



3rd Annual Meeting

November 27th - 28th, 2025

Kasteel Bloemendal, Vaals, Netherlands

Abstract Book



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Welcome

Dear Colleagues,

We are delighted to welcome you to the 3rd Annual Meeting held on November 27th & 28th, 2025 at Kasteel Bloemendal in Vaals, Netherlands. This year's conference motto is **"Advancing the 3Rs: From Innovation to Implementation"** reflecting our shared goal of translating cutting-edge 3R research into real-world improvements in animal welfare and scientific rigor.

The network has become a powerful force for innovation, fostering collaborations that are driving advancements in crucial areas like **basic biomedical research and laboratory animal science**. We have witnessed remarkable progress stemming from the diverse expertise within our community, and this meeting is designed to build on that success.

Recognizing the importance of nurturing the next generation of 3Rs leaders, we're offering a dedicated **pre-conference workshop – "Beyond Compliance: Integrating the 3Rs into Your Research Career"** – specifically **designed for Early Career Researchers** at November 26th at the University Hospital Aachen. This interactive workshop will provide practical guidance on incorporating the 3Rs into research design, navigating ethical challenges, and building a fulfilling career committed to responsible science.

The main conference program will feature a dynamic mix of an inspiring keynote presentation by Chris Barkus (NC3Rs), thematic talk sessions, and interactive poster discussions. We will be delving into the topics such as **organoids in disease remodeling, novel approaches in reduction, laboratory animal welfare including aquatic organisms, and preregistration of animal studies**. We will also have a dedicated session for **short talks selected from abstracts**, offering a platform for emerging researchers to showcase their work.

Beyond the scientific program, we will provide ample opportunities for networking and collaboration, including a **Get-Together Event** at the location CT² in the first conference evening.

Let's connect, learn from each other, and collectively build a future where ethical research and scientific excellence go hand in hand. We look forward to welcoming you in Vaals!

Best regards,

3R Competence Network NRW Organizing Team

About



Our goal: medical progress in line with the best possible animal welfare

The eight faculties of medicine of the universities in North Rhine-Westphalia have formed a 3R Competence Network. In line with the 3R principle, the network aims to enable (bio-) medical progress in NRW in line with best animal welfare and to specifically support research, innovation and training in the field.

Our vision for the state of North Rhine-Westphalia is that the structural and spatial integration of animal experimentation units in 3R centers will become a catalyst for innovation to reconcile urgently needed medical progress with the best possible animal welfare. For experimental research, direct and constant face-to-face exchange among all stakeholders is of key importance. In our view, the creation and implementation as well as consequent improvement of integrative 3R concepts is a central key to success.



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08:00 **Registration**

09:00 **Welcome Ceremony**

09:30 **Session 1: Refinement chaired by René Tolba (Aachen)**

Patrick Reinhardt (Bochum) - Refining operant behavioral assessment in an open-science homepage environment

Esther Mahabir-Brenner (Cologne) - Managing old-aged mice

Agnesa Mazrekaj (Düsseldorf) - Refinement in aortic research

Florian Alexander Dehmelt (Tübingen) - Electrical stunning as a way to kill zebrafish? Behavioural and neurophysiological effects observed in larvae

10:50 -Coffee Break-

11:15 **Session 2: New thinking in 3Rs chaired by Sabine Bischoff (Düsseldorf)**

Julia Menon (Utrecht) - Does Preregistration Work? Evidence from the First Evaluation in Animal Research

Daniel Hoffmann (Essen) - Reduction of animal numbers by computational analyses that maximize information gain per animal

Emma Pietsch (Berlin) - From 3R to 6R – Building Trust in Preclinical Models through Quality and Transparency

Speaker from participants - Presentation of results from Pre-conference Satellite Workshop for Early Career Researcher (November, 26th)

13:00 -Lunch Break-

14:00 **Session 3: Selected short talks chaired by Christine Hartmann (Münster)**

Daphne Bouwens (Aachen, Winner of the 1st Paper of the Quarter) - In vitro atlas for human fibrosis modelling using iPSC-derived kidney organoids

Alina Deipenbrock (Düsseldorf, Winner of the 2nd Paper of the Quarter) - Modelling of the multicellular tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) on a fit-for-purpose biochip for preclinical drug discovery

Dennis Roth (Düsseldorf) - Detection and Isolation of Spontaneous Human Circulating Tumor Cells in the In Vivo Avian CAM Model

14:00 Session 3: Selected short talks (continued)

Anni Feldmann (Cologne) - Development and evaluation of a dummy-based training protocol for aseptic hysterectomy to generate germ-free mice

Nina Graffmann (Düsseldorf) - Generation of a human dipeptidyl peptidase 4 (DPP4) knockout induced pluripotent stem cell (iPSC)-line to replace animal models in diabetes and fibrosis research

Esther Teitge (Münster) - An In Vitro Mechanical Stretching Device for Simulating Scratching in Human Cell Models of Chronic Itch

14:45 Session 4: Panel chaired by Valeska Stephan (Rostock) & Nicole Linklater (Marburg)

Animals and/or NAMs ? - An interactive exchange on the use and future of animals in science

15:30 Poster Session 1 (Odd Numbers) & Coffee Break**16:30 Session 5: Replacement chaired by Marieta Toma (Bonn)**

Joana Witt (Düsseldorf) - 3D Tissue Models of the Ocular Surface and Beyond

Yotam Menuchin-Lasowski (Muenster) - Developing an iPSC-Derived Optic Nerve Model for the Study of Myelin Diseases

Ivonne Vazquez (Bonn) - Generation of Vascularized Lung Organoids for Disease Modelling

Egor Dzyubenko (Essen) - Maturation and Vascularization of Human Brain Organoids for Next-Generation Preclinical Stroke Models

17:40 -Wrap-up Day 1-

End of scientific program of Day 1

19:30 Social Evening Program

19:30 Arrival at the CT² in Aachen, Germany

20:00 6th Aachen Animal Welfare Award Ceremony

20:30 Rejoin, Refresh and Rest

08:30 **Registration**

09:00 **Session 6: Keynote Lecture chaired by Philip Dammann (Essen)**

Chris Barkus (London) - NC3Rs - From Innovation to Implementation: 20 Years of Supporting the 3Rs to Pioneer Better Science

10:30 **Poster Session 2 (Even Numbers) & Coffee Break**

11:40 **Session 6: Reduction chaired by Branko Zevnik (Cologne)**

Nicole Schupp (Duesseldorf) - Use of 3R-compliant methods to investigate nephro- and hepatotoxicity

Roland Ullrich (Cologne) - Lymphoma-associated myeloid-monocytic (LAMM) cells mediate resistance against CAR-T cell therapy in aggressive B-cell lymphoma

Oliver Dräger (Bielefeld) - Establishment of a Human iPSC-Derived Nociceptive Neuron Model for the Investigation of Sex-specific Differences in Migraine Pathophysiology

Ralf Weiskirchen (Aachen) - From Bile Duct Ligation and Carbon Tetrachloride-Induced Liver Injury to Biochips: The Evolving Toolbox of Experimental Liver Models

13:00 - Wrap-up Day 2 & Closing Remarks -

13:15 End of scientific program Day 2

13:30 3R-Network Meeting for 3R centers (invitation only)

15:00 End of the 3rd Annual Meeting of the 3R-Competence Network NRW

Keynote lecture

Keynote lecture



Chris Barkus

NC3Rs (National Centre for the Replacement, Refinement & Reduction of Animal in Research)

From Innovation to Implementation: 20 Years of Supporting the 3Rs to Pioneer Better Science

The UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), established over 20 years ago, uses the 3Rs as a framework to pioneer better science. This talk will give some background on the organisation, which is independent but supported by public funds, and provide examples of successes across its wide-ranging programmes work. Many of these examples will highlight the collaborative approach taken with scientists and organisations nationally and internationally, from supporting individual researchers and acting as an honest broker to enable information exchange between organisations to driving policy change through global partnerships. This supports the application of 3Rs innovations from early development to widespread implementation, maximising the long-term 3Rs impact which remains the central priority of the NC3Rs.

Abstracts | Talks

Refining operant behavioral assessment in a self-built homecage environment

Patrick R. Reinhardt

Research Division Experimental and Molecular Psychiatry, Department of Psychiatry, Psychotherapy and Preventive Medicine, LWL-University Hospital, Ruhr-University Bochum, Germany

Neural Basis of Learning, Department of Psychology, Ruhr-University Bochum, Germany

Operant behavioral approaches are one of the most widely employed paradigms to investigate behavior in basic research and behavioral changes in preclinical animal models. Testing is mostly performed in operant chambers where the animal has a fixed timeframe to obtain a reward via correct responses to a stimulus. Benefits include a standardized and controlled environment and the possibility to perform behavioral batteries to test a variety of behavioral domains. However, time removed from the homecage and food restriction to increase motivation can cause stress to the animals and alter behavioral outcomes. In addition, the manual placement of the animal in the chamber by the experimenter holds several influencing aspects. These factors should be addressed to further refine operant behavioral tests. One possible approach for this is performing behavioral testing homecage based. Aim of the presented project is to establish a method for homecage based operant behavioral assessment by connecting an operant chamber to the animal's homecage via a transition tube to allow free access. Employing RFID- and weight sensors enables restricted access for one animal at a time and the collection of individual data. The transition system is based on affordable microelectronics and constructed with an open-science idea to enable easy and cheap implementation in other labs. It will be connected to an already existing, self-built, raspberry pi based operant chamber, running with an open-source code (Open Toolbox for Behavioral Research). Construction results of the setup will be shown and an overview of a proof of concept study to assess effects on animal welfare will be presented.

Managing old-aged mice

Esther Mahabir¹, Pia Kahnau², Beryl Eusemann³, Paul Friedemann Pohlig^{4,5}, Katharina Hohlbaum²

¹Comparative Medicine, Center for Molecular Medicine Cologne (CMCC), Faculty of Medicine and University Hospital Cologne, Cologne, Germany ² German Federal Institute for Risk Assessment (BfR), German Centre for the protection of Laboratory Animals (Bf3R), Berlin ³Leipzig University, Faculty of Veterinary Medicine, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany ⁴Cluster of Excellence Cellular Stress Responses in Aging-Associated Diseases (CECAD), Faculty of Medicine and University Hospital Cologne, University of Cologne, Germany ⁵In Vivo Research Facility, Faculty of Medicine and University Hospital Cologne, University of Cologne, Germany

Ageing is a process associated with progressive physiological decline and increased susceptibility to disease. The increase in the number of mice kept for experiments on age-related diseases and surplus animals kept over extended periods poses a challenge for animal care. In addition, veterinary and scientific staff may have difficulty in distinguishing a healthy old-aged mouse from a sick old-aged mouse. Therefore, assessing the well-being of old-aged mice requires training and experience. As such, it is necessary to define when ageing begins, what phenotype an ageing or old mouse displays, and how ageing mice should be treated and housed. To enable consistent assessment of the health status of old-aged mice, we describe a healthy old-aged mouse and specify clinical symptoms that require action and/or treatment. In this talk, we present defined age-specific characteristics including behaviour, body posture, coat and skin, eyes, body orifices, teeth, visible and/or palpable increase in mass as well as body weight, which should be monitored. The associated photographs and videos will be shown. As a result, a care sheet, firstly for old-aged BL/6 mice, was developed. It can be generally applied to old- aged mice without experiment-related, burdened phenotypes and will be evaluated in different institutions. In conformity with the 3Rs, we provide refinement measures to enhance animal welfare of these mice.

Refinement in Aortic Research

Agnesa Mazrekaj*, Joscha Mulorz, Marta Stei, Martin Mollenhauer, Per Arkenberg, Malte Kelm, Georg Nickenig, Stephan Baldus, Hubert Schelzig, Markus U. Wagenhäuser

*Clinic for Vascular and Endovascular Surgery University Hospital Düsseldorf

Modeling aortic diseases such as abdominal aortic aneurysms (AAA) is challenging due to the disease's multifactorial nature and associated comorbidities. Large animal models remain essential for translational research offering high anatomical and pathophysiological relevance. However, surgical standardization and ethical considerations remain significant hurdles.

We refined an established porcine AAA model to improve procedural consistency while prioritizing animal welfare. AAA was induced in 24 male Landrace and Cardio pigs through a combination of systemic oral β -aminopropionitrile (BAPN), intraoperative balloon dilatation, and intraluminal administration of elastase and collagenase. Key surgical refinements included replacing laparotomy with a retroperitoneal approach to reduce trauma. Also, instead of a permanent lumbar artery occlusion, a temporary occlusion was performed to minimize the risk of spinal ischemia. Additionally, a sheath (Cook Medical®) placed directly into the abdominal aorta preserved caudal mesenteric perfusion during integrity testing of clamped aortic segments. It also prevented leakage and ensured hemodynamic stability and pressure-controlled enzyme delivery to the abdominal aorta. These refinements eliminated postoperative paresis, prevented cardiovascular instability and enabled full recovery within 3–4 days without complications.

The introduced approach highlights the importance of close interdisciplinary collaboration among vascular surgeons, veterinarians and basic scientists to improve the scientific validity of aortic in vivo models while adhering to the highest standards of animal welfare.

Electrical stunning as a way to kill zebrafish? Behavioural and neurophysiological effects observed in larvae

Florian Dehmelt

Centre for Integrative Neuroscience and Institute of Neurobiology, University of Tuebingen, Tuebingen, Germany

When killing zebrafish cannot be avoided, it must be accomplished doing the least harm possible. Established methods for this species include anaesthetic overdoses and hypothermic shock through rapid cooling, but both have known limitations. The commonly used anaesthetic MS-222 (or tricaine) is aversive, slow to act, subject to inter-individual variability, and considered unreliable for larvae. While rapid cooling likely accelerates the onset of unconsciousness, it alone does not reliably cause death, and larvae in particular can survive hours of exposure. This is why killing zebrafish by electrical stunning may be a less harmful alternative. To test its suitability and to develop protocols for routine laboratory practice, we characterised both behavioural and neurophysiological effects of electrical stunning in 4-day-old zebrafish larvae. We identified the electric field characteristics and stimulus duration that reliably kill both free-swimming larvae and agarose-embedded larvae. Behavioural analysis and calcium neurophysiology show that larvae lose consciousness, and stop responding to touch and visual stimuli, in less than one second. These electrically stunned larvae no longer show coordinated brain activity. Their brains instead undergo a series of concerted whole-brain calcium waves over the course of many minutes before the complete cessation of signals. If proven successful in routine laboratory practice, this rapid and reliable way to kill zebrafish larvae could help refine the welfare of at least a part of the millions of zebrafish used in research each year.

Does Preregistration Work? Evidence from the First Evaluation in Animal Research

Julia Menon

preclinicaltrials.eu, Utrecht, Netherlands

Improving the quality and reliability of animal research is a growing priority within the scientific community, and preregistration offers a promising way forward. Preregistration, the act of recording a study protocol before the study begins, is well established in fields such as clinical research but remains uncommon in animal studies. To establish its relevance, we conducted an evaluation in two phases.

Phase 1 assessed the impact of preregistration on reporting and study quality by comparing preregistered vs non-preregistered papers. The preregistered group achieved higher scores in reporting quality and critical appraisal. Item-level analyses using the ARRIVE guidelines essentials 10 highlighted improvements in reporting for inclusion/exclusion criteria, randomisation and blinding methods, experimental procedures, and reporting results (i.e. summary/descriptive statistics of results, with a measure of variability).

Phase 2 examined adherence to preregistered protocols and the reporting of deviations by comparing protocols with their resulting publications. Preregistration encouraged consistency; however, undisclosed deviations were still observed in 21.6% of cases. The best-reported items across protocols and papers were primary outcomes, interventions, and hypotheses.

These findings provide the first empirical evidence that preregistration positively influences reporting quality in animal studies. This talk will not only present these results but also place them in the broader context of the benefits, current practices, and challenges of preregistration. In addition, we will demonstrate how existing platforms support fast, secure, and flexible preregistration, and show you how to get started with registering your own studies.

Reduction of animal numbers by computational analyses that maximize information gain per animal

Daniel Hoffmann

Faculty of Biology and Center of Medical Biotechnology, University of Duisburg-Essen

It is becoming increasingly clear that conventional null-hypothesis significance testing with p-values (NHSTp) is problematic for basic biomedical research with animals, typically characterized by small sample sizes, small effect sizes, and noisy data. Under these conditions, application of black-box NHSTp with an arbitrary, non-biological significance threshold leads to non-robust p-values and often to false positives or negatives. In other words: we often do not gain information and thus waste animal lives.

There are several ways to improve information gain per animal, for instance: (1) use of prior biological information, (2) more accurate statistical models of biological systems, (3) focus on inference of quantitative biological knowledge. These are features of Bayesian statistics, which is growing in popularity since the 1990s. I introduce the basics of Bayesian statistics and demonstrate with concrete examples from research with animals that it can indeed yield more information per animal.

There are several reasons why Bayesian statistics is not yet used by every researcher: (1) it requires familiarity with a conceptual and mathematical framework that goes beyond what is commonly taught to biologists, (2) it usually requires programming skills not common among biologists, (3) it sometimes requires more computational power. I discuss ways of overcoming these problems with new technology such as our novel BAYAS tool. In this way Bayesian statistics can be made more widely accessible and animal numbers can be reduced at scale by better planning and evaluation of experiments.

From 3R to 6R – Building Trust in Preclinical Models through Quality and Transparency

Emma Pietsch

BIH QUEST Center for Responsible Research at Charité – Universitätsmedizin Berlin

Animal models remain central to preclinical research and are widely regarded as the gold standard in many disease areas. However, they raise important ethical questions, and species-specific differences often limit their translational validity. Three-dimensional *in vitro* systems such as organoids and organ-on-chip models have therefore emerged as promising human-relevant tools to complement or, in selected cases, replace animal experimentation, thus contributing to the 3R (Replace, Reduce, Refine). For these advanced models to fulfil their potential, they must be adopted at scale, which requires that they are practical to implement, scientifically robust, and deliver results that are at least equally informative as animal experiments.

Achieving this level of trust and reliability demands methodological rigor, transparent reporting, and systematic model evaluation. The 6R framework addresses these needs by expanding the 3Rs with Robustness, Registration, and Reporting. Within the Einstein Center 3R Berlin, a cross-sectional project dedicated to the 6R develops and implements related practical measures.

As part of this effort and using tools of meta-research, we systematically assess the strengths and limitations of *in vitro* and *in vivo* models of human disease. Using Inflammatory Bowel Disease as an example, we explore how harmonized outcome measures across model systems and their alignment with clinically relevant endpoints might enhance their translational value. Collaborating with preclinical researchers, clinicians, patient representatives, and regulators, we aim to support transparent model characterization and demonstrate how *in vitro* and *in vivo* approaches might provide complementary insights.

Through our integrative efforts across research communities, complemented by public outreach activities, we seek to foster realistic expectations, informed decision-making, and ultimately more responsible and clinically meaningful biomedical research.

Insights from the Pre-Conference Workshop "Beyond Compliance: Integrating the 3Rs Into Your Research Career"

Bernhard Schermer¹, Sabine Bischoff², Leonie Tix³

¹ University of Cologne, ² University of Düsseldorf, ³ RWTH Aachen University

Session 1: From Obligation to Opportunity – Making the Most of the 3Rs by Bernhard Schermer The 3Rs are often perceived as a necessary burden - something one must deal with when conducting animal research or navigating the accompanying bureaucracy, whether during grant preparation or when submitting animal study protocols. Without downplaying the considerable - and at times disproportionately large - administrative workload, it is worth viewing the 3Rs from a more positive perspective: beyond reducing animal suffering and stress, actively engaging with the 3Rs not only has the potential to optimize research projects, but can also support researchers in coping more effectively with the emotional and practical challenges of conducting animal experiments and in communicating their work more clearly. In this session, we explore how scientists and experimenters can benefit from embracing the 3Rs. **Session 2: Lab Culture Reboot - Tools for Improvement and Transparency by Sabine Bischoff** In this workshop session, we address challenges faced by earlycareer researchers in laboratory animal science. It offers a space to reflect on lab dynamics and develop strategies for successful integration. Emphasis is placed on open communication about expectations, responsibilities, and workflows to build trust, clarity, and supportive team culture. Through shared experiences and discussion, participants learn to foster transparent, collaborative environments that encourage feedback and cooperation. They also explore tools such as CIRS-LAS (Critical Incident Reporting System - Laboratory Animal Science), SMAFIRA (Smart Feature-based Interactive Ranking), Norecopa, the Experimental Design Assistant, and the Mouse Breeding Calculator to improve study planning, transparency, reproducibility, and animal welfare. **Session 3: Paperwork for Animal Proposals - Tips and Pitfalls by Leonie Tix** Writing an animal proposal (AP) is a structured scientific and ethical self-assessment. It is a quality assurance tool that ensures that animal experiments are only performed if they are essential, ethically justifiable, and methodologically appropriate. The process itself is a crucial part of good scientific practice and ethical selfawareness in research. The approval procedure for APs in Germany is a formalized, multistage process that can vary depending on the completeness of the paperwork and the relevant state authority's requirements. Incomplete proposals affect the application deadline, and insufficient information in the proposal significantly delays the approval process. In this session, participants use the previous workshop contents to address the core requirements of an approvable AP and write better proposals in the future.

In vitro atlas for human fibrosis modelling using iPSC-derived kidney organoids (Paper of the Quarter Q1/2025 Winner)

Daphne Bouwens¹, Vivien Goepp¹, Sikander Hayat¹, Jitske Jansen¹, Rafael Kramann^{1,2}

¹Department of Medicine 2 (Nephrology, Rheumatology, Clinical Immunology, Hypertension), RWTH Aachen University Medical Faculty, Aachen, Germany; ²Department of Internal Medicine, Nephrology and Transplantation, Erasmus Medical Center, Rotterdam, The Netherlands

Chronic kidney disease is a progressive condition of deteriorating kidney function, prevalent in over 13% of the world population. Fibrosis is the common hallmark of all progressive nephropathies. Existing in vitro or in vivo models for studying CKD progression often fail to accurately replicate human disease, which contributes to the high failure rate of drugs in clinical trials. To address this, we aim to develop a comprehensive in vitro injury atlas using human-induced pluripotent stem cell (iPSC)-derived kidney organoids exposed to a variety of injury stimuli to model different flavours of fibrosis.

We generated kidney organoids according to Jansen et al. CSC 2022. Kidney organoids were exposed to eight different injury models, including Hypoxia, IL-1 β , Cisplatin, TGF β 2, proteinoverload, Diabetic injury or a combination thereof. Organoids were processed for 10x Genomics single cell RNA sequencing, proteomics and IHC, and compared to available human disease data.

Principal component analysis of human kidney organoids revealed distinct gene signatures for each injury condition. Collagen quantification and NABA scores confirmed effective fibrosis modelling. Some Differentially Expressed (DE) genes and proteins (e.g., FN1, COL1A1) were shared across injury conditions (Cisplatin, IL-1 β , Hypoxia plus IL1 β), while others were injury-specific (e.g., TNC, FBLN2 in Cisplatin), reflecting both shared and distinct gene expression patterns. Integration with human data showed overlapping gene signatures between injured organoids and human kidney disease.

Through this approach, we successfully developed human disease-specific fibrosis organoid models that reflect human biology in a data driven manner, enabling target validation and future drug research to be more human disease-specific.

Modelling of the multicellular tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) on a fit-for-purpose biochip for preclinical drug discovery (Paper of the Quarter Q2/2025 Winner)

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Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal form of pancreatic cancer. One major cause for a fast disease progression is the presence of a highly fibrotic tumor microenvironment (TME) mainly composed of cancer-associated fibroblasts (CAF), and various immune cells, especially tumor-associated macrophages (TAM). To conclusively evaluate drug efficacy, it is crucial to develop in vitro models that can recapitulate the cross talk between tumor cells and the surrounding stroma. Here, we constructed a fit-for-purpose biochip platform which allows the integration of PDAC spheroids (composed of PANC-1 cells and pancreatic stellate cells (PSC)). Additionally, the chip design enables dynamic administration of drugs or immune cells via a layer of human umbilical vein endothelial cells (HUVEC). As a proof-of-concept for drug administration, vorinostat, an FDA-approved histone deacetylase inhibitor for cutaneous T cell lymphoma (CTCL), subjected via continuous flow for 72 h, resulted in a significantly reduced viability of PDAC spheroids without affecting vascular integrity. Furthermore, dynamic perfusion with peripheral mononuclear blood cells (PBMC)-derived monocytes resulted in an immune cell migration through the endothelium into the spheroids. After 72 h of infiltration, monocytes differentiated into macrophages which polarized into the M2 phenotype. The polarization into M2 macrophages persisted for at least 168 h, verified by expression of the M2 marker CD163 which increased from 72 h to 168 h, while the M1 markers CD86 and HLA-DR were significantly downregulated. Overall, the described spheroid-on-chip model allows the evaluation of novel therapeutic strategies by mimicking and targeting the complex TME of PDAC.

Detection and Isolation of Spontaneous Human Circulating Tumor Cells in the In Vivo Avian CAM Model

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Circulating tumor cells (CTCs) are clinically relevant markers of metastasis and prognosis in gastrointestinal (GI) cancers. Yet their rarity and the limitations of existing in vivo models severely restrict our ability to study tumor cell dissemination at the mechanistic level. Here, we demonstrate for the first time that the avian chorioallantoic membrane (CAM) assay can serve as a biologically relevant and ethically advantageous 3R model for spontaneous CTC formation. Six GFP-labeled human GI cancer cell lines (colorectal, pancreatic and biliary) were xenografted onto the CAM. Solid tumors formed in all models, and GFP+ single CTCs were detected in the CAM blood using different established methods and isolated by flow cytometry.

Subsequent single-cell whole genome amplification enabled downstream single-cell STR profiling to confirm the identity of isolated CTCs, as well as the detection of tumor-specific point mutations and copy number alterations. CTCs showed a high genetic concordance with their parental cell lines, establishing the CAM assay as a novel, versatile, and scalable platform to investigate early metastatic dissemination and CTC biology.

Importantly, the CAM assay represents a powerful alternative to rodent models, aligning with the 3R principles by enabling mechanistic cancer research in a non-sentient system that is not classified as an animal experiment under current EU legislation. It allows for the reduction of vertebrate animal use, while providing a refined system with rapid tumor growth, easy accessibility, and high imaging compatibility. This model opens new avenues for preclinical CTC research, including pharmacological screening, longitudinal monitoring, and functional testing of patient-derived material—without reliance on mammalian models.

Development and evaluation of a dummy-based training protocol for aseptic hysterectomy to generate germ-free mice

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Microbiome research relies on germ free mice to study host–microbe interactions without microbial interference. These animals are produced through aseptic hysterectomy, which requires sterile transfer of fetuses for rearing under germ free conditions. The procedure demands high precision and strict sterility, as contamination invalidates animals, increases use, and wastes resources. Yet, structured training methods that allow staff to gain technical proficiency without live animals remain unavailable.

To address this, the protocol was adapted for gnotobiotic facility conditions using a glove based dummy that enabled full simulation of the hysterectomy under sterile conditions. Concurrently, microbiological monitoring was refined to enhance sensitivity, specificity, and cost efficiency. The validated workflow was then applied to in vivo hysterectomy, leading to the successful generation of germ free offspring. Insights from these simulations informed the development of a reusable silicone model produced from 3D printed molds, which was further evaluated for handling, sterilization, and contamination resistance.

This stepwise approach demonstrated that simulation based training combined with iterative sterility assessment enables reliable protocol transfer to in vivo conditions. The resulting framework supports standardization and reproducibility of germ free mouse production while promoting refinement within the 3Rs. Beyond gnotobiotic research, the silicone dummy model could be practical tool for training and procedural optimization in general hysterectomy techniques and neonatal handling.

Generation of a human dipeptidyl peptidase 4 (DPP4) knockout induced pluripotent stem cell (iPSC)-line to replace animal models in diabetes and fibrosis research

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Metabolic dysfunction-associated steatotic/fatty liver disease (MASLD/MAFLD) has a prevalence of 30% in the Western World. It is highly associated with type 2 diabetes mellitus (T2D), obesity and cardio-vascular diseases. MAFLD manifests as aberrant fat storage in the liver leading to inflammation, functional impairments and potentially fibrosis and/or carcinoma. Due to the complexity of this multifactorial disease, therapy options are still rather limited. By employing an in vitro induced pluripotent stem cell (iPSC)-based model to decipher basic disease associated molecular pathways, we detected Dipeptidyl peptidase 4 (DPP4) as a potential key mediator of the disease. DPP4 is a crucial factor in inflammation-associated diseases. Its overactivity fosters organ fibrosis, especially in the context of diabetes where it mainly affects the liver and the kidney.

To elucidate the role of DPP4 in the development of MAFLD, we inhibited DPP4 activity with Vildagliptin (VILDA) and discovered fatty acid and purine metabolism as well as inflammation response pathways to be improved upon DPP4 inhibition.

Currently, we are generating a human DPP4 knockout iPSC line to further study the cell-type specific effects of DPP4 on early and late stages of MAFLD as well as liver and kidney fibrosis.

An In Vitro Mechanical Stretching Device for Simulating Scratching in Human Cell Models of Chronic Itch

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Despite different underlying diseases, chronic pruritus (chronic itch) always leads to increased scratching behavior and thus to severe mechanical stress on resident skin cells. This is particularly pronounced in chronic nodular prurigo (CNPg), a chronic inflammatory disease which is characterized by a self-perpetuating itch–scratch cycle. Traditional studies investigating the effects of scratching often rely on animal models, which limits their relevance to humans and contradicts the 3Rs principles (Replacement, Reduction, and Refinement). To address this, we applied an in vitro mechanical stretching device and developed a stretch paradigm that applies defined cyclic strain to cultured keratinocytes, thereby mimicking scratching and skin tension under controlled laboratory conditions.

To investigate the effects of repeated scratching, primary keratinocytes from patients with CNPG were subjected to cyclic mechanical stretch for six hours. CNPG involves mechanical stress on resident skin cells as a central pathogenic driver. Integrated transcriptomic and proteomic analyses of stretched keratinocytes revealed deregulation of key genes and proteins, including mediators of type 2 inflammation, neuroinflammatory signaling, and neuronal projection. Furthermore, alterations in transcriptional regulators suggested lasting impacts of mechanical stress on gene expression programs.

By reproducing complex scratch-induced cellular responses without animal use, this device provides a robust alternative to animal pruritus models. It enables precise control of biomechanical parameters, generates human-relevant molecular insights, and offers a scalable platform for studying mechanobiology in dermatology. Overall, this in vitro stretching system represents a technically innovative and ethically responsible approach that supports the 3Rs while advancing research into chronic itch and inflammatory skin disease.

Animals and/or NAMs ? - An interactive exchange on the use and future of animals in science (Poster ID: 140 / Day 2)

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The developments of methods to replace or refine animal models (New Approach Technologies, NAMs) has been progressing rapidly over the last decade. While animal models have historically been considered the gold standard, there are challenges in terms of transferability and robustness, as well as ethical concerns regarding the use of animals in research. At the same time, NAMs are gaining importance – not only for ethical reasons, but also for scientific reasons. However, despite the progress made, animals are still being used, which gives rise to some questions regarding both approaches and future developments in these fields. In this session we want to invite the audience to an open, interactive exchange of opinions and discuss similarities, differences and chances regarding these two approaches. The aim is to reflect on the status quo of both approaches and draw lessons from it for the future developments regarding the use of animals in science.

3D Tissue Models of the Ocular Surface and Beyond

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The ocular surface, a complex system of multiple structures such as the cornea, conjunctiva, and several glands including the lacrimal gland, plays a vital role in maintaining vision and ocular health. Dysfunction of any of these tissues can destabilize the entire system, often leading to chronic ocular surface diseases such as dry eye syndrome, inflammation, or fibrosis, severely impacting patients' quality of life. To better understand and treat these conditions, there is an urgent need for physiologically relevant, human-based in vitro models that can serve as complementary alternatives to animal testing. This talk will present several ongoing projects in our lab exploring diverse scaffold strategies to construct functional ocular surface models. These include biologically derived scaffolds such as compressed collagen matrices and decellularized porcine conjunctiva as a native-like support structure. In parallel, we are advancing the field of lacrimal gland tissue engineering. By decellularizing porcine lacrimal glands, we generate extracellular matrix-derived hydrogels suitable for 3D bioprinting. Tissue-specific hydrogels preserve the native biochemical composition, enhancing cell viability and promoting physiological function. Compared to standard substrates like collagen type I and Matrigel, the decellularized lacrimal gland hydrogel significantly improved the secretory function of lacrimal gland epithelial cells. We will also discuss techniques to further enhance mechanical stability and printability for future applications in the development of a vascularized lacrimal gland model.

Developing an iPSC-Derived Optic Nerve Model for the Study of Myelin Diseases

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The optic nerve is a major site of pathology in demyelinating disorders such as multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD), where early damage can result in vision loss and long-term disability. While animal models have contributed significantly to our understanding of optic nerve demyelination, their translational value remains limited due to interspecies differences in anatomy, immune responses, and myelination patterns. Furthermore, they do not allow for the investigation of patient-specific disease mechanisms.

To address these limitations, we are developing a fully human in vitro model of the optic nerve using induced pluripotent stem cells (iPSCs). Our model incorporates iPSC-derived retinal organoids, oligodendrocytes, astrocytes, and microglia in co-culture, aiming to reconstruct the cellular complexity, spatial organization, and functional dynamics of the human optic nerve. This platform is designed to enable the induction and observation of neuroinflammatory and demyelinating processes under controlled conditions, allowing detailed analysis of cell-type-specific responses and interactions.

By capturing key aspects of human optic nerve architecture and glial interactions, the model may help identify novel pathological features and support preclinical investigations into potential therapeutic approaches.

Generation of Vascularized Lung Organoids for Disease Modelling

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In our previous published work, we developed a robust and reliable protocol for the generation of murine bronchioalveolar lung organoids (BALO). This model was generated by FACS cell sorting and co-cultivation of bronchoalveolar epithelial stem cells and resident mesenchymal cells isolated from the lung homogenate of adult mice. After 21 days, BALO spatially organized in bronchiolar and alveolar regions with airway-like structures containing basal cells, secretory, and ciliated cells while alveolar-like regions differentiate into mature alveolar epithelial cell type 1 and type 2. To achieve organoid endothelization, CD45negCD102+ endothelial cells (ECs) were flow-sorted from the lung homogenate of P9 mice. After proving EC identity and functionality, ECs were directly microinjected into BALO culture. Overall, the incorporation of pulmonary ECs into differentiated organoid cultures via microinjection leads to the formation of a complex endothelial network over the alveolar compartment of the organoids allowing study of EC responses to epithelial injury *ex vivo*. Notably, human induced pluripotent stem cells (iPSC)-derived lung bud tip organoids with an integrated endothelial compartment have been also generated. Co-culture of the sorted NKX2.1+ lung progenitors with iPSC-derived ECs not only increased organoid complexity and size significantly but also lead to the formation of EC tubes near organoids' distal areas, indicating epithelial-endothelial cell crosstalk. In summary, our laboratory's overarching objective is to establish and utilize advanced murine and human *in vitro* models to uncover novel immune cell-specific molecular signalling pathways implicated in disease development, progression, and resolution.

Maturation and Vascularization of Human Brain Organoids for Next-Generation Preclinical Stroke Models

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Human brain organoids (hBOs) recapitulate the organization of multiple brain regions and allow for developmental disease modeling¹. However, existing organoid models are unsuitable for studying mature brain disorders such as stroke because they fail to reproduce glial cell maturation, the extracellular matrix (ECM) complexity, and functional vasculature, which are key determinants of neuronal plasticity and tissue remodeling after stroke²⁻⁴. We developed an hBO that closely replicates the physiological 1:1 neuron-glia ratio and enables brain vasculature integration. By exposing these glia-enriched hBOs to a heartbeat-like acoustic stimulation during maturation, we advanced their physiological maturation including neuronal activity, cell differentiation, synapse formation, and vascular development.

Using Ca²⁺-imaging, we show that the heartbeat stimulation activates astrocytic PIEZO mechanoreceptors and triggers Ca²⁺-mediated signaling. Heartbeat increased the extracellular space volume and promoted functional maturation of hBOs, as indicated by superresolution 3D-STED-shadow imaging⁵, immunohistochemical analyses, and multi-electrode array electrophysiology. Moreover, our approach promotes cellular differentiation, ECM expression, and drives the emergence of vasculature-supportive astrocytes, as indicated by single-cell RNA sequencing and LC-MS/MS proteomics. Consequently, we were able to successfully interrogate vasculature in hBOs, as indicated by immunolabeling and 3D imaging.

These findings provide the foundation for a human-specific platform to advance the testing of novel restorative stroke treatments.

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Use of 3R-compliant methods to investigate nephro- and hepatotoxicity

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The kidneys (here, especially the proximal tubular epithelial cells (PTEC)) and the liver are constantly exposed to toxic metabolites and xenobiotics. Although intensive research is being conducted into alternatives to animal testing for investigating the hepato- and nephrotoxicity of drugs and chemicals, no fundamental breakthrough has yet been achieved. Problems with in vitro methods include insufficient metabolic (liver) or transport (kidney) capacity. We are using two strategies to try to circumvent these weaknesses. First, we are optimizing the ex vivo incubation of precision-cut tissue slices (PCTS) for reliable toxicity predictions. PCTS preserve tissue architecture, cellular heterogeneity, and metabolic capacity, offering a promising link between in vitro and in vivo models. They reduce animal numbers by allowing simultaneous testing of multiple substances with a single organ. We have tested kidney (PCKS) and liver (PCLS) slices with five prototypical toxins. PCKS showed sensitivity comparable to established kidney cell lines, while PCLS exhibited IC50 values closely matching in vivo toxicity data. High reproducibility between different experimenters was achieved, underscoring the robustness of the model. Second, we are characterizing PTEC-like cells (PTELC) differentiated from human induced pluripotent stem cells (iPSC), for their functionality and sensitivity to nephrotoxins. PTELC exhibited similar morphology to PTEC, expressed prototypical PTEC markers, and showed remarkable transport capacities. Treatment with nephrotoxins also yielded IC50 values closely matching in vivo toxicity data. With these two models, one reducing and the other with the potential of replacing animal experiments, essential prerequisites for future meaningful toxicity studies are established.

Lymphoma-associated myeloid-monocytic (LAMM) cells mediate resistance against CAR-T cell therapy in aggressive B-cell lymphoma

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CAR T cell therapy has strongly improved the outcome of patients with r/r B-NHL. Still, around 50% of patients do not durably benefit from CAR T cell therapy. To elucidate hallmarks associated with an immunosuppressive lymphoma microenvironment (LME) and CAR T cell resistance, we apply multiomic analysis to pre and post CAR T cell specimens. In patients with non-durable response, we identify a prognostically relevant lymphoma-associated myelomonocytic (LAMM) gene signature. High-dimensional profiling via scRNA-seq and IMC reveals distinct CSF1R+/CD14+/CD68+ LAMM cell population to associate with poor clinical outcome. We demonstrate that these LAMM cells inhibit CAR T cell proliferation and cytotoxicity. Mechanistically, inference analysis of cell-cell communication within our scRNA-seq data identifies a direct LAMM – T cell interaction e.g. via PGE2-EP2/EP4 axis. Strikingly, in an autochthonous DLBCL mouse model we demonstrate combined anti-CD19 CAR T cell and CSF1R blockade to convert an immunosuppressive LME and improve survival. Our data provide a strong rationale to combine anti-CD19 CAR T cells with CSF1R inhibitors in future clinical trials of patients with r/r B-NHL.

Establishment of a Human iPSC-Derived Nociceptive Neuron Model for the Investigation of Sex-specific Differences in Migraine Pathophysiology

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Migraine is a common neurovascular disorder with its underlying mechanisms still not fully decoded. Today, there is prominent evidence that the disparity in incidences between sexes is mediated by an imbalance in sex hormone levels, which was shown to affect the excitability and sensitization of trigeminal nociceptive neurons. To investigate the interplay of sex hormones, the Transient receptor potential vanilloid 1 (TRPV1) and the TWIK-related spinal cord potassium channel (TRESK) signaling in migraine pathophysiology, we established a protocol for reprogramming human dermal fibroblast into induced pluripotent stem cells (iPSCs). The iPSC-derived nociceptive neurons serve a model system to study the interplay of nociceptor-specific ion channels and sex-hormone receptors likewise. Additionally, a sex-matched migraine patient and healthy control cohort was established and used to identify novel migraine-related genetic variants by whole genome sequencing. The functional effect of the identified variants can further be analyzed in the patient-derived nociceptive neurons by electrophysiological recordings, gene and protein expression studies. Taken together, our human iPSC-derived nociceptive neuronal model can serve as an alternative to studies formerly conducted in dorsal root ganglia or trigeminal neurons dissected from rodents.

From Bile Duct Ligation and Carbon Tetrachloride-Induced Liver Injury to Biochips: The Evolving Toolbox of Experimental Liver Models

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To understand liver physiology, pathogenesis and therapeutic response, model systems are needed that can capture the remarkable metabolic complexity of the liver. Historically, researchers have relied on in vivo injury models such as bile-duct ligation (BDL) for cholestasis, carbon tetrachloride (CCl₄) intoxication for fibrosis, and acetaminophen (paracetamol) overdose for acute toxic injury. These approaches have provided valuable insight into inflammatory cascades, cellular cross-talk and fibrogenic remodeling. However, they have well-known limitations in translational relevance, ethical burden, and mechanistic resolution.

Recent technological innovations are reshaping the experimental landscape. Three-dimensional human liver organoids can recreate zonation, progenitor dynamics and patient-specific genetics in a dish, enabling high-content drug screening and personalized disease modeling. Microfluidic “liver-on-a-chip” platforms integrate primary hepatocytes, non-parenchymal cells and controlled perfusion, providing unprecedented control over oxygen gradients and shear stress while allowing real-time readouts of metabolism and toxicity. Precision-cut liver slices bridge the gap between whole-organ context and in-vitro manipulability by preserving native architecture, immune populations and extracellular matrix for up to several days in culture.

This talk will discuss the principles, advantages and caveats of each model, highlighting how the strategic combination of classical in-vivo protocols with next-generation in-vitro systems accelerates discovery. Examples will illustrate how multi-modal approaches uncover novel fibrotic drivers, predict hepatic inflammation, drug toxicity, and inform regenerative therapies. Finally, I will outline emerging directions, such as vascularized organoid assemblies, integrated multi-organ chips and AI-guided phenotyping, models that promise to close the translational gap between bench and bedside in hepatology research.

Abstracts | Posters

Replacement

ID 114 - PIBS: A Next-Generation Ex Vivo System for Surgical Bio-Sealant Evaluation- Bridging the Gap Between In Vitro Simplicity and In Vivo Complexity in Surgical Adhesive Testing

Britta Majchrzak-Stiller, Ilka Peters, Lea Skrzypczyk, David Wiedemann, Lisa Trzebiatowski, Waldemar Uhl, Johanna Strotmann, Philipp Höhn

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Biological sealants are increasingly used across several surgical disciplines to support wound closure, reduce fluid leakage, and enhance tissue regeneration. However, the evaluation of mechanical performance and interactions with tissue and body fluids under physiological conditions remains a significant challenge. Traditional in vivo models are often costly, ethically problematic, and lack standardization.

To address this gap, we developed the Physiological Incubation Biosimulator (PIBS)—a novel ex vivo platform designed to replicate key microenvironmental parameters at the surgical application site, including body temperature, humidity, pressure and contact with body fluids. The PIBS allows for reproducible and quantitative testing of sealant performance under near-physiological conditions without the need for living animals. Importantly, the PIBS model can utilize porcine pleura, which is obtained as a by-product of the food industry and would otherwise be discarded. Furthermore, collagen casing commonly used by the food industry for sausages may be used. It serves as a biomimetic substrate that closely resembles the native extracellular matrix, providing a realistic environment for sealant interaction and evaluation. Moreover, both materials are readily available and cost-effective, making them ideal materials to complement the PIBS platform by further minimizing reliance on animal experiments while maintaining physiological relevance. The PIBS significantly reduces the ethical and environmental burden of animal research, aligning with the principles of the 3Rs in biomedical testing. This platform offers a valuable alternative for preclinical assessment of surgical bio-adhesives and supports their development and validation in a more ethical, sustainable, and cost-effective manner.

ID 115 - Modelling Polycystic Kidney Disease Using Human ASC-derived Kidney Organoids

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder, yet its molecular pathway remains incompletely understood. Mutations in either of the genes PKD1 or PKD2, which encode the proteins polycystin-1 and polycystin-2 that together form a mechanosensitive calcium channel, lead to dysregulated ciliary cAMP signaling, contributing to cyst formation and loss of kidney function. Targeting this cAMP dysregulation has become a prime objective in ADPKD research. Human ASC-derived kidney organoids, known as tubuloids, offer a promising platform to model ADPKD in a human tissue-like environment. However, they inherently resemble renal cysts, making them challenging to interpret. We aimed to overcome this drawback by adapting the tubuloid culture method to optimize their utility in modelling ADPKD.

We established and characterized tubuloids from 7 donors in suspension and extracellular matrix-supported dome culture, identifying differences in morphology and protein expression through immunohistochemistry and immunofluorescence microscopy. By applying RNA-sequencing, we validated transcriptional stability, and in combination with gene set enrichment analysis, we identified a distinct transcriptional profile in dome tubuloids that resembled dedifferentiated cystic epithelium. Moreover, we demonstrated the tubuloids' applicability as ADPKD models by inducing cyst formation in response to chronic cAMP elevation.

Thus, we defined a reproducible in vitro model of human renal cystogenesis with pathophysiological relevance, highlighting the influence of extracellular matrix on tubuloid differentiation. Next, we will apply state-of-the-art optogenetic techniques to specifically modulate ciliary cAMP levels to study the molecular mechanism of ADPKD.

ID 117 - Structured cell Culture Dishes (StruCD)

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Replacing animal testing by in-vitro cell culture experiments is a prominent 3R strategy. With StruCD we plan to provide an intermediate system between classical cell culture dish and organoid culture:

The micro-environment of cells is a key factor in biological systems. For classical cell culture models, cells are grown in flat culture dishes with a smooth, hard surface, not representing well the mechanical and structural properties of the in vivo situation. Yet, these structural and geometric features directly regulate cell function and fate, e.g. in the gut, where intestinal epithelial cells grow on villi (bulges) and crypts (depressions). The idea of StruCD is to use the recently patented MicroFold technology to produce 3D-structured, stiffness-controlled, cell culture substrates to better reflect the mechanical microenvironment of the in-vivo situation. Initial tests with an intestinal epithelial cell line seeded on MicroFold multi-cell-scale structures already show that they interact with the structure and preferentially settle and grow in its “trenches”.

While structured, StruCDs remain almost planar, facilitating easy handling and microscopic observations. StruCDs are cheap to manufacture and compatible with standard lab procedures, thus offering a cost-effective way of more realistic cell culture experiments, in between the simple classic cell culture, and the more complex organoid systems.

ID 118 - Development of structured cell culture dishes

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The translation of in-vitro findings into in-vivo systems remains a major challenge in biomedical research, due to discrepancies in the cellular microenvironment. Physical and mechanical cues in particular play a decisive role in regulating cell behavior. Cells are not only responsive to biochemical stimuli but also actively sense and adapt to the geometry and stiffness of their surroundings through mechanotransduction. These parameters critically influence cell cycle progression, proliferation rates, and drug responsiveness, thereby affecting the predictive value of conventional cell culture models.

To address this limitation, we present soft, structured cell culture dishes designed to more closely mimic the mechanical and topographical properties of native tissues. Using a novel PDMS stretch-based fabrication technique, we generate surface morphologies that replicate tissues like the intestinal epithelium, while maintaining tunable geometrical control to emulate a range of physiological structures.

Cell growth experiments performed with CMT93 and MC38 cells demonstrate that proliferation on structured PDMS substrates is reduced compared to standard rigid plastic, indicating a shift toward more physiologically relevant cell cycle dynamics. Furthermore, a fully automated, in-house-developed imaging analysis pipeline reveals non-uniform cell density distributions correlated with the substrate's height profile, indicating a spatially modulated growth behavior.

Our findings highlight that structured culture systems can substantially improve the physiological relevance of in-vitro models. This approach enhances the translatability of cell culture data and supports more targeted pre-selection of candidates for in-vivo studies, ultimately contributing to the reduction of animal experiments.

ID 122 - Use of environmental health monitoring in laboratory mouse facilities and factors influencing its implementation in German-speaking countries

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The health status of laboratory animals plays a decisive role for their health and welfare as well as the validity of the study results. In recent years, numerous comparative studies demonstrated that environmental health monitoring (EHM), which detects nucleic acids of infectious agents via molecular biological methods, is superior to soiled bedding sentinels (SBS). Its implementation can reduce the number of mice used for health monitoring (HM) in conformity with the 3Rs. A survey containing 33 questions was conducted by members of the Committee for Hygiene of the GV-SOLAS to assess the prevalence of EHM use in Germany, Austria and Switzerland and to better understand factors that influence its implementation in laboratory animal facilities.

Our survey showed that 64% of 91 animal facilities predominantly use SBS for their HM programs, 20% use a hybrid method (combination of EHM and animals), and 16% implement EHM. Notably, 59% of facilities equipped with at least 50% IVCs already use EHM to varying degrees. An annual reduction of 8-1,200 animals per facility was reported when EHM was used. Beliefs on factors such as cost, reliability of the methodology, and the number of false-positive results differ among facilities that mainly use animals or EHM. The choice of HM strategy is influenced by the existing cage system and the availability of decontamination equipment. Furthermore, inclusion of EHM in the FELASA recommendations as well as more information and further training on EHM will most likely enhance acceptance of EHM in German-speaking countries in the future.

ID 130 - G-Force Guided Self-Assembly of Collagen Hydrogels for Optically Accurate Cornea in vitro Models

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The optical clarity and refractive index of the human cornea result from the highly organized architecture of its collagen type I fibrils. Collagen hydrogels have been extensively investigated for corneal tissue engineering; however, conventional fabrication methods often produce scaffolds with limited optical performance or inadequate mechanical strength. In this study, we present a scalable fabrication method for producing highly transparent, refractive collagen scaffolds with improved mechanical and biological properties. By precisely controlling the magnitude of G-force during gelation, collagen type I fibrils can be directed to self-assemble into planar ordered structures. The resulting scaffolds exhibit optical transparency comparable to that of the native cornea across the visible spectrum. The compact fibrillar organization enhances mechanical strength without compromising light transmission, closely mimicking the structure and function of the corneal stroma. The process is inherently cytocompatible and allows uniform stromal cell integration within the hydrogel matrix. In vitro culture studies further confirmed the retention of collagen's native cyto- and biocompatibility. Overall, G-force-guided fibrillogenesis offers a robust and reproducible platform for fabricating advanced corneal equivalents, where optical transparency serves as a practical and quantifiable indicator of model health, supporting future applications in corneal tissue engineering and translational research.

ID 135 - Blood toxicity in human organotypic brain slice cultures - A first step toward human ex vivo modeling of SAH

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Subarachnoid hemorrhage (SAH), mainly caused by ruptured cerebral aneurysms, affects 6–9 per 100,000 people annually. SAH still carries high mortality (30–50%) and morbidity, primarily due to early brain injury and delayed cerebral ischemia. Pathophysiologically, SAH involves toxic blood components, especially iron.

This preliminary study investigates hemoglobin's effects on ex vivo murine hippocampal slices and human slice cultures, aiming to reduce in vivo experiments and establish human ex vivo models.

Murine hippocampal slices (P4–6) were cultured for 14 days and divided into control, 20 μM hemoglobin (Hb), and 50 μM Hb groups. Propidium iodide (PI) staining and multielectrode array (MEA) recordings assessed damage and functional integrity at baseline (T0) and 72 hours post-treatment (T72). Human slice cultures, prepared from surgical tissue, underwent analogous MEA recordings post-treatment. Histological analysis is ongoing.

At 72 hours, murine slices exposed to Hb showed greater damage intensity than controls. Baseline values were similar across groups. Human slice cultures exhibited a concentration-dependent reduction in signal intensity after Hb treatment, with no signal detected at 50 μM Hb in both murine and human slices. Data for 20 μM Hb was misleading, likely due to low sample size (human: n=27; murine: n=9).

PI staining revealed significant toxicity in murine slices, indicated by increased PI intensity. Neither murine nor human slices showed relevant functional activity after 50 μM Hb treatment. Both models successfully demonstrated cerebral toxicity of blood components. These findings may represent an initial step toward developing an ex vivo human SAH model, pending incorporation of additional pathophysiological factors.

ID 138 - SMAFIRA: deep machine learning to assist researchers with retrieval of 3R-relevant literature

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Retrieval of 3R-relevant information provides the basis for the consideration of methods to replace, reduce and refine the use of animals that is required by EU legislation. Such information retrieval entails a time-consuming screening of databases that mirror the current state of knowledge in experimental biomedicine. Here, we introduce SMAFIRA, an open access online tool to facilitate a 3R-relevant information retrieval that employs state-of-the-art language models from the field of deep learning. The tool is freely available at <https://smafira.bf3r.de>, and provides 3R-relevant literature citations in a ranked order, and classified with regard to the experimental model that was used. SMAFIRA fills in a significant gap in the collection of available 3R-relevant tools.

Reduction

ID 110 - From Virtual Reality (VR) to Augmented Reality (AR) - Reduction and Replacement of Animals in Laboratory Animal Science Skill Trainings

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Virtual Reality (VR) and, more recently, Augmented Reality (AR), have emerged as transformative technologies with promising applications across various fields, including laboratory animal science education. Traditionally, training in laboratory animal science includes the transfer of theoretical knowledge and the training of practical skills using live animals. After initial qualifications, competence must be achieved and maintained through repetition and routine use in experiments. Considering the 3Rs, VR/AR teaching and learning modules could help to reduce the number of animals used for education by incorporating them into existing courses and ongoing training, providing practical skills virtually before or instead of hands-on training on living animals.

Therefore, VR-teaching/learning modules were initially implemented into the practical skill training of FELASA Function A & D courses, where practical skills are also trained on live animals. FELASA course participants were provided with 33 VR teaching/learning modules for rodents. Using an online evaluation tool, participants were asked to evaluate the quality, usability, and the 3R potential of the alternative approaches. To test AR, as the next level of immersive teaching and learning, an AR module for the use of the isoflurane anesthesia unit used for rodents was generated. Initial evaluations by 12 experienced staff members yielded positive feedback.

Through the VR teaching and learning modules, the 3Rs were further integrated into our teaching and learning concept for the laboratory animal science courses. In addition, the use of AR teaching modules showed the potential of animal- and tutor-independent skill training, especially for practical, continuous professional development.

ID 111 - CRISPR Meets hiPSCs: Driving 3R-Based Innovation at the CECAD Research Center

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Human-induced pluripotent stem cells (hiPSCs), in combination with CRISPR/Cas genome editing, provide an advanced and ethically responsible platform for modelling human diseases, conducting drug screening, and advancing regenerative medicine. At the CECAD Transgenic Core Unit (TCU), we have successfully established reliable and efficient CRISPR-based editing protocols in hiPSCs, enabling both gene knock-out and knock-in applications. Building on our long-standing expertise in generating genetically modified mouse models, this development expands the TCU toward human-based experimental systems, establishing the hiPSC facility as a complementary pillar to its animal model platform. Through this initiative, the TCU actively embraces the 3R principles (Replacement, Reduction, Refinement) within its own operations. The infrastructure enables preliminary studies in human cells, allowing researchers to avoid animal experiments or refine research questions before animal model generation, thereby directly contributing to reduced animal experimentation numbers.

The central aim is to develop a standardized, accessible pipeline for hiPSC genome engineering across the CECAD Research Center. This platform will enable researchers to request customized genome-edited hiPSC lines for their projects, fostering campus-wide collaboration and ensuring high-quality, reproducible results. The TCU will provide comprehensive support – from CRISPR strategy design and genetic validation to screening of clones and quality control of the established cell line – lowering technical barriers for groups without prior stem cell experience. In addition to conventional knock-outs and small knock-ins, the platform will continue to integrate emerging tools such as degron-tagging, allowing rapid and reversible regulation of protein levels.

This open-access infrastructure will significantly strengthen human-based disease modelling, promote 3R implementation, and enhance research capacity at the University Hospital Cologne.

ID 123 - Cancer-SOLES: A Systematic Online Living Evidence Summary platform for animal model selection in cancer research to support the 3R principle

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Achieving reproducible and credible results in cancer research is challenged by the heterogeneity, publication volume, and biases inherent in preclinical cancer treatment efficacy studies. With more than 20,000 in vivo cancer studies published annually, systematic reviews are indispensable for synthesising evidence, selecting appropriate animal models, and reducing redundant experiments. However, traditional systematic reviews are time-consuming to conduct and often outdated when completed. To overcome these limitations, we developed Cancer-SOLES (Systematic Online Living Evidence Summary), a continuously updated, open-source platform that combines automation and text-mining to accelerate evidence synthesis. Cancer-SOLES is a curated database of more than 59,000 in vivo preclinical cancer studies. Cancer-SOLES employs an automated workflow, including regularly-updating systematic searches, automated deduplication, full-text retrieval and integration of metadata from sources such as OpenAlex. In addition, Cancer-SOLES incorporates automated identification of study quality indicators such as reporting standards, bias reduction, data/code sharing practices, and implements an interoperable workflow that can be used by other SOLES projects. In this context, we are validating Regular Expression dictionaries, ensuring high specificity, sensitivity, and precision, to automatically annotate key study characteristics including: animal model type, tumour features, small-molecule interventions, and outcomes. Pre-screened and curated content is displayed in a free, interactive, web-based dashboard that allows users to navigate evidence by study design features, evaluating the translational potential of drug-model combinations, and to visualise evidence gaps. This living systematic review framework provides a scalable approach for refining in vivo preclinical cancer research, improving translational validity, and reducing unnecessary animal use, supporting the 3R principles.

ID 126 - Retrospective Analysis of Participation and Practical Use of FELASA Courses: A Survey of former course participants and Principal Investigators

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Laboratory Animal Science (LAS) is a highly regulated field within the EU and Germany, requiring competence-oriented training aligned with the 3R principle (Reduce, Replace, Refine). The Institute for Laboratory Animal Science (ILAS) at the RWTH Aachen University continuously improves its FELASA Function A and D courses to ensure high-quality education and animal welfare standards. This retrospective analysis aimed to identify areas for improvement in course registration, content, and competence development, to provide the most suitable qualification and training while limiting the use of animals. Therefore, two online surveys were conducted addressing former course participants (2011-2025) and current Principal Investigators (PIs) listed at the ILAS. Results showed that former participants mostly applied their acquired skills after the course, particularly animal handling and humane killing methods, in academic or professional contexts. However, approximately 18% did not perform further animal experiments after completing the course. PIs emphasized the importance of broad skills and flexibility in staff qualification, with the university intranet identified as the main source of course information. Both groups rated e-learning formats as effective, while Virtual Reality (VR) content demonstrated promising potential for further reduction of animals used in practical skill trainings and in reinforcing practical competencies. The findings highlight the need for a clearer registration structure and better use of alternative training options to enhance efficiency and reduce the number of animals in these courses. Integration of emerging technologies, such as VR and Augmented Reality (AR), may play a pivotal role in enhancing the current course format.

ID 127 - 3R-compliant in vitro test system for Stem cell-based therapeutic approaches for corneal clouding diseases

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Corneal endothelial dysfunction affects ~4% of the population and can lead to blindness. Currently, corneal transplantation is the only treatment, but donor shortages highlight that alternative regenerative therapies are urgently needed. This study investigates the potential of eyelid-derived neural crest stem cells (Epi-NCSCs) to differentiate into functional corneal endothelial cells (CECs). Biomimetic collagen microstructures mimicking Descemet's membrane provide a physiologically relevant environment for stem cell differentiation, offering a promising strategy for personalized therapies and in vitro testing. To assess mechanotransductive differentiation, inverted DMT molds were fabricated via two-photon lithography and cast in a novel human collagen formulation. Epi-NCSCs were isolated and cultured on these biomimetic surfaces. Collagen, a key ECM component, supports cell adhesion and differentiation. Laminin coatings showed optimal adhesion and proliferation after 14 days. Immunofluorescence staining of Nestin and CD271, along with spindle-like morphology, confirmed effective isolation and stem cell maintenance. Adhesion to DMT induced polygonal shape, monolayer formation, and upregulation of key CEC markers like ZO-1 and Na/K-ATPase. In an initial transparency ex vivo test the stem-cell-derived CEC graft showed functional corneal epithelium. Epi-NCSC differentiation into CECs using appropriate artificial extracellular matrices is a promising tool in tissue engineering, regenerative medicine and 3R compliant in vitro test system in corneal diseases.

ID 131 - Innovative statistical methods for the reduction of animal numbers in animal experiments – simulation study investigating the feasibility of calculating direct effects from small sample sizes

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Introduction

Researchers face the challenge that on the one hand, out of ethical reasons, animal numbers must be reduced, while on the other hand reliable data must be obtained. We, with a simulation study, aimed to find out under which conditions reliable data can be obtained from small n-numbers.

Methods

In a simulation study in R we varied sample size, effect size and simulated normal and non-normally distributed data. We calculated confidence intervals with Monte Carlo Simulation, Bootstrap Resampling or z-scores, combined with robust estimators and with or without bias correction. Reliability of confidence intervals was assessed by type I error and balance.

Results

The type I error was smaller for larger sample sizes, with reliable results for samples as small as $n = 30$ and larger. The balance of confidence intervals similarly was reliable for sample sizes of $n = 30$ or larger, however was better for smaller effect sizes. For non-normally distributed data, the deviations from the ideal values were larger, mainly for increasing effect sizes. The method with the best results was Monte Carlo Simulation with robust estimator MLR with and without bias correction. Also, Monte Carlo Simulation with robust estimators MLM, ML and Mplus presented with reliable confidence intervals.

Conclusion

The study suggests that for sample sizes of $n = 30$ confidence intervals for direct effects can reliably be calculated. In the next step, investigations should be performed on the standardized indirect effect, with the aim of being able to investigate more complex relationships between variables.

ID 132 - A straightforward and resource-efficient 3D-printed bioreactor prototype for ex vivo culture of full-thickness porcine skin

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Ex vivo skin culture systems represent a promising alternative to in vivo animal experiments, aligning with the 3R principles. Due to its close structural and physiological resemblance to human skin, full-thickness porcine skin serves as a translational model for investigating pathophysiological mechanisms and therapeutic strategies in cutaneous disorders, congenital skin defects, and tissue regeneration. However, maintaining tissue viability ex vivo remains challenging due to limited nutrient and oxygen diffusion, necrosis in deeper layers, and the high medium demand of conventional systems.

Existing bioreactor platforms are often complex, costly, and difficult to reproduce. Many rely on specialized materials, intricate fluidics, and large medium volumes, limiting accessibility and standardization. Therefore, there is a clear need for simple, space-efficient, and resource-saving solutions that enable robust and reproducible skin culture.

Here, we present a first prototype of a custom-made, 3D-printed bioreactor designed for the ex vivo culture of full-thickness porcine skin. The system features a low-material, modular design that minimizes medium consumption while ensuring homogeneous oxygen and nutrient supply. All components can be fabricated from commercially available materials using standard 3D printing techniques, allowing for rapid and cost-effective replication. Preliminary tests demonstrate preserved tissue viability over several days of culture with significantly reduced medium requirements.

Our prototype provides a compact and reproducible platform in accordance with the 3R principle, utilizing skin grafts from other experiments (“Reduce”) and aiming to investigate human full-thickness grafts from plastic surgery as an alternative to animal use.

ID 137 - Monitoring the uptake of alternative methods in Germany

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According to official statistics, the number of animal experiments in Germany has decreased in recent years. The reasons for this decline are not yet clear. We investigated whether this trend correlates with an increase in the use of alternative methods. To monitor the uptake of in vitro methods, we have developed an automated classifier, based on the recently published SMAFIRA tool, that predicts the method used in published studies by the abstract. We have classified publications from Germany published between 2013-2024 in the research areas that use the most animals. Our first results indicate a small increase in the uptake of cell culture methodology in neuroscience and immunology whereas no change could be detected in oncology. We will perform further analyses and manual annotations to confirm these results. Monitoring the trends in animal research can help stakeholders to evaluate the efficacy of measures fostering the implementation of in vitro approaches and identify research areas that would benefit most from the development of alternative methods.

ID 139 - Loss of epidermal aPKC λ and p53 on STAT3 signaling: analyzing microenvironmental dependence using Interfollicular Epidermis Organoids

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Squamous cell carcinoma (SCC) is one of the leading causes of death worldwide and the second most common skin cancer in humans. The stratified mammalian skin epidermis forms an essential life-long self-renewing barrier that protects against external hazards such as UV radiation, infections, and mechanical stress. To ensure long-life protection, these barriers maintain functional homeostasis and proper tissue architecture even in the presence of cancer-causing mutations without forming tumors.

However, the exact mechanism how epidermal stem cells maintain skin homeostasis, barrier function, and enable p53-mutated clones to progress to actinic keratosis, characterized by field cancerization and invasive skin SCC, is not clear. We have previously identified the polarity protein atypical protein kinase C (aPKC λ) as a key regulator of epidermal stem cell homeostasis, acting as a rheostat for cell and tissue mechanics to regulate epidermal cell fate, shape, and skin barrier function (Niessen et al., 2013, Rüksam et al., 2023). Recent work from the Niessen lab showed that epidermal aPKC λ in the absence of p53 preserves physiological skin regeneration and stem cell fate while inhibiting STAT3-driven inflammatory signaling, epithelial dysfunction, and immunosuppressive microenvironment formation enriched in tumor-associated macrophages, thereby preventing field cancerization and SCC. In agreement, reduced aPKC λ in human SCC correlates with accelerated metastasis (Persa et al. in preparation).

To dissect whether epidermal signaling is dependent on or independent of the dermal microenvironment, and how STAT3 signaling is modulated during interfollicular epidermis (IFE) organoid differentiation, we employ this organoid system to investigate the contribution of the local niche to polarity-driven signaling alterations at high cellular resolution. This approach aligns with the 3R principles by reducing animal experimentation while providing a versatile platform to study early tumorigenic changes and evaluate targeted interventions modulating microenvironment-dependent STAT3 signaling.

Refinement

ID 108 - Handle with Care: Successful Implementation of Cup and Tunnel Handling

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To minimize stress for our rodents in animal husbandry and experimentation, we regularly review and adapt our handling methods. This reduces stress for the mice and rats and makes handling them much easier, which is a major advantage, especially during application. It also makes daily interaction with the animals much easier.

Our goal was to establish cup and tunnel handling in animal housing and experimentation. To this end, various techniques and materials were tested and adapted according to the strain, individual animal and conditions of the experiment.

Implementation: Various cup and tunnel handling methods were tested in breeding and husbandry to determine their suitability for the lines and individual animals. The project was then reviewed and the team was trained.

Conclusion: The procedure for establishment, as agreed with the animal facility management and the animal welfare officers/committee, was successfully implemented in animal housing. Presenting the methods as part of a training course for animal keepers went well, and all the keepers are interested in implementing them.

Outlook: Regular training courses for employees will now follow, and the offer will also be extended to scientists, so that the methods can be used in experiments.

ID 113 - Optimizing personalized humanized mice lacking murine MHCs with busulfan preconditioning to track the diversity of human T cells

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Immunodeficient mice transplanted with human cord blood CD34+ cells support long-term development of human immune cells, including polyclonal T cells with varied T cell receptors (TCRs). However, mouse major histocompatibility complexes (MHCs) may activate human T cells through xenograft reactivity, complicating identification of antigen-specific ones. We compared development of human T lymphocytes derived from 10 cord blood donors in NSG (NOD Scid Gamma) vs NSG-DKO (NSG lacking murine MHCs) mice. Additionally, irradiation vs busulfan preconditioning protocol was assessed: Busulfan-treated mice displayed higher weight gain than irradiated mice, indicating higher overall vitality. Mice in different cohorts showed comparable huCD45+ cell engraftment and huCD3+ cell long-term reconstitution up to 20 weeks (averaging 66% and 30%, respectively). Significantly higher levels of central and effector memory T cells were found in NSG-DKO, while NSG mice had more naive T cells. Furthermore, high serum levels of human Granzyme A, Granzyme B and IFN- γ were observed in busulfan-treated NSG-DKO mice, which were further confirmed by single-cell mRNA sequencing. Moreover, human T cells with high MKI67 expression were primarily found in these mice. Furthermore, more conserved and expanded α/β TCR repertoire was found in busulfan-treated NSG-DKO in comparison to NSG mice. In summary, humanized NSG-DKO mice pretreated with busulfan exhibit more proliferative and mature T cell repertoire. This 3R-compliant model (refined to avoid xenograft reactivities, reduced to one cord blood per sample per mouse to represent one patient) can be used to interrogate human-to-human HLA-mediated T cell responses and could potentially serve as replacement for non-human primate models.

Poster | ID 113

ID 116 - Training-Based Housing Refinement Reduces Stress and Improves Welfare in Laboratory Rabbits

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Background: Rabbits are commonly used as an animal model in biomedical research and in education and training. This project aimed to refine housing-conditions and establish a training-protocol to improve welfare and reduce stress responses in rabbits under experimental conditions.

Implementation: 1. Housing refinement: Over recent years, the housing was continuously adapted to meet updated legal requirements and to enhance animal welfare. The former single-housing in wire-floor cages with pelleted feed only was replaced by group-housing in spacious cages with perforated plastic floors (FA Scanbur TYPE EC2), hiding places, and ad libitum hay provision. In addition, a silicate composite floor for group-housing was installed, windows and drains were covered, and additional shelters were provided as environmental enrichment. Animals were gradually habituated to the new housing and social environment. 2. Training protocol: To further reduce stress, a medical training protocol based on clicker training was implemented. Rabbits were trained to follow a target stick and voluntarily enter a box, allowing handling or examinations without the need for stressful capture.

Conclusion and Outlook: These measures significantly improved rabbit welfare and handling in our facility. Floor-housing is now the standard after an adaptation phase, while cage-housing is limited to specific experimental needs (e.g., postoperative monitoring). Trained animals show reduced aversive reactions and increased cooperation during handling. The poster presents examples of individual training progress and adaptations of the protocol. Future work will expand training to additional experimental procedures to further minimize stress.

ID 124 - Making Waves: Reassessing the Forced Swim Test

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The Porsolt Forced Swim Test (FST), primarily utilized for phenotyping and screening novel antidepressants in rodents, is one of the most widely used tests in depression research.

Despite its popularity, this test has received increasing criticism mainly due to welfare concerns and interpretation discrepancies. To ensure both animal welfare and a reliable interpretation of reproducible results of this test, experiments must be designed and analyzed with the utmost rigor in order to use it appropriately.

To assist with this, we provide a solid, accessible database of all experimental setups and tested drugs in rats to avoid duplication of experiments and support future study design with adequate protocols that maximize animal welfare without neglecting the research focus. While many reviews have covered the FST, none is as comprehensive as our systematic mapping review, covering the 3,907 manuscripts published on the FST in rats since 1977.

As the field remains divided over whether the FST portrays depressive-like states or adaptive coping and learning, we aim to inspire a consensus on the interpretation of the FST, to strengthen its translational validity and relevance, as potential part of a wider test battery for depression.

Our intention is to raise awareness for the critical aspects of this test, to balance the current reliance on this test with the objective to minimize animal distress until valid non-animal alternatives are developed, which also cover the behavioral dimension of depression

ID 125 - Digital, semi-automated pipeline for efficient score sheet documentation required for monitoring burdened animal strains

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Introduction

Breeding of burdened animal lines requires approval from the local authorities (“Landesamt für Verbraucherschutz und Ernährung - LAVE”) and involves regular scoring of all animals. Depending on the breeding scale and number of lines, organizing the documentation, i.e., animal scores, can be very time- and labor-consuming.

Aim

To improve the efficiency of scoring documentation, our goal was to establish a semi-automated digital pipeline.

Methods

We use exported animal data from Tick@Lab, an animal management software used in Aachen, to Excel to execute our custom-developed Excel macros. These macros execute 3 major functions: 1) generating mouse individual scoresheets; 2) generating a table listing all mice required for scoring; 3) updating the weights/scores from the summary table to each scoresheet file and inserting the electronic signature of the responsible person. At the end of breeding, the fate, e.g., experiment or sacrifice, of each animal is added, the scoresheet is reviewed, signed, and stored as a PDF file at a secure data server.

Results

The macro enables efficient documentation and data collection, storage, and accessibility. Weight, total scores, and the severity are calculated automatically. This method saves estimated around 50–80 % of the time previously required for preparing scoresheets, given that we have been monitoring approximately 80–120 mice per week.

Discussion & conclusion

Digitalization with our semi-automated pipeline helps fulfil legal documentation requirements more efficiently and was approved by the local authorities (LAVE). This approach reduces the ever-increasing administrative burden while adhering to all legal requirements, thereby strongly supporting and reducing time-burden of scientific staff, and aligns with the principles of a “culture of care.”

ID 128 - Building a Rat Playpen: A Culture of Care Project for Animals and Apprentices

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Promoting a Culture of Care in laboratory animal facilities requires initiatives that support both animal welfare and the development of the people who care for them. In this project, animal caretaker apprentices collaborated with veterinarians to design and construct a rat playpen (“Rattenspielplatz”) as an enrichment and refinement measure. The goal was to provide rats with increased opportunities for natural behaviours such as exploration, play, and social interaction, while simultaneously strengthening the apprentices’ awareness of animal welfare and fostering confidence, teamwork, and professional skills.

The apprentices were encouraged to take responsibility for every stage of the project. They explored the biological and legal background of rat husbandry, planned the playpen design, and sourced and repurposed materials by coordinating with different departments across the university, such as locksmiths and carpenters. They also considered hygiene and safety aspects. The apprentices practiced using carpentry tools, solved problems during construction, and presented their ideas to peers and senior staff. Through this process, they developed initiative, communication skills, and ownership of their work.

The outcomes were twofold. The rats gained access to a stimulating, varied environment, while the apprentices experienced personal growth and a stronger sense of belonging within the animal facility. The project built bridges between apprentices, senior caretakers, and veterinarian staff.

By combining refinement with staff engagement, the rat playpen project demonstrates how small-scale initiatives can meaningfully contribute to both animal well-being and the cultivation of a caring, welfare-oriented culture.

ID 134 - Refining Humane-Endpoint Identification with ARIMA Forecasting

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The refinement of the 3R principle requires an accurate severity assessment with robust, objective, and model-specific parameters and methods. The definition of humane endpoints is crucial to reducing suffering and pain. We want to expand the application of the RELative Severity Assessment (RELSA) procedure to predict humane endpoints in individual animals. For this, we built the foRcast tool, which is an AutoRegressive Integrated Moving Average (ARIMA)-based forecasting model to predict developments based on previous observations. We aim to identify individual animals at risk of humane-endpoint thresholds to refine experimental procedures.

To evaluate and validate the performance of this tool, we reanalyzed two models, one for sepsis and the other for colitis, focusing on individuals who reached humane endpoints.

The humane-endpoint RELSA scores ranged from 0.73 to 0.93, with predictions achieving a root mean square error (RMSE) of 0.07. The prediction interval coverage probability (PICP) reached 80 %. Only one forecast showed a larger deviation from the actual value, which we assigned to a rapid deterioration of the mouse's well-being between the measurements.

These results suggest that the foRcast tool makes accurate predictions but highlight the need for higher-resolution measurements than daily aggregations to detect changes in well-being early. The latter is underlined by the intrinsic limitation of ARIMA models of not being able to predict drastic changes. In combination with the definition of humane endpoints on the RELSA scale, this tool allows the identification of animals at humane-endpoint thresholds. It is a valuable addition to already existing refinement tools.

New Thinking in 3Rs

ID 102 - Mealworms (*Tenebrio molitor*) in veterinary preclinical education: Bridging best educational practice and ethical responsibility

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The use of animals in veterinary preclinical education remains an indispensable tool for teaching future veterinarians about critical physiological functions. However, to acknowledge animal welfare concerns and adhere to the 3R principle (Replace, Reduce, Refine), educators have implemented innovative approaches to minimize or substitute traditional animal experiments for teaching purposes. These include using patient-derived materials as well as *in silico* models. While computer simulations are valuable educational tools for demonstrating tissue responses to various physiological and pathophysiological conditions, annual student surveys show greater popularity and learning success in courses incorporating hands-on practical experiments compared to theoretical exercises. Therefore, we sought to find novel alternatives that balance educational efficacy with ethical responsibility.

In physiology seminars, veterinary students engage in practical experiments across eleven different courses to grasp physiological functions. One such method, indirect calorimetry allows investigation of an organism's metabolism and energy homeostasis by measuring oxygen consumption and carbon dioxide production. In this setting, small groups of students explore the impact of nutrients and stressors, and discuss metabolism-associated diseases (e.g., diabetes mellitus or hypo-, hyperthyroidism) across species. By integrating mealworms (*Tenebrio molitor*) into practical seminars, students gain concrete experience in handling living animals and conduct measurements and analyses of vital parameters independently. This approach enables students to meet essential learning outcomes without resorting to higher-order animals.

Overall, the use of mealworms for indirect calorimetry in veterinary education supports the dual goals of maintaining high educational standards while promoting ethical practices.

ID 106 - Are German Researchers Willing to Involve Animal Care Staff in Scientific Discussions? Building Transparent Communication Together

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Animal welfare and research integrity in laboratory settings rely on effective collaboration between researchers and animal care staff. This study investigated the positions of German researchers to include animal care staff in scientific discussions about experimental planning and conduct with the goal of developing transparent communication and organizational culture. We surveyed researchers from 23 European countries, emphasizing here the German responses on communication and willingness to involve animal care staff in scientific discussions. Findings indicate that while 84% of German participants rated internal communication between researchers and animal care staff as average or better, meaningful participation is not consistently translated into frequent joint training or engaging meetings. Notably, 38% and 50% of the researchers reported “never” as the frequency of Culture of Care and mental health meetings, respectively, highlighting missed opportunities for staff engagement. Feedback meetings and continuous education also remained infrequent despite agreement on tackling and improving communication. Despite that, German researchers recognized the positive impact of involving animal care staff, with over 70% acknowledging improvements in the quality of science and job satisfaction. The study pointed out the necessity for early involvement in experimental planning and conduct, and the recognition of contributions of animal care staff. Therefore, we suggest that improvements in institutional leadership support, transparent structures, continuing education, and the integration of every member of the staff in discussions about scientific planning and conduct are essential for incorporating a sustainable culture of care that considers overall welfare and enhances scientific outcomes.

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ID 107 - Pigeons as a model system in Cognitive Neuroscience: Achievements on Refinement and Reduction

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Despite fundamental differences in neuroanatomy between avian and mammalian brains, birds exhibit cognitive abilities on par with mammals across abilities such as learning and memory, reasoning, metacognition, and theory of mind. Understanding the neural mechanisms underlying this functional convergence remains a key question in comparative neuroscience. The Department of Biopsychology at Ruhr University Bochum, particularly through DFG-funded SFB 1280 subproject A01, has pioneered three methodological innovations that advance avian cognitive neuroscience while rigorously implementing the 3R principles (Replacement, Reduction, Refinement).

Small Animal Magnetic Resonance Imaging (MRI): Non-invasive neuroimaging enables repeated measurements within individual subjects, reducing animal requirements while enhancing statistical power through within-subject experimental designs. This approach improves data quality and eliminates interindividual variability inherent to between-subject comparisons.

Optogenetics: This technique provides unparalleled temporal precision for causal manipulation of neural circuits. By enabling direct intervention in defined neural populations within individual animals, optogenetics yields causal insights that surpass correlational findings from conventional methods, substantially reducing the number of animals required for mechanistic studies.

High-Density Electrophysiology: Silicon probes with numerous recording sites, combined with computer-guided motorized stereotaxy, increase neuronal yield per animal while minimizing surgical placement errors. These technical improvements reduce animal numbers and surgery duration. Furthermore, our laboratory has established balanced anesthesia protocols specifically optimized for avian species, exemplifying refinement principles in experimental practice.

These integrated methodological advances enable rigorous investigation of the neural basis of avian cognition while significantly reducing animal use and enhancing animal welfare in behavioral neuroscience research.

ID 109 - Gut microbiota as a decisive factor in immune regulation and stroke outcome

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Gut microbiota play a central role in directing immune cell polarization in healthy and pathological conditions. Acting as a living bioreactor, the microbiome generates not only energy precursors but also immunomodulatory molecules. Previously, we showed that gut microbiota differences in mice from different commercial breeders lead to markedly different immune responses after stroke. In addition, stroke leads to persistent dysbiosis of gut microbiota, which exacerbates brain injury. Mechanistically, this dysbiotic state promotes long-term proinflammatory T-cell polarization in both the intestinal immune compartment and ischemic brain. Importantly, polymorphonuclear neutrophils are the first systemic responders to ischemic stroke and infiltrate the brain in large numbers. In our latest work, we show that depletion of gut microbiota—either through broad-spectrum antibiotics or germ-free conditions—attenuates neutrophil activation after stroke. This disarming of neutrophil responses was associated with decreased expression of inflammatory genes in the brain, reduced brain vascular thrombosis, smaller infarct volumes, and improved behavioral outcomes. In summary, our findings reveal that gut microbiota plays a critical role in post-stroke innate and adaptive immune activation and directly aggravates inflammatory brain degeneration.

ID 120 - Advancing the 3Rs at a Medical Faculty: Pioneering Projects in Replacement, Reduction, and Refinement

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The responsible implementation of Replacement, Reduction, and Refinement is crucial for ethical and high-quality biomedical research. Charité - Universitätsmedizin Berlin, a leading institution in translational medicine, is driving three lighthouse projects within Charité 3R that significantly advance the 3Rs principles.

Replacement: Turning Clinical Waste into Scientific Gold – The Primary Tissue Pipeline: In many clinical procedures, valuable human tissue is discarded despite its potential to revolutionize biomedical research. The Primary Tissue Pipeline (PTP) addresses this gap by establishing a service platform that facilitates access to living human bioprobes for research. This initiative enhances the use of organoid models and direct tissue cultures, promoting personalized medicine and regenerative therapies. By bridging the gap between healthcare and research, the PTP provides ethically sourced, high-quality tissue samples, accelerating scientific discovery while reducing reliance on animal models.

Reduction: EPIC3R – Experimental Imaging at Charité: Preclinical imaging plays a pivotal role in reducing the number of animals required for research while increasing the scientific value of studies. EPIC3R integrates multimodal imaging technologies to refine experimental designs, enabling longitudinal studies that minimize animal use. By offering a centralized infrastructure for small animal imaging, including e.g. MRI, PET, CT, and intravital microscopy, EPIC3R supports translational research and contributes to digital in silico modeling. This initiative fosters innovative approaches that optimize preclinical studies and enhance clinical translation.

Refinement: Refinement Sharepoint – A Digital Platform for Enhanced Animal Welfare: Refinement strategies aim to improve animal welfare and experimental procedures. The Refinement Sharepoint platform at Charité provides a collaborative, in-house knowledgesharing system for researchers, veterinarians, and technicians. This internal platform offers protocols, instructional videos, and expert contacts to facilitate best practices in humane animal handling and procedural refinement. By fostering a protected and supportive environment, the Refinement Sharepoint ensures continuous improvement in animal welfare while maintaining scientific rigor.

These initiatives underscore Charité's commitment to responsible research and the advancement of the 3Rs. By implementing sustainable structures that enhance replacement, reduction and refinement, these projects contribute to ethical, innovative, and high-impact biomedical research.

ID 136 - Systematic assessment of outsourcing of animal experiments from Germany

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According to official statistics, the number of animal experiments in Germany has decreased in recent years. The reasons for this decline are not yet clear. Individual reports suggest that relocation abroad could play a critical role in this reduction, but further research is needed to understand its implication.

To quantify a potential outsourcing of animal research to other countries, we have extracted the location of performed *in vivo* experiments on rats from publications between 2014 and 2024. We only selected publications, where the first and the last author had a German affiliation. This approach allowed us to evaluate trends across different fields of research. We have screened 6226 studies, and extracted the information of the location from 3805 included studies.

Although we can observe a slow but steady increase, the number of rat experiments performed outside of Germany from our evaluated studies remains marginal. Experiments performed outside of Germany were mainly performed either in the European Union or in countries that had comparable animal protection legislation, such as the UK, Norway and Switzerland.

Using our method, it is not possible to distinguish between classical collaboration and outsourcing aimed at avoiding costs or administrative tasks related to animal experimentation. Further research and analysis are needed to understand the reasons for outsourcing, compare global developments, and analyse different areas of research. Automating the process could help provide the answers to these questions.