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ABSTRACTBAND

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REFISHMENT – REFINEMENT IN FISH RESEARCH

Abstract Number: 98

Poster Number: 00

AN INTERNATIONAL SURVEY ON ENVIRONMENTAL ENRICHMENT FOR ZEBRAFISH

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Background:
The zebrafish is one of the most commonly-used animals in research, but best practice for many aspects of zebrafish husbandry and care is unclear. One area of debate is the use of environmental enrichment. Despite growing evidence that enrichment can benefit zebrafish welfare, and wide acceptance of the importance of enrichment for other laboratory species, provision for zebrafish may be minimal.

Methods:
To better understand what enrichments are most widely used for zebrafish and the barriers that prevent wider implementation, the RSPCA conducted a survey of zebrafish research facilities. Participants were asked about various aspects of the enrichment approaches used in their facilities, including types used, whether any forms of enrichment had been discontinued, and what challenges they faced in introducing more enrichment.

Findings:
The survey received over 100 responses from around the world. Use of some enrichments, such as social housing and live food, was relatively widespread, but other forms such as substrate or artificial plants were less commonly used. Most participants stated they faced barriers to further implementation of enrichment, including concerns over impacts on water quality, and resistance from other staff.

Interpretation:
Overall, the results of the survey were encouraging, with a majority of participants stating they used some form of enrichment. However, there was a clear desire to implement more enrichment, suggesting a need for more enrichment strategies that do not compromise water quality. Zebrafish facilities may also benefit from further resources and training on possible enrichment strategies.

Abstract Number: 233

Poster Number: 01

HOW TO IMPLEMENT THE 3RS IN MOUSE ASSISTED REPRODUCTION: EXAMPLES FROM RECENT STUDIES

Kerstin E. Auer[1], *Auke Boersma*[2], *Thomas Kolbe*[3], *Thomas Rülicke*[4], *Maik Dahlhoff*[5]

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Background:
Mouse model generation relies on assisted reproductive techniques (ART) like embryo transfer (ET) and *in vitro* fertilization (IVF). Here we show how ART can be refined.

Methods:
We conducted bilateral and unilateral surgical ETs, comparing pregnancy rates and embryo survival to determine which method yields the best outcome with the least impact. We assessed the impact of selecting genetically attractive males on inducing pseudopregnancy in recipients for ET. We investigated the link between the sperm-specific protein proAKAP4 and sperm fertilization ability, using enzyme-linked immunosorbent assays and IVF assays. We examined the suitability of percutaneous epididymal sperm aspiration (PESA) for measuring sperm quality determined by computer assisted sperm analysis.

Findings:
Pregnancy rates were higher in bilateral and unilateral right-sided ETs compared to the left side. Bilateral transfers also had higher embryo survival than unilateral left-sided and did not differ from unilateral right-sided transfers. Male genotype affected mating behavior and hybrid males showed higher mating plug rates than inbred males. Sperm proAKAP4 levels impacted IVF rates, with low proAKAP4 levels resulting in lower rates than medium or high proAKAP4 levels. PESA samples showed diminished sperm quality compared to the conventional sperm collection method.

Interpretation:
Unilateral right-sided transfers can replace bilateral ETs without affecting ET success. Choosing males by genotype can enhance pseudopregnancy rates and reduce the number of required recipients. Selecting sperm donors on a proAKAP4 threshold can increase IVF rates and reduce the number of oocyte donors. PESA is not suitable to assess sperm quality.

Abstract Number: 102

Poster Number: 02

REHOME, DAS 4TE „R“ FREILASSUNG VON TIEREN UND PRIVATE UNTERBRINGUNG

Astrid Fabry[1], *Victoria Schiffer*[2], *Roberto Plasenzotti*[3], *Birgit Reininger-Gutmann*[4]

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Background:
In Österreich befasst sich der §10, des TVG mit der Unterbringung von ehemaligen Versuchstieren. Zu den Bedingungen für die private Unterbringung gehört der Gesundheitszustand der Tiere, dass keine Gefahr für Mensch, Tier oder die Umwelt besteht und dass geeignete Maßnahmen ergriffen werden, um das Wohlergehen der Tiere sicherzustellen. Ebenfalls muss die Sozialisierung der privat unterzubringenden Tiere gewährleistet sein. Dies ist jedoch eine sehr zeitintensive Aufgabe.

Deshalb ist eine Zusammenarbeit mit Tierschutzvereinen und -organisationen hilfreich und notwendig. Diese suchen für die Vermittlungstiere geeignete, private Plätze, holen die Tiere aus dem Labor, kümmern sich um Futterumstellung, eventuelle Kastrationen, Gewöhnung an ein größeres Territorium, Vergesellschaftung mit anderen Artgenossen und letztlich Auslieferung an die private Unterkunft mit Platzkontrolle und Schutzvertrag.

Im Laufe der letzten Jahre ist das Interesse sowohl bei den Versuchstiereinrichtungen, als auch bei mehreren Tierschutzorganisationen gestiegen, sich mit der Vermittlung von Labortieren zu befassen.

Um eine leichtere Kontaktherstellung und dadurch eine bessere Vermittlungsquote zu erzielen hat es sich die RepRefRed Society (Gesellschaft zur Förderung von alternativen Biomodellen; https://www.reprefred.eu), das Austrian 3R Center (A3RC; österreichisches 3R Zentrum) zur Aufgabe gemacht, sich diesem Thema zu widmen und eine zentrale Anlaufstelle für die Vermittlung von Labortieren in private Unterkünfte zu bieten.

Abstract Number: 188

Poster Number: 03

A RE-USEABLE OPEN-SOURCE PLATFORM TO UPGRADE RAT CAGES

Sophie Schober[1], *Claudia Gold*[2], *Michael Schunn*[1]

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[2] Institute of Science and Technology Austria; Preclinical Facility

Background:
Due to the 3Rs considerations based on national law (522. Verordnung, § 12. Abs 2), laboratory animals must be able to live out their natural behavior. They must shape and choose their environment. Furthermore, rats are extremely curious and like to climb to get an “overview”. 20 years ago, an animal bedding supplier came up with the idea of a second level for rat cages. This 2nd level consists of a wooden platform which fits in a commercially available cage. As these wooden platforms can be autoclaved but not washed and therefore hardly reused, we tried to develop an easy accessible and affordable, wash-, autoclave and re-useable open-source platform, which serves as refinement and improves animal welfare. Furthermore, it fulfills regulatory requirements.

Methods:
Different designs of platforms were produced and tested. The production process was kept as simple as possible to make it easily reproducible. A questionnaire was set up to assess the handling of the model by the animal caretakers as well as the acceptance of the model by the rats.

Findings:
A model consisting of aluminium and teflon was identified as being the most promising. The materials are commercially available and can be bought at low cost. The manufacturing consists of cutting the materials and screw them together. The platform can be washed, autoclaved and reused.

Interpretation:
The presented model is easily reproducible at low-costs and fulfills the criteria of cage configuration for rats.

Abstract Number: 236

Poster Number:04

IMPLEMENTATION OF ALTERNATIVE HANDLING METHODS: A EXPERIENCE REPORT

Claudia Gold[1], Rudolf Fuchs[2]

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Background:
In July 2022 the Preclinical Facility at ISTA (Institute of Science and Technology) decided to start implementing alternative handling methods for mice (tunnel handling / cup handling).

Methods:
Tunnel handling and cup handling was tested for the handling of C57Bl6, CD1 and different genetically modified mouse strains. For tunnel handling standardized cardboard tubes, red plastic tubes and transparent plastic tubes were used.

Findings:
Each method could be associated with several advantages and disadvantages. Numerous factors have to be considered: strain and age of the animals, number / commitment of animal care takers, time factor and ergonomical issues.

Interpretation:
Finding the one and only handling method that fits for all animals / all situations is a nearly impossible thing to do.

3RS & REGULATORY

Abstract Number: 109

Poster Number: 05

CAREFUL BREEDING PLANNING TO REDUCE THE NUMBER OF SURPLUS ANIMALS

Doris Schneller[1], Lena Hornetz[2], Kurt Reifenberg[2]

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Background:
For biological reasons, in breeding genetically modified mice it is in many cases not possible to breed exclusively animals with such characteristics that can then be used in planned experiments. The generation of surplus animals is unavoidable despite the most careful breeding planning because litters are not always the same size, because many animals with undesirable genetic traits must be bred, and because often only one sex is needed in the animal studies. In order to reduce the number of unusable animals as much as possible, it is important to establish a breeding strategy that meets the needs of the animals and also to make good use of the animals from the breedings.

Methods:
Different breeding calculators are available that allow optimized breeding planning. The principal characteristics, differences and applicabilities of various breeding planners are discussed.

Findings:
The “Zurich Breeding Calculator” is a cohort planner, i.e. it is designed to plan the one-time breeding of an animal cohort. The “Heidelberg Breeding Calculator” is also a cohort breeding planner. However, it assumes that the calculated breeding females are not mated in a scheduled manner, so that the desired number of animals is produced successively over a longer period of time. The “Continuous Breeding Calculator of TJL” takes into account a continuous production of animals and must therefore also represent a renewal of the breeding animals.

Interpretation:
Various breeding planners are available to help in careful breeding planning. Each has specific characteristics and can therefore be applied based on the individual needs.

Abstract Number: 111

Poster Number: 06

FOR HOW MUCH LONGER CAN PRE-CLINICAL RESEARCH GET AWAY WITH INADEQUATE STATISTICAL METHODOLOGIES?

Florian Frommlet[1]

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Background:
Preclinical research has a severe problem of replicability. Consequently, preclinical research involving animals is at risk of losing credibility, which it simply cannot afford in the current political situation. There is strong lobbying towards banning preclinical animal trials, resulting in a recent press release of the EU Commission about accelerating phasing out of animal testing for drug development. To counter this movement there should be no doubt about the quality of animal trials and their benefit for society.

Methods:
The use and abuse of statistics in animal trials is one important ingredient which contributes to the problem. One key difference between clinical research and pre-clinical research is the involvement of statisticians. In the pharma industry regulators mandate that trained statisticians are responsible for statistical work (which includes experimental design and data analysis). For pre-clinical research no such guidelines exist. Similarly, within academia there are way more statisticians involved in clinical research than in pre-clinical animal trials. In fact, there are fairly few statisticians who are knowledgeable about the particular difficulties arising within pre-clinical experiments.

Findings:
This talk will point out some of the relevant statistical problems within pre-clinical research. It will indicate where statisticians might be helpful and why it would make a difference to get more statisticians involved in pre-clinical research.

Interpretation:
Statistics is not easy and it is astonishing that so many lab researchers believe that they can do it on their own. However, this has consequences and the question is how long pre-clinical research can actually afford this.

Abstract Number: 170

Poster Number: 07

3R-SMART: INFORMATION AND TRAINING PLATFORM FOR METHODS TO REPLACE AND SUPPLEMENT ANIMAL EXPERIMENTS

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[2] Institute for Animal Hygiene; Animal Welfare and Farm Animal Behaviour

Background:
The Directive 2010/63/EU firmly strengthens the adoption of the 3R principle (replacement – reduction – refinement) for the use of animals for scientific and educational purposes.

Against this background, the BMBF-funded project 3R-SMART (https://www.3r-smart.de) was designed.

Methods:
In addition to showcasing specific examples of alternatives to animal testing, 3R-SMART also covers legal and ethical aspects of working with laboratory animals. The information is tailored to different needs, providing either a quick overview or more in-depth information in the form of video or text content.As well as providing educational content on alternative methods, the website offers news and updates, a calendar of upcoming events and a forum for exchanging ideas on the 3Rs. Interactive maps of the 3R Centres in Germany and Europe provide an overview of the 3R Centres and information on the activities and priorities of the individual 3R Centres. Furthermore, 3R-SMART supports the 3R research activities of various stakeholders by enabling them to present their latest 3R findings on 3R-SMART in order to increase the reach of their research results. In this way, 3R-SMART is constantly being expanded and developed.In order to disseminate and transfer knowledge about the 3Rs, 3R-SMART will make open educational resources (OER) available and also is planning to offer 3R seminars and other learning opportunities. In this context, work is being done in cooperation with LAS interactive (https://las-interactive.de) on a combined continuing education portal on laboratory animal science and alternatives to animal experimentation (fee-based) for continuing professional development.

Abstract Number: 242

Poster Number: 08

OVARIECTOMY IN SURPLUS FEMALE MICE TO GENERATE CAGE MATES FOR EXPERIMENTAL MALES – A REFINEMENT TO IMPROVE BIOMEDICAL SCIENCE

Christoph Töscher[1], Andreia Joana Miguel Madalena[2], Sophie Schober[3], Michael Schunn[1]

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[2] Institute of Science and Technology; Preclinical Facility; Preclinical Facility,
[3] Ista; Preclinical Facility

Background:
Different experimental procedures may require male experimental animals to be housed alone. Given that adult males can no longer be pooled with others once they have been separated, it is common practice to keep single-housed males when cage mates are used for experiments. We generated sterile female mice to serve as cage mates, as a measure to avoid single housing and thus improving general welfare of male experimental animals.

Methods:
Ovariectomy surgeries were performed in 51 females aged between 8 to 10 weeks according to Nagy et al. (2013). The procedure was approved by the federal ministry under license GZ: BMBWF-66.018/0029-V/3b/2019.

Findings:
Since a single sterilized female can be paired with multiple males, more than 51 single housed males have been avoided. For this pilot study, no additional laboratory mice had to be purchased/ generated. The animals used for this procedure were surplus animals (wild type) from ISTA's internal facility. Furthermore, a reminder system which requests scientists to act if males are sitting alone for more than 3 weeks was implemented.

Interpretation:
Providing otherwise single housed males with a sterile female can enable species-typical social behavior (Olsson et al., 2007) and therefore might improve the welfare of these animals according to the 3Rs. Biomedical Science could be improved because social housing reduces stress, improves health outcomes and therefore the quality of the model (Pham et al., 2010). Further studies to confirm the effect are planned.

EXPERIMENT SPECIFIC 3RS

Abstract Number: 97

Poster Number: 09

ENHANCING ANIMAL WELFARE AND BIOMARKER DISCOVERY THROUGH NOVEL HOME CAGE MONITORING (HCM) VIA DIGITAL VENTILATED CAGES (DVC®)

Stefano Gaburro[1], Daniela Pollak[2], Torben Hager[3]

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[2] Medical University of Vienna; Department of Neurophysiology and Neuropharmacology (Center for Physiology and Pharmacology),
[3] Tecniplast Deutschland GmbH

Background:
The significance of animal experimentation in biomedical research demands advanced welfare standards for animal models. The rise of novel home cage monitoring (HCM) technologies, particularly DVC®, offers the promise of addressing this challenge.

Methods:
DVC®, a standout HCM system located within the animals' natural habitat, was used to provide 24/7 monitoring in mostly stress-free environments using micro-electromagnetic fields (mMF). The system's scalability supported monitoring from one to over a thousand cages, without necessitating running animal license alteration. The welfare and spontaneous behaviour of different mouse models were observed in three experimental settings described below.

Findings:
The continuous monitoring provided by DVC® highlighted several benefits. The automated night welfare assessment, utilizing artificial intelligence, preemptively detected potential health issues, evident in a Covid-19 mouse model where it anticipated clinical symptoms indicated by weight losses. The technology pinpointed polyuria as a marker for prolonged hyperglycemia in diabetes types 1 and 2. Ultimately, an AI-based approach revealed hidden aggressive interactions in group-housed settings.

Interpretation:
The introduction of DVC® in the HCM domain signifies a pivotal shift towards methodological precision and resource optimization in animal welfare. By aligning with the 3Rs principles, the system not only minimizes induced unspecific stressors but also fortifies the validity of non-invasive monitoring protocols. The findings suggest that DVC® augments both the welfare standards for experimental subjects and the methodological accuracy of animal model studies in the biomedical sector

Abstract Number: 110

Poster Number: 10

NEW NON-INVASIVE, LABEL-FREE MONITORING APPROACH FOR 2D AND 3D CELL CULTURE

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Background:
Two major issues of cell-based toxicological and drug response assays are the lack of the temporal component of endpoint assays, and the strong dependency of reproducibility and significance on the quality and condition of the cells used. Thus there is a tremendous need to provide insight into the usually inaccessible processes inside the incubator.

Methods:
We developed a novel lensfree imaging method exploiting the optical properties of the cell itself for imaging inside the incubator, which allows non-invasive, super compact, label-free, live-cell monitoring. By applying AI to determine key cell culture parameters such as confluence, proliferation, and cell motility, high-quality, automated, objective, and real-time data can be collected.

Findings:
Applying our lensfree microscopy (LM) method, we find that memory effects from heterogeneous cell culture conditions lead to an increase of variance during subsequent assays like e.g. omics-readouts or other cell based assays, like wound healing assays, motility and proliferation assays significantly. Furthermore, our LM is also suitable for 3D applications and will enable quantification of organoid growth dynamics and interactions.

Interpretation:
Our approach dramatically increases control and processing speed. In the context of the reproducibility crisis, we hope to make a contribution in the direction of standardization of cell-based research in the future.

Abstract Number: 178

Poster Number: 11

ANALYZING BLOOD OF LONG COVID PATIENTS INN MOTION

Martin Hermann[1], Regine Gerth[2], Anna-Lena Wyremblewsky[3], Dietmar Fries[4], Nikolaus Wick[2]

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Background:
Patients who recovered from Covid-19, but are still experiencing symptoms such as shortness of breath, cognitive dysfunction (brain fog) or chronic fatigue, suffer from a condition known as "long-COVID". Patients suffer for several months, a year and some even longer, without a proper treatment for this condition.

The underlying nature of this illness, still remains well hidden behind it´s plethora of signs and symptoms making it difficult to find a specific treatment option. How can we learn something about this relatively new illness, in the best case without the need of animal experiments?

Methods:
In the past, we developed an approach referred to as "biopsychronology", which combines the use of indicator dyes for cellular function and integrity with live cell confocal imaging to gain rapid insight into the functional status of tissues and organs. Herein, we apply this method using a drop of blood of long-COVID patients, which is incubated under static conditions with live stains such as HOECHST, Wheat-Germ Agglutinin and ffbp (fibrin binding protein) in order to visualize nuclei, cell morphology and fibrin/microclots.

Findings:
With the above mentioned imaging modality, we were able to get a grip on the heterogenic nature of long-COVID. Each patient´s blood tells its own story. Differences in cell morphology, the presence of microclots, high amounts of granulocytes, NET formation, platelet interactions, Rouleaux Effects or even the presence of bacteria can be visualized live in real time.

Interpretation:
The basis for every treatment is the understanding of the underlying problem. Our live confocal imaging approach gives us a picture of our blood InN motion responding to challenges such as long-COVID.

ADVANCING ANIMAL TESTING ALTERNATIVES: FOCUS ON REFINEMENT & REDUCTION

Abstract Number: 139

Poster Number: 13

MODULATION OF L-TYPE CALCIUM CURRENTS ASSOCIATES WITH CHANGES OF DENDRITIC GROWTH IN HIPPOCAMPAL NEURONS

Stefano Lanzetti[1], Pietro Mesirca[2], Alessandra Folci[3], Rosina Maier[1], Eleonora Torre[4], Fabian Rinner[1], Cornelia Ablinger[5], Sabrin Haddad[6], Gerald J. Obermair[6], Marta Campiglio[5], Matteo E. Mangoni[7], Valentina DI Biase[1]

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Background:
L-type voltage-gated calcium channels (L-VGCCs) are upstream of competing pathways underpinning the promotion or inhibition of dendritic growth. However, how L-VGCCs implement signaling specificity is unknown. We hypothesize that modulation of I_{CaL} is critical to trigger distinct processes controlling dendritic growth.

Methods:
1. Murine hippocampal neurons were transfected with soluble eGFP (DIV4), treated for 48h with L-VGCC drugs (DIV5-7), and processed for Sholl analysis (DIV7).
2. Voltage-patch clamp of the neurons upon treatment (DIV 5-7).
3. Transfection of STAC2-HA (DIV4), which suppresses calcium channel inactivation while increasing I_{CaL} density, and subsequent processing for Sholl analysis (DIV7).
4. We treated the neurons at DIV5 and performed biotinylation assays (DIV7) followed by western blots.

Findings:
1. Dihydropyridine (DHP) blockers suppressed dendritic growth, and overexpression DHP-insensitive $Ca_V1.2$ mutant recovered it. Agonist FPL 64176 enhanced dendritic growth while agonist BayK-8644 had no effect. Immunostaining experiments showed that Bay-K 8644 boosted activated CamKII levels (pCamKII), unlike FPL 64176.
2. DHPs block L-VGCC currents, while BayK-8644 and FPL 64176 both increase I_{CaL} density. Only, FPL 64176 slowed I_{CaL} inactivation.
3. STAC2-HA overexpression increases dendritic growth while keeping pCamKII at basal levels.
4. FPL 64176 but not Bay-K 8644 reduced membrane-expressed $Ca_V1.2$.

Interpretation:
We propose that calcium entry through $Ca_V1.2$ is necessary for controlling dendritic growth and that regulation of L-VGCC kinetics may be essential for modulating $Ca_V1.2$ membrane levels to restrict CaMKII signaling and regulating dendritic tree development.

Abstract Number: 217

Poster Number: 14

DOES ABERRANT GLUTAMATE METABOLISM IN MELANOMA AFFECT DENDRITIC CELL FUNCTION?

Sophie Dieckmann[1], Christoph Tripp[2], Helen Strandt[1], Florian Hornsteiner[1], Herbert Oberacher[3], Natascha Kleiter[4], Johannes Wölk[5], Patrizia Stoitzner[6]

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Background:
Tumor immunity is affected by metabolites in the tumor tissue. The transgenic melanoma mouse model tg(Grm1)EPv spontaneously develops melanoma due to an overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. We study the metabolic changes in progressing EPV melanoma and potential effects on dendritic cell (DC) and T cell responses. The aim is to intervene with this metabolic pathway to block tumor growth. To minimize animal usage, we are evaluating inhibitors on melanoma cell lines.

Methods:
To screen for metabolic changes, we used LC-MS. We performed detailed analyses of myeloid subsets with multi-color flow cytometry. Currently, we are screening glutamate pathway inhibitors in vitro to find the ones with the highest potential for blocking tumor cell proliferation while not impacting DC.

Findings:
We detected a decrease in levels of amino acids, ATP as well as metabolites of the TCA cycle and glycolysis in progressing tumor lesions. We observed a percentual shift towards glutamate and glutamine during tumor progression. Our FACS measurements showed a decrease of cDC2 and macrophages, but an increase of neutrophils and monocytes in the tumor tissue. First results with the inhibitors BPTES (glutaminase inhibitor) and BAY-36-7620 (mGlu1R antagonist) allude to a higher effect of BPTES on the Grm1 melanoma cells. At the same time, BAY-36-7620 seems to negatively affect BMDC.

Interpretation:
Combination therapies with inhibitors of the glutamate pathway and immunotherapy might improve response rates in melanoma patients and therefore be essential for the design of novel therapeutic strategies for cancer patients.

Abstract Number: 226

Poster Number: 15

THE DILEMMA OF ANIMAL TESTS IN ACADEMIC SUBSTANCE DEVELOPMENT

Markus Nagl[1]

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Background:
Investigation of new substances is a common issue in research. In case of promising in-vitro studies, unambiguously the question of tolerability and efficacy in vivo arises and therefore the question of animal testing. The more the development proceeds to clinical dimensions, the more regulatory questions come up.

Methods:
Taking the example of more than 30 years academic development of N-chlorotaurine, an endogenous well tolerated antiseptic that can be used in multiple body regions for topical treatment of infections, the questions of 3R (refinement, reduction, replacement) of animal tests are discussed here.

Findings:
The goal of 3R is not only an academic issue, but it is in part clearly counteracted by permanently increasing safety regulations of drug development. Demonstration of tolerability, safety, and efficacy are required in two different animal species for each indication, before clinical trials in humans can be initiated, which appears not to be justified in all cases. Problematic is the diverse development of the academic and regulatory world with different standards and aims. For instance, academic studies may not be approved by authorities but standard tests by GLP (good laboratory practice) laboratories required, which may lead to disputable repetitions of animal tests. Due to significant costs in this context, hidden conflicts of interests can be conceived occasionally.

Interpretation:
To consider, discuss, and answer the good reason of animal tests is more laborious, but frequently better than simply to comply with checklists. Animal studies are always a compromise and, regarding requirements in drug development, a balancing act between too much and too less regulation.

Abstract Number: 249

Poster Number: 17

PRIORITIZING ENVIRONMENTAL PROTECTION OVER TOXICITY PREDICTION TO ELIMINATE VERTEBRATE ANIMAL TESTING: DAPHNIDS AS SENTINELS TO ENABLE SAFE USE OF ALTERNATIVE METHODS FOR ACUTE FISH TOXICITY.

Martin Paparella[1]

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Background:
Traditionally, assessing chemical impacts involves acute aquatic toxicity tests using diverse organisms representing the trophic levels. To align with 3R goals, alternatives like the acute fish RTgill-W1 cell line test

and zebrafish embryo test were developed to replace juvenile fish tests. However, these alternatives exhibit lower sensitivity, particularly for neurotoxic chemicals and allylalcohol, which is biotransformed in fish. Further investigation was required to assess their suitability in replacing the acute fish test.

Methods:
We gathered EnvironTox database data aligned with OECD Test Guidelines 202 (daphnids) and 203 (fish) for acute toxicity studies. Variability and ratios between daphnid and fish toxicities were plotted, categorized by various neurotoxicity modes of actions.

Findings:
Daphnids displayed higher sensitivity than fish for neurotoxic chemicals, except for certain outdated cyclodienes. Additionally, daphnids were more sensitive than fish to allylalcohol toxicity. These results emphasize the regulatory standard daphnids test's adequacy in addressing earlier identified outliers in alternative method performance.

Interpretation:
This research redirects focus from predicting fish toxicity to prioritizing environmental protection. This shift allows replacing juvenile fish tests in OECD standardized testing approaches and facilitates further reduction of vertebrate animal tests in environmental toxicology.

ADVANCING QUALITY CELL CULTURE PRACTICES & NEW APPROACH METHODOLOGIES

Abstract Number: 149

Poster Number:18

THE ROLE OF ETHER LIPID METABOLISM IN FERROPTOSIS: HOW CAN ANIMAL EXPERIMENTS BE REPLACED?

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Background:
Polyunsaturated fatty acids (PUFAs) are prone to lipid peroxidation, which ultimately culminates in ferroptosis, a cell death mechanism inhibited by GPX4. PUFAs fuel intestinal inflammation but their exact role remains debated. Recent research implicates ether lipid metabolism in ferroptosis, namely the enzyme PEDS1 and the AGMO cofactor tetrahydrobiopterin synthesized by GCH1. We want to study the intricate relationships between these three players in ferroptosis in the gut by crossing PEDS1, AGMO, GCH1 into a GPX4 knockout mouse model. In order to reduce and replace mouse experimentation, extensive *in vitro* testing is currently ongoing to establish which cell types should be targeted.

Methods:
Cell lines were cultured according to established protocols. Enzyme activities in cells and human tissues were analyzed using established HPLC-based assays. Gene expression of these enzymes in human tissues was determined by qPCR. CRISPR/Cas9 technology facilitated enzyme knockouts, followed by genetic complementation through plasmid transfection.

Findings:
In order to understand which cell types in the gut express AGMO, PEDS1 and GCH1 we tested various cell lines and found that only a macrophage line harbored the three activities and will be used for our *in vitro* studies, including enzyme knockouts and rescues. Also in human biopsies, only one of the three enzymes was reproducibly active.

Interpretation:
Our *in vitro* results allow us to narrow down our experimental plan towards a macrophage-specific knockout in mouse thereby adhering to the 3R principles.

Abstract Number: 171

Poster Number: 19

STRUCTURE-BASED MODELLING OF THYROID PEROXIDASE

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Background:
Neurodevelopmental disorders have been reported to become more prevalent in recent years. Exposure to environmental chemicals plays a significant role in the pathogenesis. Hence, an urgent need for test methods capable of detecting such substances exists.

The adverse outcome pathway concept has been established to allow a better understanding of adverse events without using animal experiments. It starts with a molecular initiating event (MIE) in which a stressor binds to a protein, leading to a series of key events that result in an adverse outcome, such as the herein-discussed developmental neurotoxicity (DNT).

Several reported MIEs leading to DNT are involved in thyroid hormone synthesis, transport, or metabolism. The objective of this study is to create computational models of MIEs leading to DNT through disruption of thyroid homeostasis.

Methods:
First, thyroid peroxidase (TPO) inhibition was modelled. Homology models were generated and drugs co-crystallized with templates were re-docked for structure validation. Subsequently, a virtual screening workflow was executed in which about 2000 *in vitro*-tested substances were docked. Ultimately, a congeneric series of flavonoids of known activities was docked by applying an induced fit protocol.

Findings:
The virtual screening workflow allowed a ranking of active vs inactive compounds. Docking of the congeneric series demonstrated a reasonable correlation with in vitro results.

Interpretation:
The correlations between the rankings allowed to establish a binding hypothesis for flavonoids. The created models can be utilised to elucidate the binding modes of additional compounds and to uncover other TPO inhibitors.

Abstract Number: 173

Poster Number: 20

ENHANCING REPRODUCIBILITY AND AFFORDABILITY OF CELL CULTURE VIA MEDIUM OPTIMIZATION

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Background:
Cell culture experiments are an emerging alternative to large-scale experiments with animal models. Their major limitations are high cost and low reproducibility. Available serum-free media are often very costly and underperforming as compared to FBS-based medium. One of the major reasons for that is the instability of growth factors and cytokines contained therein. We propose an approach to address this issue.

Methods:
We applied a DoE - based approach to test potential stabilizers in a high-throughput manner for their ability to support muscle stem cells' short- and long-term proliferation in serum free medium. We evaluated the influence of these stabilizers on myogenic potential of the muscle stem cells by comparing fusion indexes upon differentiation. We examined the ability of stabilizers to prolong half-life of certain growth factors in cell culture medium, monitored via ELISA. We further used microscale thermophoresis to assess direct interaction of stabilizers with two growth factors by measuring their binding affinities.

Findings:
We demonstrate that certain stabilizers consistently support high proliferation and differentiation rates of muscle stem cells, compared with FBS-containing medium. They also significantly raise the half-life of critical growth factors. One of the stabilizers was also shown to directly interact with GF, changing conformation of the latter.

Interpretation:
We show that optimization and stabilization of serum-free media not only leads to compositions that are as efficient as FBS-containing media, but also significantly (up to 50%) drops the media price. This finding should greatly improve applicability and reproducibility of *in vitro* experiments.

Abstract Number: 175

Poster Number: 21

FIRST APPROACHES TO SIMULATING A BLOOD FLOW VESSEL WITH AN ANEURYSM TO EVALUATE ITS DANGEROUSNESS WITH COMPUTATIONAL FLUID DYNAMICS

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Background:
In this paper, the first approaches and simulation results of a blood flow vessel with an aneurysm are presented based on computational fluid dynamics (CFD). The goal is to calculate the velocity vector field, pressure field, and wall shear stresses to find out whether the aneurysm is dangerous.

Methods:
The segmented blood vessel with aneurysm is provided by the openLB package (version 1.6-0). This is a surface geometry in stl file format (filename: aneurysm.stl) and is loaded in the Space Claim design modeler. There, the surfaces of the inlet and the two outlets are defined as groups (named selections) so that the appropriate boundary conditions can be applied. The fluid domain is spatially discretized with 20 cells and 5 boundary cells around the cross-section and solved with an inflow velocity of 0.45 m/s until the solution converges to scaled residuals of 1e-6.

Findings:
Results show a maximum velocity of 1.55 m/s, a static pressure drop of 1387.277 Pa, and a maximum wall shear stress of 57 N/m^2. The maximum wall shear stress is not at the location of the aneurysm; therefore, it might not be dangerous.

Interpretation:
Compared to real blood, there are some simplifications. First, blood is used as a Newtonian fluid. Second, there is a constant flow boundary condition set, which is not to heartbeat. However, the simplified model is a nice digital twin to support the surgery decision process.

Abstract Number: 176

Poster Number: 22

PREDICTING DRUG-INDUCED CHOLESTASIS WITH SYSTEMIC FINGERPRINTS

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Background:
The increasing importance of data science in Next-Generation Risk Assessment (NGRA) stems from the growing volume of data within the field of life sciences. It enables researchers to predict toxicity, that ultimately entails adverse events (AEs). One prevalent AE is drug-induced liver injury (DILI), which manifests as damage to the liver following drug administration. Within the spectrum of DILI, cholestasis is a non-idiosyncratic (dose-dependent) subtype, which is characterized by the impairment of bile flow in the liver. Understanding the mechanisms of these conditions is crucial to mitigate the hepatotoxic effects of potential drug candidates. This study aims to depict cholestasis, emphasizing the significance of systemic fingerprints.

Methods:
The retrieval of compound-interaction profiles was conducted for a cholestasis dataset, in an open-source KNIME workflow called "Path4Drug". Compound-target interactions with IC_{50} values were retrieved through 5 publicly available databases (ChEMBL, Drugbank, IUPHAR/BPS, PharmGKB and TTD) and annotated to pathways using Reactome pathway analysis API service. Subsequently, binary matrices were created for the compound-target and -pathway interactions. These matrices served as input for different machine learning algorithms and a feature importance analysis was performed.

Findings:
For the target descriptor set, the highest Matthews Correlation Coefficient of 0.35 was reached in an XGBoost model. The feature importance analysis ranked the Bile Salt Export Pump (BSEP) as top feature.

Interpretation:
Inhibition of the top 1 ranked transporter BSEP causes toxic accumulation of bile acids in the liver and is considered one of the primary reasons for cholestasis.

Abstract Number: 190

Poster Number: 23

THE BEST OF BOTH WORLDS: A COMBINATION OF IN SILICO AND VITRO METHODS FOR TOXICITY PREDICTION OF NEONICOTINOID PESTICIDES AND THEIR MAJOR METABOLITES

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Background:
Neuronal nicotinic acetylcholine receptors (nAChRs) are considered target proteins for neonicotinoid pesticides. Originally, they were designed to achieve selectivity on insect receptors. However, concerns have arisen regarding their selectivity on a human level. Therefore, we propose a combination of in vitro and in silico methods, that investigate the potentially toxic effects of these compounds and their main metabolites on human neurons. This study aims to advance next-generation risk assessment and toxicity prediction since there is a paradigm shift to implement novel and human-centred methods in this field.

Methods:
As in silico method, we applied an ensemble docking study on structures of human nAChR isoforms α7 and α3β4. This elucidates crucial protein-ligand interactions that give more structural insights into this molecular initiating event (MIE). For refinement of the representative docking poses, 50ns molecular dynamics simulations are conducted, and additionally, binding energies are calculated. Collaboration partners from the Risk-Hunt3R project conducted a calcium imaging assay on human LUHMES neurons, that confirmed a key event (KE) downstream of the MIE.

Findings:
The in silico methods predicted an agonistic effect of the neonicotinoid metabolite descyano-thiacloprid, where little data was known previously. Furthermore, the triggering of the MIE was confirmed via assessment of the subsequent KE on human neurons.

Interpretation:
This study contributes to paving the way for the implementation of novel methods in future toxicity risk assessments, which aligns with the 3R concept.

Abstract Number: 222

Poster Number: 24

LIFTING THE BURDEN FROM ANIMALS AND THE HEART: A COMPREHENSIVE APPROACH TO CARDIOTOXICITY EVALUATION

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Background:
Cardiovascular diseases remain the predominant cause of death globally, with indications that environmental pollutants play a significant role in their exacerbation. Despite this, cardiotoxicity is not yet a primary focus in the prevailing regulatory standards for chemicals, biocides, and pesticides, which lean heavily on animal testing-based evaluations.

Methods:
In the context of the EU-H2020 Project ALTERNATIVE (www.alternative-project.eu), our mission is to rethink the evaluation of cardiotoxicity by: (1) Spotlighting the existing regulatory constraints, (2) combining diverse lines of evidence related to heart failure induced by environmental pollutants using the Adverse Outcome Pathway (AOP) framework (3) identifying non-animal testing methods and Biomarkers of Effect (BoEs) to evaluate key events within the AOP, and (4) conceptualizing an Integrated Approach to Testing and Assessment (IATA).

Findings:
Our research has highlighted gaps in the current cardiotoxicity evaluation process, the limited predictability of both animal and in vitro methods as per existing regulatory guidelines, and the importance of considering the older, more susceptible population. We've established a novel AOP network (incorporating AOPs #479 & #480) that systematically integrates human epidemiological data (from 15 studies) and experimental evidence (from 362 studies) on heart failure triggered by environmental pollutants.

Interpretation:
These AOPs are set to form the foundation for an IATA addressing organ toxicity, including cardiotoxicity, aiming to reduce the reliance on animal testing.

Abstract Number: 240

Poster Number: 25

REDUCING THE USE OF ANIMALS IN DRUG DEVELOPMENT: AN EXAMPLE OF MISSED REGULATORY OPPORTUNITIES WHILE WORKING TOWARDS AN ORPHAN DRUG FOR TGASE-1 DEFICIENT ICHTHYOSIS PATIENTS.

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Background:
Various excellent 3D full-thickness skin models mimicking monogenic skin conditions have been described in the past years, for instance for congenital ichthyosis and epidermolysis bullosa. Lately we have been able to show that extensively characterised and validated disease-specific skin models are an excellent platform for individualised drug testing when evaluating the effects of protein replacement methods for Tgase-1 deficient ichthyosis patients.

Methods:
We routinely isolate dermal and epidermal cells from patient and control donors skin tissue; subsequently cells are cultured on feeder cells or feeder free, as required and then employed for the generation of 3D full-thickness skin models (patented). Models are subjected to topical protein replacement treatment followed by functional, histological, expression, genomic and toxicological analyses.

Findings:
Our 3D full-thickness skin models can mimic diseased and healthy skin. Nanoparticle (nanogel) mediated topical protein replacement therapy was shown to successfully restore the impaired epidermal barrier in patient 3D skin models and to also restore the Tgase-1 enzyme activity. We could not detect any cell and tissue toxicity. We feel saddened by the requirements to complete animal-based preclinical animal studies, as requested by EMA.

Interpretation:
3D skin models can fully replace animals in drug development and (early-phase) pre-clinical drug testing, thus significantly reducing the use of animals in academic and industrial pharmaceutical research while advancing orphan drug developments for children and infants, an underrepresented patient group in the EU.

INN MOTION: CELL CULTURE 3.0 > 3D BIOPRINTING, MPS & BEYOND

Abstract Number: 108

Poster Number:26

UTILIZING MECHANICAL STIMULATION AS A METHOD TO INVESTIGATE MECHANOTRANSDUCTION IN CHONDROCYTES AFFECTED BY OSTEOARTHRITIS

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Background:
Mechanotransduction is a complex cellular process in which mechanical forces or stimuli are converted into biochemical signals within cells. In osteoarthritis (OA) research, this is of particular interest to understand how mechanical forces and stresses affect chondrocytes and thus contribute to the progression of OA.

Methods:
Cyclic tensile strain was applied in terms of three different intensities by the Flexcell™ tension system. Influence on catabolic parameters such as MMPs, ADAMTS, and IL-6 were assessed by qPCR. Changes in phosphorylation of FAK, STAT3 as well as MAP kinases were verified by western blot analysis. GTPase isoforms associated with MAPK/ERK signaling and integrin regulation were examined for their expression behavior. To translate the results to signal transduction associated with mechanical stimulation integrins, the purinergic receptor P2Y2, and downstream regulators were tested to gain initial insight.

Findings:
Moderate-intensity tensile strain, specifically in the SM/SA profile, demonstrated the highest effectiveness in reducing the production of matrix-degrading enzymes and the expression of IL-6. Additionally, SM/SA stimulation led to a decrease in the phosphorylation levels of FAK and STAT3, with a more pronounced effect observed in chondrocytes affected by OA. When the data was normalized to unstimulated cells, variations in the relative gene expression of P2Y2, Src, H-Ras, and K-Ras were evident.

Interpretation:
The findings from the study of mechanotransduction in chondrocytes contribute to a better understanding of the cell biology of healthy and diseased cartilage cells.

Abstract Number: 114

Poster Number: 27

HEALTHY AND DISEASED BARRIER MODELS FOR PRECLINICAL AND TOXICOLOGICAL TESTING: AN EXAMPLE OF THE GUT AND DISTAL AIRWAYS

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Background:
The drug development process and toxicological testing of molecules and chemicals are highly leaning on animal testing. This is not only time and cost-consuming but also ethically questionable. Therefore, new reliable, and cost-effective alternatives to the animal models are needed.

Methods:
Here, we describe various qualified biomodels developed on the [AX]Barrier-on-chip system. Their preclinical relevance is demonstrated via translation of important parameters such as target identification, pharmacodynamic studies, and vascular leak risk assessment using different readouts.

Findings:
For the gut we used a co-culture model (Caco-2/HT29 cells) with strain considering circadian rhythms to model gut peristalsis. While the proinflammatory cocktail induced decreased barrier functionality (TER, permeability) and increased cytokine release (IL-8), the approved drug Azithromycin showed a protective effect on barrier integrity and inflammation.

For the distal airways we established models based on primary cells and immortalized cell lines. With fit-for-purpose models we showed a protection of overexpressed ECM proteins upon Nintedanib treatment on primary-cell derived fibrosis model

(gene and protein level), confirmed safety concerns of Proleukin® on an immune-competent system (increased inflammation, immune cell recurrent, barrier disruption) and investigated the effect of environmental molecules and toxin (CdCl2 and LPS) in a co-culture model (immune cell activation, vascular leak, increased toxicity).

Interpretation:
The [AX]Barrier-on-chip models show translational potential and can assist in decision-making within the drug development pipeline while minimizing animal models.

Abstract Number: 151

Poster Number: 28

IMMUNE-MODULATORY EFFECTS ON VESSEL BARRIER FUNCTION AND METASTASIS IN 3D BIOPRINTED, VASCULARIZED NEUROBLASTOMA-ON-CHIP MODEL

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Background:
The progression and metastatic potential of neuroblastoma relies on shaping the tumor environment and stimulating vascularization. Understanding how immune cells affect vessel barriers and migration during metastasis is a vital clinical question, challenging to investigate using conventional 2D cell cultures or tissue samples.

Methods:
We developed a method to 3D-bioprint vessel-containing tissues with tumor spheroids into fluidic chips to study cancer metastasis in multi-organ-on-chip devices. In the neuroblastoma-on-chip model we will investigate the impact of macrophages, dendritic cells, and cytokines on vessel permeability and tumor cell migration. This approach employs iPSC differentiation, confocal live cell fluorescence imaging, flow cytometry, and RNA sequencing of metastasizing cells.

Findings:
Neuroblastoma tumor will be integrated into a multi-cell type vascularized connective tissue equivalent and connected to bioprinted liver and kidney tissue surrogates. Influence of macrophages on tumor migration and the impact of anti-angiogenic medications on metastasis will be explored.

Interpretation:
The project will develop a novel 3D-bioprinted, perfused "tumor metastasis-on-chip" model to study vessel barrier function during metastasis-associated intra- and extravasation processes and how immune cells modulate different phases of metastasis, thus providing a platform for drug screening and personalized in vitro drug testing in precision medicine approaches.

Abstract Number: 152

Poster Number: 29

DEVELOPMENT OF AN IMMUNOCOMPETENT 3D-BIOPRINTED SKIN-ON-CHIP MODEL

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Background:
Due to the complexity of the skin, the development of in vitro models to study skin biology is challenging. Commonly used human skin equivalents consist of collagen with fibroblasts topped with epidermal layers composed of keratinocytes. We are interested in developing a 3D-bioprinted skin-on-chip model that will be complemented with immune cells. For a start we tested how dendritic cells (DC) can be incorporated into the dermal compartment.

Methods:
For this purpose, we embedded monocytes or monocyte-derived DC (moDC) together with fibroblasts into a gelatin-methacrylate mix that upon blue light exposure polymerizes forming stable hydrogels. With flow cytometry analysis we investigated viability, differentiation and maturation of DC in hydrogels.

Findings:
We observed that moDC stayed viable in hydrogels and displayed an immature phenotype when analyzed by flow cytometry. Moreover, these moDC could be activated within hydrogels by addition of a maturation cocktail consisting of TNF-alpha, IL-1beta, prostaglandin E2 and IL-6. When CD14+ monocytes were embedded into hydrogels and differentiated by GM-CSF and IL-4, they upregulated HLA-DR and downregulated expression of CD14.

Interpretation:
These preliminary results suggest that in hydrogel embedded moDC or monocytes are viable and can be further differentiated in hydrogels. In the next step we will now implement these cells into our 3D-bioprinted skin-on-chip model to establish an immunocompetent skin model. In future we hope to use the immunocompetent human skin model for drug testing and vaccine developments.

Abstract Number: 154

Poster Number: 30

3D BIOPRINTED TISSUE TO STUDY B-OXIDATION DEFECTS

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Background:
3D bioprinting technologies are revolutionizing tissue engineering, especially in studying metabolic processes using patient-derived cells. Long-chain-3-hydroxy-acyl-CoA-dehydrogenase-deficiency (LCHADD) and Very-long-chain-acyl-CoA-dehydrogenase-deficiency (VLCADD) are rare disorders of the oxidation of long-chain fatty acids (LC-FA). Therapy mainly involves a diet restricted in LC-FA, supplement substitution, and fasting avoidance. Innovative strategies are needed to reduce mortality and improve quality of life.

Methods:
The study examines mitochondrial rearrangement in β-oxidation-defective fibroblasts by exposing them to various rescuers in 2D. Mitochondrial morphology was analyzed using live cell fluorescence microscopy and quantified by counting the number of branches and dots. To mimic tissue physiology, a 3D-bioprinted, vascularized tissue model with healthy and patient-derived fibroblasts was developed.

Findings:
Analysis of mitochondrial morphology in patient fibroblasts revealed significant alterations of mitochondrial morphology, reduced oxidative phosphorylation, but increased glycolysis and significantly increased intracellular ROS levels caused by NOX2. NOX2 inhibitors and other rescuers led to mitochondrial network refusion. 3D-bioprinted tissue surrogates showed impaired vessel formation in the presence of patient fibroblasts.

Interpretation:
Our findings will improve the understanding and therapy of LCHADD/VLCADD, as there are no direct therapies for elevated ROS levels and mitochondrial dysfunction. 3D-bioprinted patient tissue models will be used for testing novel treatment modalities and tissue response during physiologic stress.

Abstract Number: 166

Poster Number: 31

HOSEC MODEL OUTSIDE THE INCUBATOR

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Background:
The ex vivo skin model hOSEC (human organotypic skin explant culture) maintains resident immune

cells and the structure of the skin. It provides information on the immunogenic response to an agent. A potential application is studying the anti-inflammatory effect of a treatment by analyzing the suppression of the release of inflammatory cytokines. Another application is the prediction of the sensitizing potential by the migration of Langerhans cells, which can be analyzed by IHC.

Methods:
Foreskin obtained from circumcisions of healthy adolescent donors with ethical approval are punched, placed in transwells, which are limited by a confinement ring, and cultured with a defined cultivation medium without serum or antibiotics. The created medium allows cultivation at room temperature without CO_2 supply.

Findings:
Cultivation for the specific conditions was verified for up to four days. Tissue integrity was assessed by HE staining analysis. Viability/Proliferation was monitored by LDH release, proliferation marker ki-67, and staining of caspase-3. A steady increase in IL-8 release over the days in culture indicated an active system. IHC staining for different cell types of the immune system was performed and showed that immune cells remain.

Interpretation:
The hOSEC model was cultivated outside the incubator at room temperature. This cultivation provides more flexibility for applications of testing effects of different conditions on native skin.

Abstract Number: 182

Poster Number: 32

STUDYING SIGNAL REWIRING IN DC SUBSETS IN 3D BIOPRINTED SKIN CANCER-ON-CHIP

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Background:
Research in tumor immunology heavily relies on mouse cancer models, however translation of these findings into the patient situation is difficult. Therefore, there is an urgent need to develop skin cancer models that reflect most closely the human situation. High content microscopy of tumor patients samples give only a snapshot of the tumor-immune cell interactions, 3D bioprinted skin cancer models would allow longitudinal and more detailed investigations of cellular interactions in cancer tissue.

Methods:
Disease-on-chip model for melanoma skin cancer and cutaneous metastases will be developed. For this purpose, they will seed tumor cell lines from human melanoma into the epidermal layers between keratinocytes of 3D bioprinted skin. By introducing LC and dermal DC into the epidermis and dermis, respectively, we will investigate the interactions between these immune cells and tumor cells by high content confocal live cell imaging. Subsequently, we will also try to use skin cancer organoids/spheroids generated from patient material for implementation into the 3D bioprinted skin model. Phenotypical changes on the protein level will be revealed by multi-color flow cytometry. Functional consequences can be tested by cytokine measurements (Bioplex assays) and DC-T cell cocultures ex vivo. Moreover, autologous T cells can be introduced into the skin cancer on-a-chip model to study by live cell imaging how tumor growth affects DC-T cell interactions in situ in tumor tissue

Findings:
With the expected results, the tumor immunology field will gain novel insights into interactions between skin DC subsets and tumor cells that will ultimately lead to the development of novel immunotherapeutical strategies.

Abstract Number: 215

Poster Number: 33

DIGITAL LIGHT PROCESSING OF GELATIN DERIVATIVES: TOWARDS NOVEL SMALL INTESTINAL IN VITRO MODELS

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Background:
In vitro models can provide a fast and inexpensive alternative for in vivo studies to perform preclinical or fundamental research. In this study, we aim to improve the physiology of intestinal in vitro models by the development of constructs that mirror the villi and crypts of the digestive tract and exhibit physiological stiffness.

Methods:
Gelatin-methacryloyl-aminoethyl-methacrylate (gel-MA-AEMA)- and gelatin-methacryloyl-norbornene (gel-MA-NB)-based biomaterial inks were developed to fabricate 3D constructs, mimicking villi or a combination of villi and crypts, with digital light processing (DLP). To assess biocompatibility of the constructs, a Caco-2/HT29-MTX co-culture in a 9:1 ratio was maintained for 21 days.

Findings:
Both hydrogels exhibited physiologically relevant stiffness, but only the gel-MA-AEMA based biomaterial ink could be successfully utilized for printing constructs with villi and crypts. On all construct designs, cell confluency was reached and paracellular permeability of small sized marker molecules in combination with TEER measurements suggested the formation of a functional barrier over time. The gene expression of enterocyte differentiation markers suggested the superior differentiation of Caco-2 cells on the 'villi' and 'villi and crypts' constructs compared to flat hydrogels, collagen type I coating or uncoated tissue culture plastic.

Interpretation:
Although both hydrogels promoted functional barrier formation and enterocyte differentiation, gel-MA-AEMA was more suited for DLP than gel-MA-NB. In addition, culturing intestinal epithelial cells on the 3D villi-like constructs ameliorated cell differentiation compared to conventional 2D setups.

Abstract Number: 219

Poster Number: 34

A HUMAN INTESTINAL MODEL TRILOGY: EXPLORING BARRIER INTEGRITY THROUGH BACTERIA INSPIRED NANOPARTICLES

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Background:
It is imperative to achieve an in-depth understanding of *in vitro* models when considering replacement of animal testing. Taking biophysical properties into account, we present the application of three human intestinal cell models to modulate the cell membrane and barrier integrity. To this aim, SiO_2-based rod-shaped nanoparticles (NPs) were employed to simulate bacteria in conjunction with the mycotoxin fumonisin B_1 (FB_1) and palmitic acid (PA) which can naturally co-occur in the intestine through dietary consumption.

Methods:
Membrane fluidity was determined in HCEC-1CT and HCT116 cells using pyrene labelled dodecanoic acid (PDA). Intercellular distances (*i.e.*, barrier integrity) were assessed in a Caco2/HT29-MTX-E12 co-culture model using images obtained with the Lionheart FX at 20x magnification.

Findings:
PA significantly lowered membrane fluidity in both cell lines whereas FB_1 counteracted this effect. In

the co-culture model, the incubation with NPs in controls significantly reduced the intercellular distance after mucus removal. When treating the cells with PA and FB_1 in advance, intercellular distances increased significantly upon incubation with NPs. These effects were not observable in the presence of mucus.

Interpretation:
Models implemented for experimental purposes need to be refined to mimic *in vivo* conditions. Fittingly, the bacterial simulation experiment demonstrates the importance of mucosal lining of the gut, which is featured in the human Caco2/HT29-MTX-E12 co-culture model. Furthermore, even in a non-toxic concentration range both PA and FB_1 modulate the plasma membrane of intestinal cells, underlining the importance of elucidating biophysical properties of model application.

Abstract Number: 237

Poster Number: 35

GENERATION OF A SODIUM ALGINATE SCAFFOLD TO BE USED AS AN EXTRACELLULAR MATRIX FOR AN ANIMAL-FREE 3D SKIN MODEL

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Background:
This project involved pioneering work to replace animal origin materials with animal-free supplements, for skin modelling. Classic skin models are generated by using bovine collagen I as an extracellular matrix. In this project, a sodium alginate scaffold replaced collagen I. A sodium alginate bilayer scaffold consists of a sponge layer designed to support growth and proliferation of dermal fibroblasts and a film layer theorised to function as a basement memebrane for epidermal kertinocytes. The sodium alginate matrix was ionically cross-linked with Ba^{2+}.

Methods:
The methods of this study were the generation of a sodium alginate matrix, composed of a sponge layer and a film layer. Production of the sodium alginate matrixes was enabled through a process of freezing and vacuum drying. The matrix was cross-linked with $BaNO_3$. Multiple cross-section of the matrix was imaged using an JSM-IT700HR InTouchScope™ Scanning electron microscope (JOEL).

Findings:
Scanning electron microscope (SEM) imaging of the sodium alginate dermal matrix showed the formation of a small number of pores in the matrix in the sponge layer. These pores were analysed to identify the pore length.

Interpretation:
This sodium alginate matrix allows for an animal-free 3D skin model. In the future, this could direct industries and researchers to use a sodium algiante matrix leading to an animal free 3D skin model for drug and cosmetic testing and promoting animal-free research on a large scale.

INN MOTION: OPPORTUNITIES & CHALLENGES OF PATIENT-DERIVED MODELS

Abstract Number: 148

Poster Number: 36

IFN-I SIGNALING IN TUMOR ASSOCIATED ENDOTHELIAL CELLS (TECS) IN NSCLC

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Background:
Endothelial cells (ECs) in the tumor microenvironment are not only essential for cancer growth but as well execute distinct pathological functions that promote tumor-progression and immune evasion. Unravelling the function of TECs in NSCLC may help to understand underlying processes, driving the referred pathogenesis. In a 3R-setting, our setup is designed to use as few animal-related products as possible while receiving highly translational data from patient-derived material essential for targeted findings in oncology.

Methods:
Primary EC-culture from NSCLC patient-derived healthy and malignant tissues were generated and used for in-depth characterization of TEC-features by bulk RNA sequencing, molecular- and functional assays. Large-scale experiments were performed using commercially available ECs. Prognostic relevance, differential gene regulation and pathway activation were investigated using bulk NSCLC datasets. In parallel, single-cell data of 182 patients and 158 controls will be used to further confirm and elaborate obtained *in vitro* and *ex vivo* findings.

Findings:
Our analyses link a pathological TEC-phenotype with upregulation of IFN-I mediated innate immune response and identified a novel marker involved in this pathway, IFI27. TECs show enhanced migration, permeability and increased IFN-I induction-potential compared to normal lung ECs. In clinical data we link IFI27 plus IFN-I activation with worse patient survival. Our data suggest that activation of IFN-I, especially of TLR3 in ECs, is directly involved in inducing a tumor-promoting phenotype.

Interpretation:
Here, we propose for the first time, an association of TEC-driven pathogenesis with the induction of IFN-I signaling in NSCLC.

Abstract Number: 160

Poster Number: 37

PATIENT-DERIVED CULTURE MODELS IN HEAD AND NECK CANCER

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Background:
Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous tumor, where the utility of the cell-lines based research is limited. In order to achieve personalized therapy, reproducible patients-derived tissue culture models are required.

Methods:
During pan-endoscopy tissue biopsies were achieved and used for compresstome sectioned slice cultures, spheroid cultures and two dimensional cultures. Slice cultures were treated with immune stimulation. The culture medium was used for immune dot blot detection of Granzyme B, and the cultured tissue was evaluated immunohistochemically. Spheroid cultures were achieved on Sarstedt BioFloat plates, and evaluated by flow cytometry. Adherent two dimensional cultures were passaged, cryopreserved and reactivated.

Findings:
In slice cultures the tumor (immune) microenvironment, tumor cells and tissue architecture were optimally represented. We stimulated the local immune system and detected the activation of Granzyme B in tissue and in culture medium. In spheroid cultures three-dimensional structure was achieved. Tumor cells were well preserved, CD8+ lymphocytes were lost from day 4. In adherent cultures cancer cell nests and carcinoma-associated fibroblasts were present. After passage, fibroblasts remained, nevertheless, by mRNA and protein analysis both tumor-epithelial and mesenchymal markers were detected.

Interpretation:
Patients-derived cultures allow personalized research and therapy of head and cancer.

Abstract Number: 197

Poster Number: 38

MODELING ULTRA-RARE SARCOMA IN VITRO

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Background:
Sarcomas are a group of solid tumors, which represent only 1% of all malignancies and encompass over 70 subtypes. A main obstacle for drug development and preclinical trials portrays the lack of suitable *in vitro* models due to the rare incidence of each subtype. To elucidate, the largest cell culture collection in the world, the American Type Culture Collection (ATCC), offers approximately 50 human sarcoma cell lines with a very limited selection in pathological subtypes. Novel cancer cell lines and normal cells from the same patient are therefore required for improvement of both basic and preclinical research.

Methods:
Cell lines were established from surgical specimens of sarcoma patients utilizing primary cell culture techniques like enzymatic dissociation, differential detachment and subculturing to high passages. Established cell lines underwent characterization through assessments of their morphology, immunocytochemistry, as well as quality control analyses involving short tandem repeat profiling and mycoplasma PCR.

Findings:
Of 31 obtained tissues from various subtypes, 25 formed adherent monolayers after dissociation, of which only six became continuous cell lines. These cell lines include two myxofibrosarcoma, two clear cell sarcoma, one extra skeletal mesenchymal chondrosarcoma and one *CIC::DUX4* sarcoma, the latter three being depicted as ultra-rare. Cancer associated and/or dermal skin fibroblasts were isolated, whenever possible, to create complex cell culture models.

Interpretation:
These new cell culture models are valuable contributions to the limited pool of sarcoma cell lines allowing functional assessment of potential molecular targets as well as deciphering of disease mechanisms.

Abstract Number: 203

Poster Number: 39

PATIENT-DERIVED 3D CO-CULTURE MODELS FOR GASTROINTESTINAL CANCERS

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Background:
In pancreatic ductal adenocarcinoma (PDAC) and cholangiocarcinoma (CCA), signaling from stromal cells is implicated in metastatic progression. Approximately 80% of their tumor mass consist of non-tumor components, predominantly cancer-associated fibroblasts (CAFs). This so-called tumor microenvironment (TME) and its molecular interactions with tumor cells impact tumor development and drug resistance. Cell lines alone therefore do not recapitulate the in vivo situation, usually requiring animal experiments even for basic research. Patient-derived cell cultures harbor all cells normally found in tissues. By generating tumor cell lines and CAFs, optimal co-cultures can be produced and donor-related differences avoided.

Methods:
Tumor tissue was enzymatically and mechanically dissociated yielding in primary cultures. CAFs were separated from epithelial tumor cells by differential trypsinization and immortalized for long term used by lentiviral transduction of the hTERT gene. Resulting tumor and CAF cell lines were characterized and validated using the same diagnostic tools as for the patient.

Findings:
We successfully isolated the epithelial tumor cells from tumor tissue and established two continuous cell lines derived from one PDAC and one CCA patient. In addition to the tumor cells we were able to isolate the stromal component out of the same tissue, yielding in matched tumor-CAF pairs of the same donor.

Interpretation:
These innovative *in vitro* models and the concept of matching cells from one donor allows comprehensive research ranging from drug screenings, cell-to-cell communication to disease mechanisms and provides a more feasible approach for translating basic research into clinical studies.

Abstract Number: 208

Poster Number: 40

MUG LUCIFER - AN AUTOLOGOUS CELL MODEL ENABLES TRANSLATIONAL RESEARCH

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Background:
Translocation-related sarcomas (TRS) represent a particular sarcoma subtype arising from specific gene fusions and often lack genomic aberrations besides the characteristic translocation. Therefore, the role of epigenetic alterations in tumorigenesis and metastasis in TRS is being investigated to identify urgently needed new therapeutic strategies.

Methods:
Our recently established patient-derived TRS model MUG Lucifer consisting of primary and metastatic tumor cells and skin fibroblasts from the same patient, presents significant genotypic and epigenetic characteristics and enables translational research. Nuclear magnetic resonance (NMR) spectroscopy was employed to investigate arginine methylation (ArgMet) in MUG Lucifer cells as part of the examination of epigenetic characteristics. Based on these results, antitumor effects of selected inhibitors were elicited in using cell viability and apoptosis assays.

Findings:
NMR spectroscopy showed elevated ArgMet levels indicating enhanced PRMT activity in MUG Lucifer tumor cells. The PRMT inhibitors used showed significant influences on ArgMet levels and the viability of MUG Lucifer cancer cells. The fibroblasts were not affected with the IC50 used, further supporting their potential as promising treatment options in the combat against CCS.

Interpretation:
The results indicate that PRMT inhibitors could be an appropriate novel treatment option for TRS patients with elevated ArgMet levels, substantiating the importance of tailored therapies in the advanced metastatic stage of TRS. MUG Lucifer represents an ideal *in vitro* model to explore tumor biology, disease progression and the metastatic process without the use of excessive animal experiments.

Abstract Number: 223

Poster Number: 41

PATIENT-DERIVED HEAD AND NECK TUMOR SLICE CULTURES – A VERSATILE TOOL TO STUDY ONCOLYTIC VIRUS ACTION

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Background:
Preclinical data is mostly dependent on cell-based in vitro systems and syngenic mouse tumor models and translating this to the clinically heterogeneous ecosystem of human tumors is a challenging task. Especially head and neck squamous cell carcinomas (HNSCC) display a complex architecture which makes the prediction of a treatment outcome quite difficult. To bridge this gap we have established a patient-derived HNSCC slice culturing system to assess permissivity and oncolytic virus (OV) action.

Methods:
HNSCC biopsies were sectioned using a vibratome and cultured for 48h. Tumor content and viability of the cultured slices was assessed by pathologists and the tumor microenvironment was characterized by immunofluorescence staining's. Presence and activation of T-cells upon stimulation was analyzed by flow cytometry and measuring IFNγ secretion. Permissivity of an oncolytic virus (VSV-GP) in these HNSCC slices was tested using a GFP-tagged OV. Co-immunofluorescence staining's were performed to analyze which cell populations could be infected by the OV.

Findings:
The complex morphology of a human tumor could be retained in these slice cultures including the preservation of cell types like tumor cells, immune cells and Cancer associated fibroblasts. Upon stimulation the cytotoxic T-cells showed functionality and could be activated. In addition, more than half of the HNSCC slice cultures were permissive to VSV-GP and the virus could infect a broad spectrum of tumor associated lineages including epithelial and stromal cells.

Interpretation:
This human tumor ex vivo platform might complement pre-clinical studies to eventually propel cancer immune-related drug discovery and ease the translation to the clinics.

Abstract Number: 224

Poster Number: 42

CONSIDERATION OF SEX AS A BIOLOGICAL VARIABLE IN STEM CELL-BASED ORIGINAL RESEARCH PUBLICATIONS

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Background:
Sex matters - not only in clinical studies, but also in preclinical research. Stem cell-based research offers new possibilities to include sex as a biological variable without resorting to animal experimentation. The SAGER guidelines provide scientists with a checklist to include sex as a crucial parameter into preclinical experimental design and publications. This study analyzed original research articles in the stem cell research community for reporting the sex of the cell model and adherence to these guidelines.

Methods:
To find out, if the stem cell research community is aware of the chances to use their models to study sex differences, a systematic analysis of 200 consecutively published articles in 4 high impact scientific journals in the stem cell field was conducted comprising articles from 2020 and 2021.

Findings:
It was retrieved that all 4 journals with speciality in stem cell research required, directly and/or indirectly through publishers, that the sex of the cells should be named. Of the 200 articles analyzed, the sex of the stem cells used was not disclosed in 64 % of the publications. The SAGER guidelines were not followed in 99 % of the articles. Furthermore, a male bias was clearly visible. Comprehensible explanations for not mentioning sex or using male cells only were not detected in any of the articles.

Interpretation:
Despite existing requirements of journals, inclusion of sex as a biological variable is still largely ignored in preclinical studies. Improvement in the field of cell-based research has not been visible in recent years. The results collected suggest that significantly more effective steps need to be taken to implement sex and gender in biomedical research.

Abstract Number: 228

Poster Number: 43

HUMAN SEX SPECIFIC IPSC-DERIVED PROXIMAL TUBULAR LIKE EPITHELIAL MODEL TO STUDY PERFUSATE VARIABLES IN MACHINE PERFUSION OF KIDNEYS IN VITRO

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Background:
Machine perfusion is a technique used in kidney transplantation as improved outcome alternative to static cold storage. The process involves ex situ circulation of a specially formulated preservation solution through the organ's vascular system. A sex specific human induced pluripotent stem cell (iPSC) -derived proximal tubular cell-based model was developed to study the mechanistic basics of influencing perfusion variables such as temperature (normothermia versus hypothermia) and perfusate composition.

Methods:
Female and male human iPSC cells were differentiated into proximal tubular like cells (iPSC-PTLs) with and without the addition of oestrogen and testosterone. Differentiation was assessed by studying morphological development and expression of stem cell and differentiation markers.

Findings:
The differentiated cultures showed characteristic cell morphology and dome formation consistent with proximal tubular epithelial characteristics. The gene expression level of the stem cell marker Oct4 showed a steady decrease during the first days of the differentiation process. The differentiated cells were shown to express megalin, Cldn2 and ACE2, all gene products known to be present in proximal tubular cells with no influence of the cells' sex or sex hormones.

Interpretation:
Male and female iPSC-PTLs were equivalent according to a set of differentiation markers. Sex hormone treatment did not influence differentiation. Therefore, these human sex specific iPSC-PTLs will be used as models to study mechanisms and sex specific effects of modulated perfusion variables in ex situ kidney machine perfusion to improve organ transplantation outcomes including organ conditioning and targeted treatment.

INN MOTION: DISEASE MODELING AND ADVANCED LAB-ON-CHIP SYSTEMS

Abstract Number: 116

Poster Number: 44

AN INNOVATIVE HUMAN IPSC-BASED CO-CULTURE MODEL FOR IN VITRO STUDIES OF NEURO-CARDIAC INTERACTIONS

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Background:
The cardiac autonomic nervous system maintains the cardiac homeostasis and alterations of it can be involved in cardiac disorders. However, these mechanisms remain poorly understood due to the lack of proper human cell models.

Methods:
A neurocardiac co-culture model was created using exclusively cells derived from human induced pluripotent stem cells (iPSCs) in a 2 chambers silicon insert.

Findings:
iPSC-cardiomyocytes (CMs) and iPSC-sympathetic neurons (SNs) were co-cultured in two separate chambers and, after insert removal, iPSC-SNs formed axons projecting towards the CMs, and visualized with immunostaining. The beat rate of iPSC-CMs was measured using the MEA system and was stable after 7 days of co-culture. A significant increase in the beat rate of iPSC-CMs was observed after nicotine treatment that had no effect on iPSC-CMs in monoculture. On the contrary, after treatment with α-bungarotoxin, which bind to nicotinic receptors blocking neural transmission, the beat rate of iPSC-CMs in co-culture was unaffected. iPSC-derived CMs responded positively to isoproterenol treatment with a significant increase of the beat rate and the subsequent propranolol administration decreased the beat activity of the cells. After 7 days of co-culture a significant decrease of the number of vesicles stained with FFN270 was observed in nicotine treated cells in comparison to the co-culture at baseline resulting in an effective release of the neurotransmitters, showing the functional exocytosis process.

Interpretation:
The proposed neurocardiac model recapitulates the neuro-cardiac axis and provides a promising modelling tool for the study of molecular mechanisms involved in the in health and disease. Funding: ITAT1047

Abstract Number: 117

Poster Number: 45

IN VITRO INHALATION AEROSOL EXPOSURE SYSTEM TO MIMIC HUMAN BREATHING

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Background:
Persistent injury to the alveolar epithelium induced by inhaled irritants is a hallmark event in the pathogenesis of chronic obstructive pulmonary disease. Animal inhalation studies present species-specific variations and makes it difficult to draw conclusions in human. Hence, there is an urgent need to develop “new approach methodologies” which are able to reproduce the alveolar barrier, breathing dynamics and inhalation mechanism.

Methods:
Here, we have employed the novel Cloud α AX12 platform to mimic realistic inhalation exposure in the distal lung. A triple co-culture system was established on-chip at the air-liquid interface (ALI) with breathing-like (BR) stretch conditions. We exposed the alveolar barrier to toxic doses of aerosolized nanoparticles (NPs; ZnO, TiO2), a well characterized toxic chemical (PHMG) and an inhaled corticosteroid (FL).

Findings:
ZnO and TiO2 NPs under ALI+BR conditions incited a pro-inflammatory cascade leading to disrupted alveolar barrier (TEER decreased ~3 fold), increased cytotoxicity (~2.5 fold) and increased pro-inflammatory gene expression. Additionally, exposure to aerosolized PHMG resulted in significant cytotoxic effects, including barrier breakdown , epithelial-mesenchymal transition, and elevated gene expression of inflammation-associated cytokines. Furthermore, our results showed that nebulized FL effectively alleviated the toxic effects of PHMG, including EMT and inflammation.

Interpretation:
Our results strongly support the use of the Cloud α AX12 platform as an alternative to animal models in inhalation toxicity and drug efficacy testing, particularly in pre-clinical and precision medicine studies.

Abstract Number: 125

Poster Number: 46

FREE FLOATING RAT TAIL TENDON FASCICLES: AN EX-VIVO MODEL OF TENDINOPATHY

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Background:
Tendinopathy is a painful musculoskeletal disease potentially leading to tendon rupture. The cause is poorly understood, but both mechanical overload as well as chronic immobilization are known risk factors. Animal models used for studying this disease involve forced treadmill running as well as injections of collagenase. Rat tail fascicles are similar to other tendons and can be isolated from cadaver material, allowing ex vivo tissue culture of tendon cells within their matrix. Here we show the pathologic cellular response to unloaded ex vivo culture, in regard to tendinopathy associated hallmarks.

Methods:
Rat tail fascicles isolated from male SD-rats were cultured for 5 days free floating and subsequently analyzed by immunohistochemistry, qRT-PCR and western blot analysis regarding the expression of markers associated with tendinopathy. Also structural changes were examined

Findings:
Rat tail fascicle tendon cells respond to ex vivo culture by upregulation of inflammatory markers such as IL1, IL6, TNF-alpha and of tissue degrading factors such as various matrix metalloproteinases. Also pain- and angiogenesis associated factors like COX2 and VEGF are upregulated. There is a significant increase in cell number and a loss of nuclear orientation and roundness.

Interpretation:
The observed inflammatory response accompanied by fibrosis and pain associated events together with cellular hyperplasia very much resembles the pathology described in tendinopathy. Considering the high yield of fascicles from one rat tail, this seems a suitable, animal saving ex vivo model for studying basic mechanisms of this common and yet poorly understood disease.

Abstract Number: 130

Poster Number: 47

CONNECT PROJECT OVERVIEW: COMPARING DYNAMICS OF EMERGING AND EXISTING RESPIRATORY VIRAL CHALLENGES WITHIN IMMUNE-COMPETENT LUNG-ON-CHIP MODELS

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Background:
Dendritic cells (DCs) represent the bridge between the innate and the adaptive immune system and reside at sites with a high chance of encountering pathogens, such as the skin or the mucosa. The aim

of this project is to include immune cell types such as T and B cells to the already established human respiratory 3D model and to investigate differences in the activation, migration as well as the inflammatory mechanisms and downstream signaling of these cells upon viral infections.

Methods:
Normal human bronchial epithelial cells (NHBE) are used in air-liquid interphase (ALI) system and human DCs are added from the basolateral side of the Transwell. In addition, autologous lymphocytes are included in the system and viremia, inflammation, tissue damage and lymphocytes activation will be studied upon infection with SARS-CoV-2 and Influenza.

Findings:
Studying the dynamics of infectious diseases within a complex 3D lung tissue model in co-culture with DC will allow to compare the effects of different viruses on DC, and allows to analyze the interactions of immune cells with infected epithelial cells.

Interpretation:
A better understanding of the similarities and differences in the early steps of viral entry and the interaction of the immune system with both the pathogen and the respiratory tissues will improve our understanding of the infection process and may open new ways to prevent or reduce respiratory infections.

Abstract Number: 132

Poster Number: 48

CHARACTERIZATION OF DC ACTIVATION FROM INFECTED RESPIRATORY TISSUE

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Background:
Dendritic cells (DCs) represent the bridge between the innate and the adaptive immune system. They reside in the periphery as immature cells specialized for pathogen recognition and antigen uptake. Upon pathogen detection, they take up and process antigens, which leads to cell activation and migration to the draining lymph nodes, where they present the antigen to naïve lymphocytes. In this work, the activation and migration of DC incorporated in a respiratory tissue model after SARS-CoV2 infection was analyzed.

Methods:
Normal human bronchial epithelial cells (NHBE) are used in air-liquid interphase (ALI) system and human DCs are added from the basolateral side of the Transwell, to allow the DC to adhere to and migrate through the Transwell, then the tissue was infected with SARS-CoV2. DC activation was analyzed by FACS. NHBE health by TEER measurement and nucleus count after 72h

Findings:
Preliminary data from the still ongoing experiments indicated that a higher number of activated DCs migrate out of the respiratory tissue after viral infection compared to the uninfected controls. These cell demonstrated elevated expression of maturation, co-stimulatory and migration markers, CD83, CD86 and CD197 respectively. As previously shown, SARS-CoV-2 infection leads to severe tissue damage of the respiratory model, which was not seen by sole addition of DCs to the respiratory system.

Interpretation:
Here we show that human DCs added to this respiratory model did not damage the respiratory model, that DCs migrated out of the tissue, and that these DCs were more activated in infected samples compared to controls.

Abstract Number: 140

Poster Number: 49

A NEW PERSPECTIVE ON CELLULAR COMPLEMENT AND HOW OPSONIZED HIV-1 ENHANCES DENDRITIC CELL SURVIVAL AND MATURATION VIA MCL-1 STABILIZATION REGULATED BY ANAPHYLATOXIN RECEPTORS

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Background:
The complement (C) system is one of the oldest components of innate immunity. It has long been thought that the only functions of this system are the recruitment of other immune cells and the recognition/destruction of invading pathogens. However, studies in recent years have shed new light on the multiple roles of C in immune regulation and crosstalk with other cellular effector systems. Recent evidence suggests that complement has additional functions within the immune system than just pathogen recognition and immune recruitment.

Methods:
Monocyte-derived dendritic cells (moDCs) were infected with HIV or complement-opsonised HIV (HIV-C) in the presence/absence of specific inhibitors for 24h. We measured expression levels of complement factors and cathepsins, intra- and extracellular anaphylatoxin generation, regulation of anti-apoptotic Bcl-2 family members as well as cell stress, maturation and survival.

Findings:
We could show that HIV-C promotes elevated anaphylatoxin production, maturation and increased survival by stabilizing the anti-apoptotic Bcl-2 family member Mcl-1, via phosphorylation at threonine 163. This effect is regulated by the anaphylatoxin C5a and the interaction with its receptor (C5aR), as upon inhibition, this stabilization was impeded. Upstream pathways controlling this specific phosphorylation event are dependent on MAP kinase and to a lesser extent protein kinase C, since inhibition of either way resulted in reduced Mcl-1 stabilization.

Interpretation:
We demonstrated a complement-dependent mechanism that caused improved DC survival and maturation, which could represent a novel approach for enhanced antigen presentation and protective immunity against viral infections.

Abstract Number: 143

Poster Number: 50

INVESTIGATION OF HUMORAL AND CELLULAR IMMUNE RESPONSE AGAINST SARS-COV-2 BA.1 AND BA.2 OF TWICE-VACCINATED AS WELL AS BOOSTED INDIVIDUALS

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Background:
Due to novel emerged SARS-CoV-2 sublineages, vaccine-induced immunity has to be re-evaluated to determine potential loss of protection, which is still critical for individuals with immune suppression or relevant pre-existing illnesses.

Methods:
Here, we investigated SARS-CoV-2 RBD IgG levels, neutralization against wild type (WT), Delta, Omicron and specific T cell activation of vaccinated individuals 1 and 6 months after 2 doses, but also 1 month after booster vaccine or vaccination and COVID-19 recovery.

Findings:
Our studies demonstrated that antibodies wane over time and neutralization against variants is very heterogenic depending on the type of vaccine or previous infection. After booster immunization or infection, a substantial increase of IgG antibodies and neutralization was observed. Vaccinated and convalescent demonstrated the highest neutralization except against BA.1, which was neutralized best by 2x ChAdOx1 vaccinated individuals boosted with BNT162b2. Longer lasting T cell immunity was detected for convalescent patients and fully ChAdOx1 vaccinated individuals. After third immunization, similar numbers specific T cells were found for all groups.

Interpretation:
Overall, our data support the recommendation of booster vaccines due to weak protection against certain variants as well as lower T cell immunity induced only after two vaccine doses. Our findings also highlight that heterologous vaccination with ChAdOx1 and BNT162b2 booster vaccine provides effective serum neutralization against omicron variants. Moreover, these studies illustrate the importance of using human material testing humoral and cellular immune components rather than in using genetically manipulated animal models.

Abstract Number: 145

Poster Number: 51

HPL AS SUBSTITUTE TO FCS TO CULTURE PRIMARY MONOCYTE-DERIVED CELLS?

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Background:
Given the recent advancements in immunotherapy and vaccination, there is a growing interest in culturing dendritic cell (DC) subsets in an FCS (fetal calf serum)-free environment for further therapeutic, personalized approaches in the clinics. Still, monocyte-derived DCs cultured in FCS-based media are used as gold standard, but to being as close as possible to clinical use of DCs, also in research settings this component should be replaced by a human alternative. Since safety is essential when applying DC vaccine therapy, use of xeno-free reagents is mandatory to avoid potential risks to infect cells with animal pathogens.

Methods:
Thus, we here optimized conditions for primary DC culture using human platelet lysate (hPL). Such differentiated DCs were directly compared to FCS-generated DCs also in terms of their susceptibility to HIV-1 using flow cytometry, imaging analyses as well as p24 ELISA to monitor productive infection.

Findings:
While FCS-generated DCs illustrated a higher survival rate compared to hPL-differentiated DCs as analyzed by flow cytometry and imaging analyses, higher cell death was observed after infection with HIV-1 or complement-opsonized HIV-1 (HIV-C). Phenotypic characterization of immature DCs (iDCs) and DCs following bacterial (lipopolysaccharide, LPS) or viral (HIV-1, HIV-C) stimulation revealed similarities among differently cultured DCs in regard to expression of characteristic DC markers (e.g. DC-SIGN, HLA-DR, CD80, CD83, CD86).

Interpretation:
Still optimizations are needed to increase the yields of *in vitro*-hPL-generated DCs, nevertheless we here describe a novel protocol to generate functional monocyte-derived DCs in absence of FCS.

Abstract Number: 172

Poster Number: 52

THE ROLE OF METABOLIC IRON CHANGES IN MACROPHAGES UPON SARS-COV-2 INFECTIONS

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Background:
The COVID-19 pandemic has raised questions about the role of iron metabolism in severe cases, linking it to hyperferritinemic syndrome and severe conditions like macrophage activation syndrome and septic shock. Thus, in this study we aimed to investigate changes in macrophage iron metabolism in a human primary system, avoiding animal-derived components where possible. We analyzed key regulators of iron metabolism - TfR, FPN1, Hepcidin, Ferritin, IL-6, IL-1b and IL-10. We examined gene expression, virus neutralization, protein expression, and localization

Methods:
For this, M1 macrophages from healthy donors were exposed to SARS-CoV-2 variants of concern (VoC). After 4–24 hours, we performed neutralization assays and analyzed cytokines and iron-regulating proteins. Virus copy numbers were determined and virus localization, TfR and FPN-1 levels and were illustrated by confocal microscopy.

Findings:
SARS-CoV-2 VoCs exhibited different cytokine and iron-regulating protein profiles. While Omicron sub-variants BA.5 and XBB1.5 showed low levels of inflammatory signal and Iron regulation, the VoC Delta induced both significantly higher and thus exhibited an altered iron regulation.

Interpretation:
Within a primary, human macrophage model, we demonstrated that SARS-CoV-2 variants influence pro- and anti-inflammatory signals, crucial for cellular iron balance. Blocking the transferrin receptor (TfR) reverses this effect, offering therapeutic potential.

Abstract Number: 181

Poster Number: 53

CHARACTERIZING SEX-DEPENDENT DC SENSING MECHANISMS AND IMMUNOMETABOLISM AT THE TISSUE BARRIER DURING SARS-COV-2 INFECTION

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Background:
COVID-19 patients suffered from decreasing levels of CD1c+ DCs and respiratory issues. The mechanisms behind this are largely unknown, but male sex has been identified as a risk factor. Thus, gaining insight into alterations of SARS-CoV-2 infected DCs metabolism via mucosal barrier models is important.

Methods:
Female and male immune-competent respiratory barrier models will be used to study molecular mechanisms, immunometabolism and signal rewiring of DC functions in an in vivo-like setting of primary cells during SARS-CoV-2 infection. The maturation, migration, inflammation and signaling will be analyzed. Microscopic and quantitative analyses for virus/host interactions within complex cell culture models using the Operetta CLS HCS system, TEER measurements, mucociliary clearance evaluation and virus quantification, high content flow cytometry, DC migration assays, Luminex for detailed cytokine analyses, RNAseq and in silico analyses for characterizing signal rewiring in infected mucosal/immune barriers (female, male) will be performed.

Findings:
The effect of SARS-CoV-2 on DC will be investigated with an intact ciliated, mucus-producing epithelium of both sexes. The subsets of DCs within the mucosal barrier upon infection are to be characterized. The changes in mTOR in DCs within mucosal tissue of both sexes and the stimulation/antagonism of complement during SARS-CoV-2 transfer reprogramming in DC function will be determined.

Interpretation:
This project to focus on DCs exposed to SARS-CoV-2 within a native ciliated pseudostratified respiratory barrier is highly innovative and has great potential to impact our understanding of SARS-CoV-2-mediated microenvironmental changes dependent on sex.

Abstract Number: 200

Poster Number: 54

COLDZYME® PROTECTS AIRWAY EPITHELIA FROM INFECTION WITH BA.4/5

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Background:
Vaccines against SARS-CoV-2 protect from critical or severe pathogenesis also against new variants of concern (VOCs) such as BA.4 and BA.5, but immediate interventions to avoid viral transmission and subsequent inflammatory reactions are needed.

Methods:
Here we applied the ColdZyme® medical device mouth spray to fully differentiated, polarized human epithelium cultured at an air-liquid interphase (ALI).

Findings:
We found using VOCs BA.1 and BA.4/5 that this device effectively blocked respiratory tissue infection. While infection with these VOCs resulted in intracellular complement activation, thus enhanced inflammation, and drop of transepithelial resistance, these phenomena were prevented by a single administration of this medical device.

Interpretation:
Thus, ColdZyme® mouth spray significantly shields epithelial integrity, hinders virus infection and blocks in a secondary effect intrinsic complement activation within airway cultures also in terms of the highly contagious VOCs BA.4/5. Crucially, our in vitro data suggest that ColdZyme® mouth spray

may have an impact to protect against SARS-CoV-2 transmission, also in case of the Omicron BA.1, BA.4 and BA.5 variants.

Abstract Number: 204

Poster Number: 55

A NOVEL 3D IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER

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Background:
Blood-brain barrier (BBB) breakdown enables auto-antibodies (-Abs) in many central nervous system (CNS) autoimmune diseases to reach their targets, causing inflammatory tissue damage and neurological deficits. Research is ongoing to understand how these Abs cross the BBB and human tissue culture BBB models could help bridge the gap in translating animal models to humans.

Methods:
Our 3D bioprinted BBB model consists of a hydrogel with astrocytoma cells overexpressing target antigens and pericytes, and a monolayer of endothelial cells. We use a transwell model with the same cellular components for comparison. Methods include human cell culture, 3D bioprinting, immunocytochemistry, permeability assays, flow cytometry, RT-qPCR, ELISA, and hydrogel cryosectioning.

Findings:
We have characterized all utilized cell types regarding cell-specific markers. We also established a protocol for cryosectioning of hydrogels, which facilitates us investigating the cross-section of the model for different markers. Currently, we are optimizing the hydrogel composition are establishing the transwell co-culture model.

Interpretation:
We plan to test various inflammatory conditions by adding different cytokines and immune regulators in order to understand BBB- and CNS tissue damage, and auto-Ab infiltration. These insights could aid in developing of new therapies for autoimmune and neurological diseases by enabling the transfer of therapeutic Abs across endothelial barriers.

Abstract Number: 205

Poster Number: 56

GLYPERA™ EFFECTIVELY SHIELDS AIRWAY EPITHELIA FROM SARS-COV-2 INFECTION AND INFLAMMATORY EVENTS

Viktoria Zaderer[1], Stefanie Dichtl-Zweimüller[2], Wilfried Posch[3], Lukas Alfons Huber[4], Doris Wilflingseder[5]

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Background:
New SARS-CoV-2 variants of concern (VOCs) and waning immunity illustrate that quick and easy-to-use agents are needed to prevent infection.

Methods:
To protect from viral transmission and subsequent inflammatory reactions, we applied GlyperA™, a novel antimicrobial formulation that can be used as mouth gargling solution or as nasal spray, to highly differentiated human airway epithelia prior infection with Omicron VOCs BA.1 and BA.2.

Findings:
This formulation fully protected polarized human epithelium cultured in air–liquid interphase (ALI) from SARS-CoV-2-mediated tissue destruction and infection upon single application up to two days post infection. Moreover, inflammatory reactions induced by the Omicron VOCs were significantly lowered in tissue equivalents either pre-treated with the GlyperA™ solution, or even when added simultaneously.

Interpretation:
Thus, the GlyperA™ formulation significantly shielded epithelial integrity, successfully blocked infection with Omicron and release of viral particles, and decreased intracellular complement C3 activation within human airway epithelial cell cultures. Crucially, our in vitro data imply that GlyperA™ may be a simple tool to prevent from SARS-CoV-2 infection independent on the circulating variant via both, mouth and nose.

Abstract Number: 212

Poster Number: 57

THE ROLE OF ENOXAPARIN IN RESPIRATORY VIRUS INFECTIONS AND ITS THERAPEUTIC IMPLICATIONS

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Background:
It is very likely that new respiratory viruses will emerge in time due to further globalization and high chance of zoonosis of viruses, meaning that we need more general methods to prevent respiratory virus infections. We have proved that infection by SARS-CoV-2 can be prevented by precisely targeting heparin drugs to the respiratory tract. Low molecular weight heparins (LMWH) are known to act as general inhibitors of virus infection, because they prevent virus attachment to cells, a prerequisite to infection.

Methods:
We have developed an animal-free 3D respiratory model to investigate the mechanism and efficacy of LMWH to block Influenza A/B infections. These studies are crucial to translate our findings into a clinical trial with the goal to develop a quick and simple prophylactic intervention against Influenza viruses.

Findings:
Exposure of the 3D epithelial tissue model to Influenza A/B caused tissue integrity destruction, facilitating infection and promoting local complement activation and pro-inflammatory cytokines. Our exciting data show that using LMWH inhibited infection with Influenza A/B as well as reduced the secretion of the pro-inflammatory cytokines, indicating a protective effect against excessive immune response and inflammation.

Interpretation:
Our study illustrated that our primary 3D human respiratory model is relevant to studying Influenza as well as other respiratory viruses or challenges and in studies investigating mechanisms of Influenza infections and immunity, as well as efficacy of potential interventions according to the Three Rs principle–Replacement, Reduction and Refinement-of animal experimentation. Our proposed studies will significantly reduce animal testing.

Abstract Number: 214

Poster Number: 58

THE ROLE OF ENOXAPARIN IN RESPIRATORY VIRUS INFECTIONS AND ITS THERAPEUTIC IMPLICATIONS

Marta Bermejo Jambrina[1], Viktoria Zaderer[2], Julia Eder[3], Killlian Vlaming[4], Teunis Geijtenbeek[4], Doris Wilflingseder[5]

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[5] Medical University of Innsbruck; Institute of Hygiene and Medical Microbiology; Institute of Hygiene and Medical Microbiology

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INN MOTION: ORGANOIDS, SPHEROIDS, IPSCS & BEYOND

Abstract Number: 131

Poster Number: 59

ANIMAL-FREE CULTIVATION OF HUMAN LUNG ORGANOIDS

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Background:
3D cultures of primary human cells like organoids are used in many research fields due to a poor translatability from animal data to humans. These models provide physiologically relevant systems to study a wide variety of research questions. The use of human lung organoids faced an extensive boost during the COVID-19 pandemic. To investigate e.g. respiratory infections of the upper respiratory tract the lung organoids have to be cultivated in a matrix like Geltrex and proximal differentiated to show all characteristics of the upper respiratory tract like ciliated cells.

Methods:
We studied the influence of the matrix and the cultivation on the presence of proximal differentiated characteristic cell types. Therefore, we used the animal-based Geltrex, which consists of matrix proteins purified from murine Engelbreth-Holm-Swarm tumor cells, Vitrogel, which is a Xeno-free tunable hydrogel, and the Clinostar system. The clinostar system is an incubator, which allows cultivation of the organoids under constant rotation and therefore allows a physiological cultivation without the need of a matrix.

Findings:
The use of an animal-free matrix or cultivation system enables an increased presence of proximal-allocated cell types analyzed by immunofluorescence and qRT-PCR compared to the state-of-the-art protocol using the animal-based Geltrex. These results suggest that the solid Geltrex matrix displays a disadvantage in the cultivation of proximal differentiated lung organoids.

Interpretation:
The use of new Xeno-free models allows to cultivate human lung organoids in the absence of animal-derived products. This results in increased expression of proximal cell types in lung organoids.

Abstract Number: 137

Poster Number: 60

P301S AGGREGATED TAU SPREADS TO THE VENTRAL PARTS OF HIPPOCAMPAL ORGANOTYPIC MOUSE BRAIN SLICE CULTURES

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Background:
Tau is a microtubule-associated protein, which can be hyperphosphorylated leading to the formation of neurofibrillary tangles. Misfolded forms of tau can spread from cell to cell and seed aggregation of native tau, which is reminiscent of the pathological spread of prion proteins. However, the details of the general spreading characteristics are incompletely understood. The present study aims to examine the spreading of P301S aggregated tau, a mutation that is implicated in tauopathies, using organotypic slices.

Methods:
Coronal hippocampal organotypic brain slices (170 µm) were prepared from postnatal (day 8–10) C57BL6 wild-type mice. Collagen hydrogels loaded with P301S aggregated tau were applied to slices and the spread of tau was assessed by immunohistochemistry after 8 weeks in culture.

Findings:
Collagen hydrogels prove to be an effective protein delivery system subject to natural degradation in 14 days and release tau proteins up to 8 weeks. Slices with un- and hyperphosphorylated P301S aggregated tau demonstrate significant spreading to the ventral parts of the hippocampal slices compared to empty collagen hydrogels after 8 weeks. The spread of P301S aggregated tau occurs in a time-dependent manner, which was interrupted when the neuroanatomical pathways are lesioned.

Interpretation:
We illustrate that the spreading of tau can be investigated in organotypic slices using collagen hydrogels to achieve a localized application and slow release of tau proteins. P301S aggregated tau significantly spreads to the ventral areas of the slices, suggesting that the disease-relevant aggregated tau form possesses spreading potential. Thus, the results offer a novel experimental approach to investigate tau pathology.

Abstract Number: 141

Poster Number:61

LONG-TERM ORGANOTYPIC BRAIN SLICES CULTURED ON COLLAGEN-BASED MICROCONTACT PRINTS: A PERSPECTIVE FOR A BRAIN-ON-A-CHIP

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Background:
In three-dimensional organotypic brain slices, all complex cellular connections are preserved with high viability, which enables the ex vivo culturing of brain cells for several weeks. This technique markedly reduces the number of animal experiments since multiple slices can be obtained from one brain.

Methods:
Brain slices (postnatal day 8–10, wild type mice, C57BL/6) were connected to microcontact prints to develop a simple brain-on-a-chip model. Using the microcontact printing technique, many peptides or proteins can be printed onto a semipermeable membrane with µm precision.

Findings:
The microcontact prints supported the directed growth of brain-derived nerve fibers, brain vessels and microglia along the lanes of the prints.

Interpretation:
Such a brain-on-a-chip model could make it possible to test new drugs and develop a diagnostic method for neurodegenerative diseases.

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Poster Number: 62

IL-4 SHAPING GLUTAMATERGIC SYNAPSE LIKE STRUCTURES IN IPSC DERIVED NOCICEPTORS

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Background:
The prototypical pleiotropic anti-inflammatory cytokine IL-4 not only acts on immune cells but also has important roles in the nervous system mediating antinociception and neuroregeneration.

Methods:
We explored the expression of IL-4, its receptors IL4ra and IL13ra1 and downstream signaling components together with morphological assays and transcriptomic profiling in human nociceptors differentiating from induced pluripotent stem cells (iDNs).

Findings:
IL-4 induced de novo formation of boutons immunoreactive for vGLUT1 in iDNs which express both, components of the IL-4 receptor complex (IL-4ra and IL-13ra1) and signaling machinery (Jak1,2, STAT5, PKC isoforms, translation factor EiF4E) during differentiation. Pharmacological inhibition of translational and cellular signaling components reduced the synaptogenic effect. Il-4 induced distinct transcriptomic changes in iDNs with 932 significantly up- and 1577 downregulated genes. GO-analysis revealed biological process ontologies for “neuron projection development”, “axonogenesis” and “synapse”, “cellular process involved in reproduction in multicellular organism”, “regulation of membrane potential” and “calcium ion transmembrane transport”.

Interpretation:
The findings partially reflected injury-induced transcriptional changes from mouse nerve injury models contributing to regenerative processes in peripheral nerve but possibly also to reconnecting primary afferent neurons to their projections in the spinal dorsal horn. IL-4 led to massive changes in transcript levels of proteins that are essential for the structure of glutamatergic synapses indicative of a more general role of IL-4 in the control of developing neuronal networks.

Abstract Number: 183

Poster Number: 63

MODELLING HUMAN DENDRITIC CELL AND MONOCYTE FUNCTION IN VITRO.

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Background:
Dendritic cells (DC) serve as critical mediators in the communication between innate and adaptive immune responses. They sense signals from tissue insults, process antigens, and migrate to lymph nodes to activate antigen-specific T-cell responses. Employing *in vitro* models modelling DC biology is essential for dissecting these complex processes and exploring potential therapeutic avenues for cancer and autoimmune diseases.

Methods:
Recently, a novel protocol to generate human DC subsets from CD34+ hematopoietic stem and progenitor (HSPC) *in vitro* was developed at Newcastle University in the Haematopoiesis and Immunity Laboratory by V. Bigley and her team. Subsequently, three functional assays were set up using *in vitro*- differentiated DC, and the results were compared to their blood-derived counterparts and monocyte-derived DC (moDC) as a reference. A migration assay, TLR agonist assay and EBNA1 T cell assay were performed. Possible limitations or benefits of using *in vitro*- differentiated DC subsets compared to blood or moDC were specially investigated.

Findings:
We successfully set up several protocols and confirmed that human cDC1 and cDC2 generated from CD34+ precursors are valuable substitutes for blood DC subtypes for experimental but also immunotherapeutic approaches.

Interpretation:
In the future, these *in vitro*-differentiated DC subtypes will be used to complement a 3D-bioprinted skin-on-chip model that will be further advanced into a melanoma-on-chip model. This approach will allow us to test different melanoma therapy options and better understand DC‘s role in tumor immunity

Abstract Number: 213

Poster Number: 64

ACOUSTOFLUIDIC PLATFORMS ENABLING NON-CONTACT AND NON-INVASIVE MANIPULATION AND TOMOGRAPHY OF BIOLOGICAL SAMPLES

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Background:
3D cellular models such as spheroids and organoids have become an important tool in biomedical sciences, with the potential to narrow the gap to *in vivo* studies, provide alternatives to animal testing and paving the way for personalized medicine. Current methods to grow these samples usually include contact to surfaces or gels, which have been found to influence cells. Solutions offering non-contact handling of these models in suspension and non-invasive monitoring for long-term studies are therefore in demand.

Methods:
We have developed acoustofluidic platforms for trapping and rotational manipulation of biological samples to collect optical tomographic data. For imaging of single cells and small spheroids, we have made platforms compatible with Optical Diffraction Tomography. For larger millimeter-sized samples, we have combined acoustic reorientation with Optical Coherence Tomography (OCT). We developed a model-based algorithm that fuses the multi-angle OCT data by performing a joint recovery of the object’s position parameters, reflectivity-, refractive index- and attenuation maps.

Findings:
We demonstrate acoustic trapping platforms suitable for handling and collection of tomographic data for samples ranging from 10 micrometer to millimeters in size. We have used our method of multi-angle OCT on zebrafish embryos and our algorithm can reconstruct the 3D objects at an enhanced penetration depth, and reduced attenuation artifacts compared to single-angle OCT.

Interpretation:
Our strategies enable non-contact, non-invasive and label-free handling and imaging of biological samples, with the potential to permit detailed and long-term monitoring of developing biological models.

Abstract Number: 216

Poster Number: 65

SATB2-DEPENDENT MECHANISMS IN HUMAN COGNITIVE ABILITY AND NEUROPSYCHIATRIC DISORDERS

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Background:
SATB2 is genetically associated with human intelligence and SCZ. Individuals with SATB2 haploinsufficiency suffer from SATB2-associated syndrome (SAS), defined by developmental delay, severe intellectual disability, and absent/limited speech. We have extensively characterized SATB2-dependent mechanisms in higher brain functions using mouse cKO models, demonstrating an important role for SATB2 in late-LTP and long-term memory consolidation. At molecular level, our data have established SATB2 as a 3D epigenome organizer, which sets up the chromatin landscape of pyramidal neurons for cognitive processes.

Methods:
Here, we aim to generate hiPSC-derived SATB2 gain- and loss-of-function cellular models to gain insights into human SATB2-dependent disease mechanisms in SAS and SCZ. To this aim, we will employ established protocols for differentiation of hiPSCs into neuronal progenitor cells and into cortical glutamatergic neurons by NGN2 overexpression. To generate SATB2 gain-of-function model, neurons will be further transduced with a lentivirus, mediating ectopic expression of SATB2.

Findings:
Functional genomics and proteomics assays will uncover effects of SATB2 on multiple genomic modalities in human pyramidal neurons and allow direct comparison between mouse and human SATB2-dependent chromatin interactions.

Interpretation:
We will test the hypothesis that SATB2-directed 3D genome folding as a mechanism of transcriptional control in cortical neurons has evolved between the two species as a correlate of the evolution of higher cognitive ability. Furthermore, our data will provide novel insights into gene regulatory mechanisms associated with human intelligence and neuropsychiatric disease.

Abstract Number: 239

Poster Number: 66

PATHOPHYSIOLOGICAL REGENERATION FOLLOWING SUSTAINED GROWTH PLATE INJURY: INSIGHTS FROM AN EX VIVO RAT FEMUR ORGANOTYPIC CULTURE MODEL

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Background:
Postnatal bone growth relies on chondrocyte proliferation and osteogenic differentiation in the growth plate through endochondral ossification. GP injuries (GPI) affect 15-30% of bone fractures, leading to growth anomalies and decreased quality of life. With no current biological therapy to prevent bone bridge formation, new investigation models are essential.

Methods:
Here, we explored pathophysiological regeneration after sustained GPI using an ex vivo rat femur organotypic culture (OTC) model, focusing on postnatal endochondral ossification. Utilizing 300 µm thick OTC with a 2 mm horizontal GPI, we EM, gene expression analysis, live/dead, histological, and immunohistochemistry stainings.

Findings:
In the OTCs, regeneration began at 3 days in vitro (DIV), with stem cell, fibroblast, and chondrocyte infiltration at 7 DIV. Further, elongated cells migrated from the zone of Ranvier to the injury site at 7 DIV, forming a network by 15 DIV. GPI-induced disruptions in endochondral ossification include altered expression patterns of ECM marker. qPCR analysis revealed a significant increase in Sox9 expression and an altered Ihh-PTHrP feedback loop, promoting chondrocyte proliferation and maturation.

Interpretation:
The data highlights GPI-induced structural and chondrocyte maturation changes in the ex vivo organotypic GPI model. This model holds potential for advancing our understanding of GPI repair mechanisms and aiding in tissue engineering and disease research.

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