelatin hydrogels enzymatically crosslinked by transglutaminase (TG). To prevent the cytotoxicity of H_2O_2, catalase was also incorporated into hydrogel because catalase is an enzyme which decomposes H_2O_2 into oxygen and water. In this study, oxygen releasable hydrogels are applied to cell culture under hypoxia condition to evaluate the effects of oxygen supply from the materials.

Scanning electron microscopy, energy dispersive X-ray spectroscopy and X-ray diffraction confirmed HAp coating of CaO_2 by PB treatment. For the preparation of oxygen release hydrogel, gelatin, TG and catalase were dissolved in Dulbecco's Modified Eagle Medium and then untreated-CaO_2 or HAp-CaO_2 was added to obtain the hydrogels. The hydrogel with untreated-CaO_2 showed burst release of oxygen and oxygen release was stopped at day 3. On the other hand, the hydrogel with HAp-CaO_2 showed sustained oxygen release over 10 days. Since sustained oxygen release from the hydrogel improved cell proliferation under hypoxic environment, this biomaterial is expected to be applied for 3D thick tissue construction.

[1] K. Park *et al*., *Biomaterials* **2018**, *182*, 234.

10 - SFB/TRR225 Project A07: Influence of anisotropic fiber-reinforcement on cell behavior and printability of bioinks

Zan Lamberger[1], Nicoletta Murenu[2], Margitta Büchner[3], Dr.-Ing. Gregor Lang[1], Dr. Natascha Schäfer[2], Prof. Dr. Dirk Schubert[3]

[1]University Hospital Würzburg, Department of Functional Materials in Medicine and Dentistry, [2]University Hospital Würzburg, Institute for Clinical Neurobiology, [3]Friedrich-Alexander Universität Erlangen-Nürnberg, Lehrstuhl für Polymerwerkstoffe

The aim of project A07 is to apply fiber-reinforcement to tailor rheological and mechanical properties of bioinks and simultaneously introduce biological functions. The development of printable short fibers with cell-biologically attractive surfaces, such as via collagen-coating, has demonstrated the high potential of such composite approaches to address the issue of the biofabrication window. Furthermore, experimental methods were developed that allow a systematic correlation of rheological properties and printability by using viscoelastic models. Biological function of dumbbell-shaped fibers was shown in vitro, where promoted integrin-based cell adhesion was observed. Based on the established methods, it is now necessary to further develop the biological potential of this system as a tissue construct. Various cell types will be tested and the influence of 2D vs. 3D culture, as well as fiber alignment on adhesion, proliferation, orientation, and migration will be examined. Moreover, fibers will be equipped with additional temporally controllable functions such as a drug delivery and biodegradation.

9 - SFB/TRR225 Project C06: Biofabrication of a glomerular ex vivo model by stepwise mimicking functional core components

Camilla Mussoni[1], Anna Laptii[2], Janina Müller-Deile[2], Taufiq Ahmad[1]

[1]Department of Functional Materials for Medicine and Dentistry, University of Würzburg, Würzburg, Germany, [2]Department of Medicine 4 – Nephrology and Hypertension, University Hospital of Erlangen, Germany

Kidney failure affects a large population and is often associated with glomerular diseases. Glomeruli are the functional units of the kidney responsible for selective ultrafiltration of blood through the glomerular filtration barrier. The current gold standard for *in vitro* glomerular models remains limited to recapitulate the structural complexity of glomerulus tissue. Therefore, there is a great demand to develop a glomerular *in vitro* model with all relevant cell types and functional components. This project aims to fabricate an *ex vivo* 3D model of the glomerulus by recapitulating its functional core components by using bottom-up and top-down biofabrication techniques. The model will be utilized to investigate cell-cell communication and renal (patho-) mechanisms to develop effective personalized treatment strategies with lower side effects using patient derived reprogrammed glomerular cells.

The project is split into four different work packages. First, a PLLA electrospun nanofibrous membrane is functionalized to mimic the glomerular basement membrane and offer support to a co-culture of monolayered podocytes and glomerular endothelial cells (GEC) to recapitulate the glomerular filtration barrier. These membranes are assembled in bioreactors to study the cell response under different flow conditions, to analyze filtration functions and cell-cell communication. Learning from the previous membrane in terms of materials and functionalization, extracellular matrix-like nanofibers will be incorporated into glomerular spheroids. In order to provide vascularisation to the model we proceed with a Polyoxazolines Melt Electro Writing printed capillary-like structures seeded with GEC and podocytes. Finally, all the aforementioned components will be bioassembled in a complex three-dimensional model.

8 - SFB/TRR225 Project B06: Reporter-conjugated Alginate dialdehyde (ADA) to investigate cell metabolism in biofabrication

Benedikt Gantert[1], Lukas Hahn[1], Rainer Detsch[2], Tessa Lühmann[1]

[1]Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg (Germany), [2]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany

A major challenge in biofabrication is to monitor cell behaviour 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 leads to cell death or changes in cell viability, development of reporter systems for cellular activity is important. To address this problem, we are developing hydrogels with a protease-sensitive peptide reporter system that can be site-specifically incorporated into alginate-based bioinks and subsequently provide insights into cell interactions. Förster resonance energy transfer (FRET) responsive peptides were designed and used as read-out system. The peptide reporters with different protease specificities were synthesised and afterwards evaluated for protease sensitivity. The reporter was incorporated into ADA using aldehyde-amine chemistry and the resulting bioink was either casted or printed together with C2C12 cells. The fluorescence change was determined after crosslinking with plate reader which leads to high applicability in everyday lab life. This reporter system enables a qualitative and, above all, simple quantitative evaluation of cell activity in bioprinted ADA constructs. In future, the several reporter peptides are easily adaptable for different bioink platforms. In summary, by adding novel reporter peptide to ADA prior to bioprinting, a quantitative analysis of protease activity in bioprinted constructs can be performed.

7 - SFB/TRR225 Project A06: Cell-Loaded Microgels as Mechanical Protection and Controlled Microenvironment for Cells in Bioinks

Sophia Löffelsend[1, 2]

[1]FMZ, University of Wuerzburg, [2]SFB/TRR225

The aim of this project (A06, SFB/TRR225) is to generate cell-loaded microgels that protect cells from mechanical forces and deformation during bioprinting. Thereby, the biofabrication window is supposed to be shifted towards bioinks with higher viscosity and higher shear forces.

This poster gives an overview of the current status of the project by describing (1) the main preliminary results and (2) the future steps. In the past, microgels were mainly based on polyoxazolines and cross-linked via a thiol-ene reaction. In the future, this polymer system is aimed to be shifted towards (1) a hybrid system of synthetic and biopolymers and (2) light-free cross-linking methods. Further, the (bio)degradability of the microgels is to be established. Apart from using these cell-loaded microgels as additives in bioinks, this project also focuses on a direct in-flow 3D printing method of the microgels right after they left the microfluidic chip. Thereby, stable filaments with a microporous structure shall be obtained. In addition, the encapsulation of spheroids and tumor cells as well as an application in cancer and vascularization research are discussed.

6 - Selected talk and poster: Helicobacter pylori shows tropism to gastric differentiated pit cells dependent on urea chemotaxis.

Carmen Aguilar [1], Mindaugas Pauzuolis[1], Malvika Pompaiah [1], Ehsan Vafadarnejad [2], Panagiota Arampatzi[3], Mara Fischer[1], Dominik Narres [1], Mastura Neyazi [1], Özge Kayisoglu[1], Thomas Sell[4], Nils Blüthgen[4], Markus Morkel[4], Armin Wiegering [5], Christoph-Thomas Germer[5], Stefan Kircher[6], Andreas Rosenwald [6], Antoine-Emmanuel Saliba[2], Sina Bartfeld[1]

[1]1Research Centre for Infectious Diseases, Institute for Molecular Infection Biology, Julius Maximilians University of Würzburg, [2]2Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz-Centre for Infection Research (HZI), , [3]Core Unit Systems Medicine, University of Würzburg, [4]Institute of Pathology, Charité Universitätsmedizin Berlin, [5]5Department of General, Visceral, Vascular and Paediatric Surgery, University Hospital of Würzburg, [6]6Institute of Pathology, Julius Maximilian University of Würzburg and Comprehensive Cancer Center Mainfranken

The human gastric epithelium forms highly organized gland structures with different subtypes of cells. The carcinogenic bacterium *Helicobacter pylori* can attach to gastric cells and subsequently translocate its virulence factor CagA, but the possible host cell tropism of *H. pylori* is currently unknown. Here, we report that *H. pylori* preferentially attaches to differentiated cells in the pit region of gastric units. Single-cell RNA-seq shows that organoid-derived monolayers recapitulate the pit region, while organoids capture the gland region of the gastric units. Using these models, we show that *H. pylori* preferentially attaches to highly differentiated pit cells, marked by high levels of GKN1, GKN2 and PSCA. Directed differentiation of host cells enable enrichment of the target cell population and confirm *H. pylori* preferential attachment and CagA translocation into these cells. Attachment is independent of MUC5AC or PSCA expression, and instead relies on bacterial TlpB-dependent chemotaxis towards host cell-released urea, which scales with host cell size.

5 - Selected talk and poster: SFB/TRR225 Project A01: Traction forces of migrating immune cells

David Böhringer[1], Lars Bischof[1], Tina Czerwinski[1], Sibel Kara[2], Anja Lux[2], Silvia Budday[3], Richard Gerum[1, 4], Ben Fabry[1], Christoph Mark[1]

[1]1Department of Physics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, [2]2Department of Genetics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, [3]3Department of Technical Mechanics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, [4]4Department of Physics and Astronomy, York University, Toronto, Canada

To reach their target outside the bloodstream, immune cells have the ability to migrate with high speeds of several µm/min through dense connective tissue. Immune cells are thought to migrate in a so-called amoeboid mode with limited or no proteolytic activity and with limited adhesion to the extracellular matrix (ECM). Therefore, these cells do not need to generate substantial traction forces for their migration in 3D, but this has never been directly experimentally confirmed. Here, we measure the traction forces of primary immune cells (B-,T-, NK-cells, monocytes and neutrophils) and of a natural killer cell line (NK-92) during migration in an in-vitro fabricated 1.2 mg/ml collagen gel (pore size 4.4 µm, shear modulus 100 Pa). Around each cell, we record one confocal reflection image stack of the collagen matrix at high speed every minute for 24 min. From these 24 image stacks, we estimate the undeformed reference state from the median deformation averaged over the observation period. Cell forces at each time point are then computed from the current matrix deformations relative to the undeformed reference state using a nonlinear finite element network solver (Steinwachs et al, NatMeth 2016) adapted for fast migrating cells. From the cell positions and cell shape, we also measure cell velocity and aspect ratio. We find that all immune cell types migrate with low traction forces <5 nN for most of the time, but the majority of the cells exhibit at least one short, highly-contractile burst of up to 40 nN during the 24 min measurement period. These force bursts are comparable in magnitude to traction forces generated by mesenchymal cells. Further, we find that cell speed and force are correlated, suggesting that the cells increase their traction forces when they encounter confinement. In line with this, we find that myosin inhibition with blebbistatin or Rock inhibition reduces traction forces and the fraction of migrating cells, whereas stimulation with interleukin-2 (IL-2) increases traction forces and the fraction of migrating cells. These findings demonstrate that immune cells can switch to a mesenchymal-like migration strategy to overcome the high steric hindrance of dense connective tissue.

4 - Selected talk and poster: Sacrificial templating via thermoresponsive polymers for simple, reproducible, and accurate in vitro vascularization

Matthias Ryma[1], Ali Nadernezhad[1], Hatice Genc[2], Kristina Andelovic[1], Vincent Mair[1], Amelie Reigl[3], Carina Blum[1], Iwona Cicha[2], Jürgen Groll[1]

[1]University Hospital Würzburg, Department for Functional Materials in Medicine and Dentistry, [2]University Hospital Erlangen, Section of Experimental Oncology and Nanomedicine (SEON), [3]University Hospital Würzburg, Tissue Engineering and Regenerative Medicine

Functional 3D tissue models are a potentially valuable tool to provide more transferable test results regarding basic research, drug development or other applications than classic 2D cell culture. However, the biggest bottleneck in the generation of functional 3D tissue models is the incorporation of a reproducible and perfusable vascularization, mimicking the in vivo hierarchy accurately and keeping bigger 3D tissue models alive.

An interesting approach to generate microchannel networks in 3D tissue models is the utilization of prefabricated sacrificial scaffolds. These scaffolds can be introduced into cytocompatible hydrogels and, after gelation of the hydrogel, dissolved to generate perfusable channels. Current approaches utilize sacrificial carbohydrate scaffolds, which only provide a short and non-controllable dissolution timeframe. To overcome this issue, their stability in aqueous solutions must be increased via laborious post-fabrication processes like coatings. However, this introduces non-dissolvable artificial materials acting as barriers into the in vitro tissue.

In this work, we introduce biocompatible poly(2-oxazoline)s as material for sacrificial scaffolds. These polymers are thermoresponsive in aqueous solutions, meaning that they dissolve on demand by simple temperature reduction. In contrast to currently comparable approaches, this leads to an increased dissolution timeframe without the need to increase stability via post-processing. Furthermore, the scaffolds can be dissolved on-demand without introducing non-dissolvable remnants. This enables the utilization of many currently used hydrogels (e.g., methacrylated gelatin or collagen) for vascularized tissue engineering approaches.

Interestingly, poly(2-oxazoline)s can be fabricated via the modern additive manufacturing technologies Melt Electrowriting and Freeform Printing to generate highly accurate microfiber networks in 2D and 3D. This enables the generation of interconnected channel networks with bifurcations, resembling the natural vascularization. Combined with specialized perfusion chambers, the simple addition of a cell-laden hydrogels and temperature reduction mediates the generation of microchannel-networks and their leakage-free connection to media-reservoirs. This allows the direct cultivation and endothelialization of the perfusable tissue construct in the perfusion chamber and its connection to perfusion devices.

3 - Selected talk (no poster): Development of a three-dimensional blood-brain barrier network with opening capillary structures for drug transport screening assays

Marie Piantino[1], Michiya Matsusaki[1]

[1]Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Japan.

The blood-brain barrier (BBB), a highly selective barrier between the circulatory system and the central nervous system, is composed of brain microvascular endothelial cells (BMEC), pericytes and astrocytes. This barrier prevents therapeutics to penetrate into the brain. Current models fail to replicate the structural complexity of the native BBB and do not exhibit enough functionality of specific transporters for drug assessment. The transferrin receptor (TfR), one of the main routes for iron transport in the brain, shows great potential for drug transport due to its high expression level by both brain BMEC and brain cancer cells, but is poorly investigated in the current BBB models. Assessing the functionality of TfR-mediated transcytosis in three-dimensional (3D) BBB models is therefore highly desired, as it could create opportunities for the screening of novel treatments for brain cancers.

We developed a 3D self-assembled microvascular network formed by BMEC, pericytes and astrocytes in a fibrin gel [1]. It exhibited perfusable capillary opening structures on the bottom of the hydrogel, as originally reported for 3D blood-/lymph-capillary networks [2]. It also demonstrated size-selective permeation of different molecular weights of dextran, which highly correlated (R^2=0.973) with the permeability values found with *in vivo* rodent brain. The TfR functionality was also confirmed by permeability assays. The efficient permeability coefficient (P_e) of transportable anti-TfR antibody (MEM-189) was about 4.77 x 10^{-6} cm/s, being seven-fold higher than those of isotype antibody (IgG1) and low transportable anti-TfR antibody (13E4) which are respectively 6.83 x 10^{-7} and 6.12 x 10^{-7} cm/s. These results suggest our model displayed a better capacity to discriminate antibodies based on their TfR-mediated permeation than previously reported [3]. This BBB model could be useful for the screening of therapeutics based on their TfR-mediated transport efficiency.

References

[1] M. Piantino *et al.*, *Mater. Today Bio.* **2022**, 15, 100324.
[2] D. Hikimoto *et al.*, *Adv. Healthc. Mater.* **2016**, 15, 1969.
[3] N. R. Wevers *et al.*, *Fluids Barriers CNS* **2018**, 15, 23.

2 - Water Soluble Polyurethane Inks for Digital Light Processing 4D Bioprinting Under Visible Light

Hossein Goodarzi Hosseinabadi[1], Leonid Ionov[1]

[1]Bayreuth University

Digital light processing (DLP) bioprinting is a fast and robust technique for production of biomimetic tissue models. The technique is mostly limited by the availability of a resin that satisfy the biological, physical and mechancial properties of the required tissues. Here, we introduce the synthesis and characterization of a PU-PEG biocompatible resin showing remarkable gradient crosslinking capabilities under near visible light digital light processing. A microfluidics platform is designed to enable selective curing and multimaterial 4D bioprinting with the resin through 405 nm irradiation at intensities as low as 1 mW/cm^2 using a low cost LCD-DLP. A design of experiments was conducted to find optimized parameters for printing the resin formulation. Cell studies with inclusion of glass beads in the resin during the printing confirmed the biocompatibility of the resin as well as the bioprinting DLP platform. The power of this approach is showcased through 4D biofabrication of bioinspired soft joints and mathematicalz defined porous scaffolds.

LIST OF PARTICIPANTS (16 Nov 2022)

First name	Last name	Company/Institution	Country
Carmen	Aguilar	**Julius-Maximilians-University Würzburg**	Germany
Taufiq	Ahmad Ahmadi	**University Hospital Würzburg**	Germany
Anahita	Soufivand	**Friedrich-Alexander-Universität Erlangen-Nürnberg**	Germany
Mona	Alzheimer	**Julius-Maximilians-Universität Würzburg**	Germany
Kristina	Andelovic	**University Hospital Würzburg**	Germany
Mateo	Andrade Mier	**University Hospital Würzburg**	Germany
Sina	Bartfeld	**TU Berlin**	Germany
Michael	Bartolf-Kopp	**University Hospital Würzburg**	Germany
Petra	Bauer-Kreisel	**University Hospital Würzburg**	Germany
Morris	Baumgardt	**Charité – Universitätsmedizin Berlin**	Germany
Kevin	Berg	**Julius-Maximilians-Universität Würzburg**	Germany
Ariane	Binder	**Helmholtz Institute for RNA-based Infection Research Würzburg**	Germany
Torsten	Blunk	**Julius-Maximilians-University Würzburg**	Germany
Francesco	Bocellato	**University of Oxfort**	UK
David	Böhringer	**Friedrich-Alexander-Universität Erlangen-Nürnberg**	Germany
Johannes	Braig	**University Hospital Würzburg**	Germany
Thea	Bruchhardt	**Julius-Maximilians-Universität Würzburg**	Germany
Margitta	Büchner	**Friedrich-Alexander-Universität Erlangen-Nürnberg**	Germany
Paula	Büttner	**University hospital Würzburg**	Germany
Nathaly	Chicaiza Cabezas	**University Hospital Würzburg**	Germany
Cindrilla	Chumduri	**Julius-Maximilians-University Würzburg**	Germany
Alessandro	Cianciosi	**University Hospital Würzburg**	Germany
Alba	Cisneros Martín	**Julius-Maximilians-University Würzburg**	Germany
Yi-Yu Robin	Dai	**University Hospital Erlangen**	Germany
Spyridon	Damigos	**Julius-Maximilians-University Würzburg**	Germany
Thomas	Dandekar	**Julius-Maximilians-University Würzburg**	Germany
Mahshid	Danesh	**Julius-Maximilians-University Würzburg**	Germany
Thomas	Däullary	**Julius-Maximilians-University Würzburg**	Germany
Laura	De Laporte	**DWI Leibniz-Institut für Interaktive Materialien e.V. / RWTH Aachen**	Germany
Rainer	Detsch	**Friedrich-Alexander University Erlangen-Nürnberg**	Germany
Oliver	Dietrich	**Helmholtz Institute for RNA-based Infection Research Würzburg**	Germany
Lysanne	Dietrich	**Julius-Maximilans-Universität Würzburg**	Germany
Leyla	Dogan	**Julius-Maximilians-University Würzburg**	Germany
Ali	Doryab	**Helmholtz Forschungszentrum für Gesundheit und Umwelt München**	Germany
Daniela	Duarte Campos	**Universität Heidelberg**	Germany
Franziska	Dusi	**University Hospital Würzburg**	Germany
Regina	Ebert	**Julius-Maximilians-University Würzburg**	Germany
Carolin	Eckert	**Friedrich-Alexander University Erlangen-Nürnberg**	Germany
Leo	Endres	**Julius-Maximilians-University Würzburg**	Germany

Markus	Engstler	**Julius-Maximilians-Universität Würzburg**	Germany
Süleyman	Ergün	**Julius-Maximilians-University Würzburg**	Germany
Tilman	Esser	**University Hospital Erlangen**	Germany
Andrea	Ewald	**University Hospital Würzburg**	Germany
Jessica	Faber	**Friedrich-Alexander University Erlangen-Nürnberg**	Germany
Christina	Fey	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Mara	Fischer	**Charité - Universitätsmedizin Berlin**	Germany
Kshitija	Gadekar	**Julius-Maximilians-University Würzburg**	Germany
Benedikt	Gantert	**Julius-Maximilians-Universität Würzburg**	Germany
Debby	Gawlitta	**University Medical Center Utrecht**	Nether-lands
Eileen	Gentleman	**King's College London**	UK
Csaba	Gergely	**University Hospital Würzburg**	Germany
Hossein	Goodarzi Hosseinabadi	**Bayreuth University**	Germany
Florian	Groeber-Becker	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Jürgen	Groll	**University Hospital Würzburg**	Germany
Dieter	Groneberg	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Marcel	Groß	**Julius-Maximilians-University Würzburg**	Germany
Maria	Guedes	**Julius-Maximilians-University Würzburg**	Germany
Eliise	Haljas	**Julius-Maximilians-University Würzburg**	Germany
Laura	Hauf	**Julius-Maximilians-University Würzburg**	Germany
Sven	Heilig	**University Hospital Würzburg**	Germany
Christina	Heinritz	**Bayreuth University**	Germany
Frederik	Hellert	**Julius-Maximilians-Universität Würzburg**	Germany
Florian	Hofmann	**University Hospital Würzburg**	Germany
Pargev	Hovhannisyan	**Julius-Maximilians-University Würzburg**	Germany
Ivana	Jeremic	**Bayreuth University**	Germany
Marvin	Jungblut	**Julius-Maximilians-Universität Würzburg**	Germany
Tomasz	Jüngst	**University Hospital Würzburg**	Germany
Juliane	Kade	**University Hospital Würzburg**	Germany
Lina	Kafuri	**Julius-Maximilians-University Würzburg**	Germany
Tannaz	Karimi	**University Hospital Erlangen**	Germany
Richard	Kellnberger	**Bayreuth University**	Germany
Daniel	Kelly	**Trinity College Dublin**	Ireland
Paul	Köhling	**Julius-Maximilians-University Würzburg**	Germany
Fabian	König	**Julius-Maximilians-University Würzburg**	Germany
Vera	Kozjak-Pavlovic	**Julius-Maximilians-University Würzburg**	Germany
Christine	Krempl	**Julius-Maximilians-University Würzburg**	Germany
Kirilo	Krjukov	**Julius-Maximilians-University Würzburg**	Germany
Yvonne	Kulicke	**University Hospital Erlangen**	Germany
Zan	Lamberger	**Julius-Maximilians-University Würzburg**	Germany
Isabell	Lang	**University Hospital Würzburg**	Germany
Anna	Laptii	**University Hospital Erlangen**	Germany
Anton	Leopold	**Julius-Maximilians-University Würzburg**	Germany
Sophia	Loeffelsend	**University Hospital Würzburg**	Germany

Hsuan-Heng	Lu	**Friedrich-Alexander-Universität Erlangen-Nürnberg**	Germany
Tessa	Lühmann	**Julius-Maximilians-University Würzburg**	Germany
Matthias	Lutolf	**University of Florida**	USA
Gohar	Mädler	**Helmholtz Institute for RNA-based Infection Research Würzburg**	Germany
Gohar	Mädler	**Helmholtz Institute for RNA-based Infection Research Würzburg**	Germany
Jos	Malda	**University Medical Center Utrecht**	Nether-lands
Bita	Massih	**Helmholtz Institute for RNA-based Infection Research Würzburg**	Germany
Thabang	Matseke	**Friedrich-Alexander-Universität Erlangen-Nürnberg**	Germany
Michiya	Matsusaki	**Osaka University**	Japan
Helene	Mehling	**Julius-Maximilians-University Würzburg**	Germany
Marco	Metzger	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Anna	Meyer	**DWI Leibniz-Institut für Interaktive Materialien e.V. / RWTH Aachen**	Germany
Franz	Moser	**University Hospital Würzburg**	Germany
Nicoletta	Murenu	**Julius-Maximilians-Universität Würzburg**	Germany
Camilla	Mussoni	**Julius-Maximilians-University Würzburg**	Germany
Masahiko	Nakamoto	**Osaka University**	Japan
Mastura	Neyazi	**Julius-Maximilians-University Würzburg**	Germany
Xuen Jen	Ng	**Bayreuth University**	Germany
Naveen Kumar	Nirchal	**Julius-Maximilians-University Würzburg**	Germany
Fatemeh	Noratabadi	**Julius-Maximilans-Universität Würzburg**	Germany
Niklas	Pallmann	**Julius-Maximilians-University Würzburg**	Germany
Mindaugas	Pauzuolis	**Julius-Maximilians-University Würzburg**	Germany
Ana Paula	Pego	**University of Porto**	Portugal
Marie	Piantino	**Osaka University**	Japan
Christina	Popp	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Pon Ganish	Prakash	**Julius-Maximilians-University Würzburg**	Germany
Shiva	Rahmani	**Julius-Maximilians-University Würzburg**	Germany
Ravisha	Rawal	**Julius-Maximilans-Universität Würzburg**	Germany
Amelie	Reigl	**Julius-Maximilians-University Würzburg**	Germany
Jennifer	Ritzer	**University Hospital Würzburg**	Germany
Jonas	Röder	**Friedrich-Alexander-Uniersität Erlangen-Nürnberg**	Germany
Saskia	Roth	**Bayreuth University**	Germany
Thomas	Rudel	**Julius-Maximilians-University Würzburg**	Germany
Matthias	Ryma	**University Hospital Würzburg**	Germany
Jochen	Salber	**Ruhr Universität Bochum**	Germany
Evelin	Sandor	**University Hospital Erlangen**	Germany
Joachim	Schenk	**Julius-Maximilians-Universität Würzburg**	Germany
Meike	Scheuring	**Julius-Maximilians-University Würzburg**	Germany
Johann	Schorzmann	**Bayreuth University**	Germany
Jonas	Schubach	**Marschall Medien Gruppe**	Germany
Mikael	Sellin	**Uppsala University**	Sweden

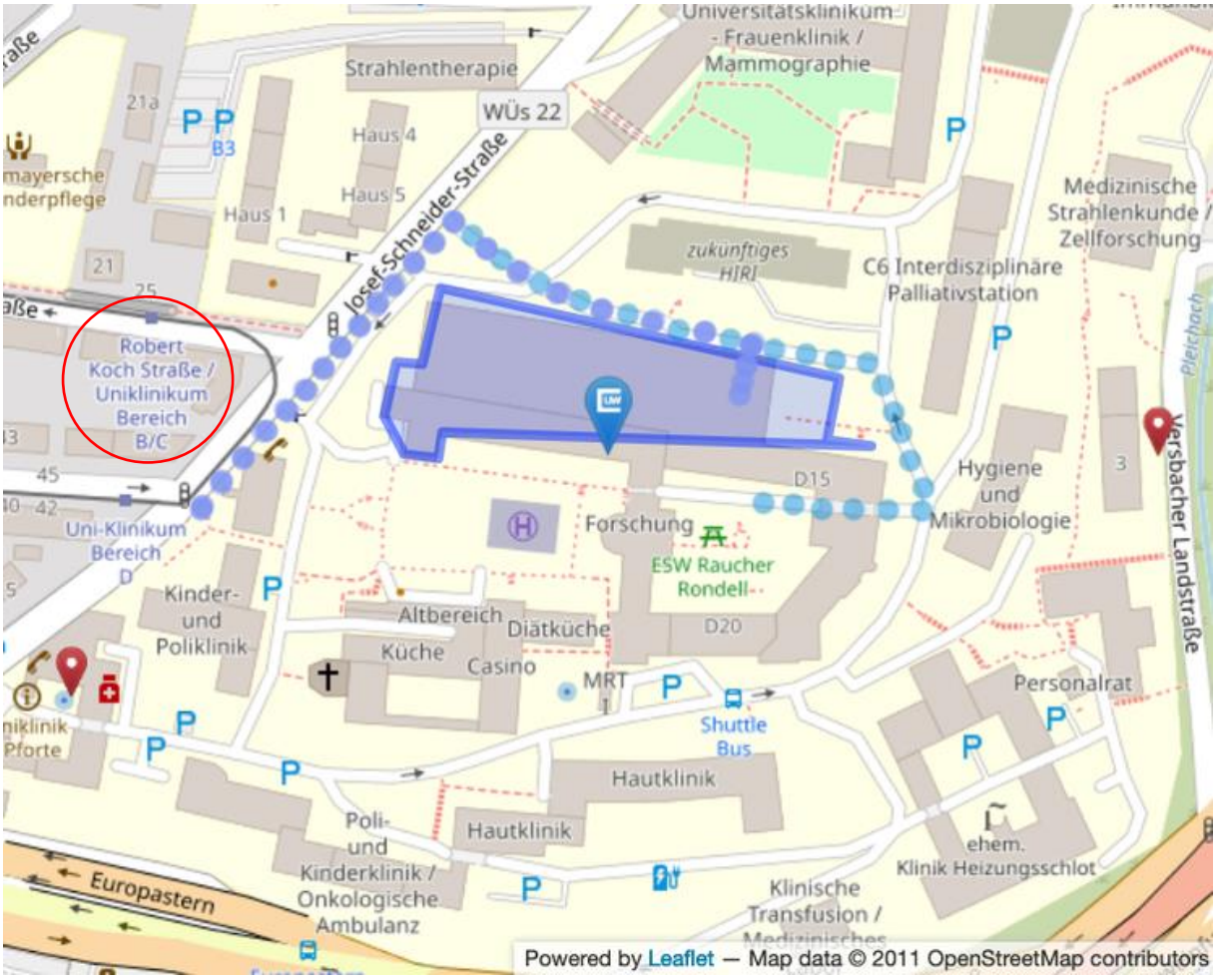
Cynthia	Sharma	**Julius-Maximilians-University Würzburg**	Germany
Dalia	Sheta	**University hospital Würzburg**	Germany
Heungsoo	Shin	**Hanyang University Seoul**	South Korea
Alexander	Sieberath	**University Knappschaftkrankenhaus Bochum**	Germany
Rinu	Sivarajan	**Julius-Maximilians-University Würzburg**	Germany
Lys	Sprenger	**Bayreuth University**	Germany
lys	sprenger	**Bayreuth University**	Germany
Sabrina	Stecher	**University Hospital Würzburg**	Germany
Maria	Steinke	**University Hospital Würzburg**	Germany
Jörg	Tessmar	**University Hospital Würzburg**	Germany
Katinka	Theis	**Julius-Maximilians-University Würzburg**	Germany
Andrew	Thorley	**Genentech**	USA
Daisuke	Tomioka	**Osaka University**	Japan
Leonhard	Topel	**Julius-Maximilians-University Würzburg**	Germany
Steffen	Trippmacher	**Bayreuth University**	Germany
Li Wen	Tseng	**Julius-Maximilians-University Würzburg**	Germany
Esra	Türker	**University Hospital Würzburg**	Germany
Fabiola	Vacca	**Julius-Maximilians-University Würzburg**	Germany
Viola	Vogel	**ETH Zürich**	Switzer-land
Tamara	Weidemeier	**University Hospital Würzburg**	Germany
Jeanette	Weigelt	**University Hospital Würzburg**	Germany
Daniela	Zdzieblo	**Fraunhofer-Institut für Silicatforschung ISC Würzburg**	Germany
Jinfeng	Zeng	**Osaka University**	Japan

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.



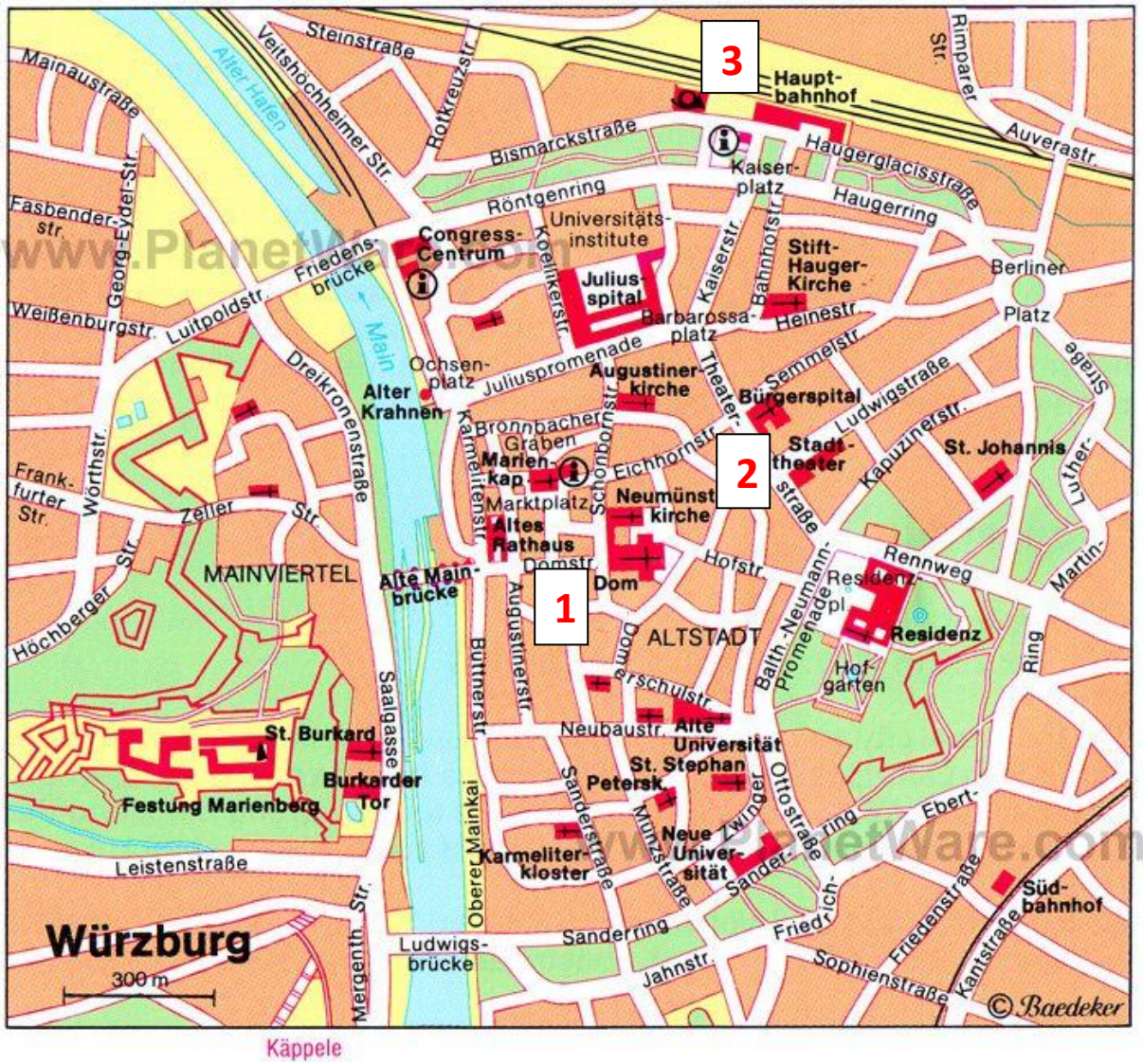
By car

You can drive onto the campus after building D15 until you reach the courtyard of house D15/D16. *'Klinikringstraße'* will be interrupted until probably 2023 due to construction work near building D20. This closure will eliminate numerous parking spaces in the UKW area C-D (near building D15). If you are unable to find a parking space, 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. Night-watchmen meeting point: ***St. Kilian Dom (Cathedral)***
Domstraße 40, Würzburg (from RVZ use Tram line 1 or 5, exit at *'Dom'* station)

2. Conference Dinner: ***Bürgerspital***
Theater Strasse 19, Würzburg

3. Würzburg main station/Würzburg HBF

4. G-Hotel
Schweinfurter Str. 3, Würzburg