Kompetenznetzwerk NRW

1st Annual Meeting

December 7th - 8th, 2023

Kasteel Bloemendal, Vaals, Netherlands

Abstract Book

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Refinement

New Thinking in 3Rs

Welcome

Dear participant,

Our network officially started in December 2022 with the kick-off event in Bonn. Since then, we, as a whole network, have been taking steps towards our aim of "**medical progress in line with the best possible animal welfare**" in four main activity areas: science management, networking, education and communication. After a successful first year of 3R-Competence Network, we are happy to welcome you to the 1st Annual Meeting.

Our network member Medical Faculty of the RWTH Aachen University is hosting the 1st Annual Meeting at Kasteel Bloemendal in Vaals, Netherlands, on the December 7th – 8th, 2023.

At this 2-day event, you will not only hear from the recent advances in the field of 3R research but also have a chance to network with your colleagues in the field. The meeting program offers sessions themed around replace, reduce, refine. There will be many scientific talks including the keynote speaker, David Morton, Professor Emeritus of Biomedical Science and Ethics, will share his lecture in the fields of animal welfare and 3Rs.

In the evening, we invite you to the Get-Together social event at Das Liebig in Aachen. The prestigious Animal Welfare Prize of RWTH Aachen University is awarded at this event. It is a special opportunity to acknowledge outstanding contributions to animal welfare while strengthening our network.

We are very excited to convene at the very first network-wide meeting. There are many reasons to be part of it. We are very much looking forward to seeing you among us!

Best wishes,

René Tolba
- Representative of Network Member Aachen -
&
Michael To Vinh, Antonia Henderson, Lena Kistermann, Burak Bali
- Office of the 3R Competence Network NRW -

About

3R Kompetenznetzwerk NRW

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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www.3r-netzwerk.nrw

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Program

Day 1 | Thursday, December 7th, 2023

08:00 **Registration**

09:00 **Welcome Ceremony**

09:30 **Session 1:** Presentations of the network members
Aachen, Bielefeld, Bochum, Bonn, Düsseldorf, Essen, Cologne, Münster

11:15 -Coffee Break-

11:30 **Session 2:** Reduction

3R by Non-invasive Magnetic Resonance Techniques
Ulrich Flögel (Düsseldorf)

Pain research in a petri dish? - Chances and limitations of cell culture models in pain research
Stephan Leisengang (Giessen)

Living up to the 3Rs – Non-invasive imaging in experimental animal research
Lydia Wachsmuth (Münster)

13:00 -Lunch Break-

14:00 **Session 3:** New thinking in 3R's

On the way to 3Rs with the EQIPD Quality System
Björn Gerlach (Mannheim)

CIRS-LAS – Redefining animal welfare: the impact of critical incident reflection
Sabine Bischoff (Jena)

3R in kidney research
Bernhard Schermer (Cologne)

15:30 Poster Session

16:30 **Session 4:** Refinement

Refinement research - State of Affairs?!
Stephanie Krämer (Giessen)

Components for Establishing Refinement Strategies in Large Animal Models: A practical example
Lisa Ernst (Aachen)

Refinement and Validity of a Cardiological Surgical Mouse Model
Marta Stei (Bonn)

18:00 **End of scientific sessions**

Program

Day 1 | Thursday, December 7th, 2023

19:00 **Bus transfer to Das Liebig from Kasteel Bloemendal**

19:30 **Get-Together Evening with fingerfood and drinks**

20:00 **Award ceremony of the "Aachener Tierschutzpreis 2023"**

20:30 **3Rs for you - Rejoin, Refresh & Rest**

from **End of the first day**
22:00 **Bus transfer to Aachen city center from Das Liebig**

Day 2 | Friday, December 8th, 2023

08:30 **Registration**

09:00 **Session 5: Keynote Lecture**

The use of clinical signs to determine the severity of experimental procedures on an animal
David Morton (Birmingham, GB)

10:30 -Coffee Break-

11:30 **Session 6: Replacement**

Human Microglia Models: An Alternative to Animal Testing?
Johannes Wurm (Bielefeld)

Do engineered human tissue constructs for in vitro biocompatibility analysis achieve sufficient complexity to replace animal testing? – Painful and instructive lessons from HyMedPoly and EVPRO
Jochen Salber (Bochum)

Adding two P's to the three R's – How about prospective prevention in animal research?
Björn Scheffler (Essen)

13:00 **Wrap-up and Closing Remarks**

13:15 **End of the 1st Annual Meeting**

Keynote lecture

Keynote lecture

David Morton

University of Birmingham

My talk will show how severity can be measured in many types of experiments, particularly those that show recognisable graded physiological responses to the experimental procedures, such as a change in body weight or body temperature. These experiments would most likely be classified as sub-acute or chronic studies. This approach would be more difficult, but not impossible, for acute and hyper-acute changes. In order to use this approach, I will describe the sorts of clinical signs of abnormality that can be selected and the underlying hypothesis that the greater the degree of change from normal, the more likely it is that an animal will be suffering in some way or another. The clinical signs shown by an animal will vary according to the type of work being undertaken, but in all cases they must be objective, reliably observable, and reliably measurable on a continuous or discontinuous (categorical) scale. I will illustrate with worked examples when a severity limit has been breached as well as how to calculate the overall severity of a project to comply with humane and legislative requirements. An additional advantage is that at the same time as scoring these adverse effects, this approach can be used to assess the effectiveness of any analgesic therapy or any other form of pain and distress relief. The approach can also be used to determine a humane endpoint or a humane intervention point for an experiment, rather than wait for an animal to die in lethality experiments or suffer greatly.

Replacement

Human Microglia Models: An Alternative to Animal Testing?

Johannes Wurm

AG Anatomie und Zellbiologie, Bielefeld University, Bielefeld, Germany

Objective

Microglia are the resident immune cells of the central nervous system and play crucial roles in maintaining brain homeostasis, defending against pathological insults and participating in brain development and repair. The differentiation of microglia as well as their homeostatic signaling in the developing and adult brain is regulated by several secreted factors, such as IL-34, M-CSF, IL-6 and TGF-β. However, most studies have been conducted in primary rodent cells due to the lack of availability of healthy human microglia. New in vitro cell models to study human microglia have been established recently including “induced microglia-like cells” (MGLC) differentiated from peripheral blood monocytes.

Methods

In order to investigate the effects of cytokines on human microglia we isolated human peripheral blood-derived monocytes by gradient centrifugation followed by consequent in vitro differentiaton into MGLCs for 14 days. Cytokine-dependent changes in gene expression were analyzed using a customized “human microglia” nCounter® profiling panel by nanostring that includes 144 genes.

Results

Morphological, functional and transcriptional analyses showed significant changes between monocytes and MGLC underlining the differentiation and transition into microglia like cells. Our data show a direct influence of different cytokines such as TGF-β on the differentiation of MGLCs with implications for future in vitro differentiation protocols.

Conclusion

Despite the different origin of microglia and monocytes, human peripheral blood monocytes can be transitioned into MGLCs that show similarities to animal derived microglia in cell culture and may serve as a model for the investigation of the biology of human microglia including the effects of signaling molecule TGF-β.

Do engineered human tissue constructs for in vitro biocompatibility analysis achieve sufficient complexity to replace animal testing? – Painful and instructive lessons from HyMedPoly and EVPRO

Jochen Salber

Department of Experimental Surgery, Centre for Clinical Research, Ruhr University Bochum, Bochum, Germany

Arthroplasty in patients with advanced osteoarthritis carries two critical complications that can lead to loosening and require at least one revision operation: septic or aseptic endoprosthesis loosening. The undisputed cause of the septic variant is based on an implant-associated infection. In the aseptic variant, the cause has not really been resolved, but a strongly held hypothesis focuses on an abrasion particle-induced, proinflammatory cause of osteolysis at the implant-bone interface. Resistant microorganisms have led to a global resistance crisis in recent decades. This must be seen as extremely critical, especially in the context of modern medicine and the use of foreign materials in patients. The development of new complementary prevention and antimicrobial treatment strategies is imperative. In aseptic, "particle"-induced osteolysis, a local, immunomodulatory approach should be taken. Before antimicrobial or immunomodulatory biomaterials can be used as release systems, implant coatings or temporary scaffolds in humans, their biocompatibility must be preclinically proven. Using today's biofabrication and information processing technologies, biocompatibility testing of such biomaterials in 3D tissue equivalents will be more comprehensive, faster, less expensive and significantly reduce the use of experimental animals. Over the past six years, the Experimental Surgery Unit at the University Hospital Bochum has focused its research on improved, preclinical in vitro biocompatibility analysis using human cell-based 3D tissue constructs within the framework of two EU-funded consortial projects (HyMedPoly, EVPRO), taking into account the European 3Rs initiative. This short talk will give the auditorium an insight into the ups and downs of this arduous but meaningful journey.

Adding two P's to the three R's – How about prospective prevention in animal research?

Björn Scheffler

[1]DKFZ Division Translational Neurooncology at the West German Cancer Center (WTZ), University Hospital Essen and University Duisburg-Essen, Essen, Germany
[2]German Cancer Research Center (DKFZ), Heidelberg Germany

Translational oncology is cancer research that bridges the gap between laboratory research and patient care. It is a two-way connection where forward translation implements basic research ideas into practice and reverse translation refers to the scientific deciphering of clinical conditions in model systems of disease. It also involves the spotting and critical interrogation of innovative treatment concepts. One of these is radioligand therapy of solid tumors, representing a rapidly evolving clinical approach in the field of precision medicine. In a recent effort to develop a reverse-translational platform technology for the molecular study of radioligand therapy in context-dependent human tumor samples, we realized that comparable animal models for benchmarking of our approach do not exist. With this talk, I would like to stimulate a discussion on the prospective design and needs of animal research in selected circumstances of innovative reverse translational oncology.

Application of human full-thickness skin models as an in vitro replacement in dermatological research

Marquardt, Yvonne; Huth, Sebastian; Heise, Ruth; Huth, Laura; Jansen, Manuela; Baron, Jens Malte

RWTH Aachen University, Aachen, Germany

Regarding the EU guidelines on animal protection and the “3R concept”, human skin models offer a good alternative for in vivo experiments as they represent many aspects of the physiological and anatomical aspects of human skin. Since the skin of laboratory animals differs from human skin in its biological properties, the use of animal models represents a limited alternative. Epidermal skin models have long been used in the cosmetic industry as a replacement for animal testing. However, three-dimensional human full-thickness skin models offer more complex structures that are comparable to skin in vivo and are established as an in vitro test system in biomedical questions. They consist of a dermis with fibroblasts and an overlying epidermis made of keratinocytes. The cultivation of the skin model at the air-liquid interphase induces the stratification of the epidermis and the physiological development of the stratum corneum. A well-developed basement membrane zone with cell interaction between the dermal and epidermal part, as well as an epidermal barrier function are given. Optimization of full-thickness skin models by integrating additional cell types offers even more complex study models for scientific research. These include inflammatory cells such macrophages, tumor cells or microvascular endothelial cells. Chronic inflammatory skin diseases such as psoriasis and atopic dermatitis are among the most common inflammatory skin diseases. In order to understand the effects of novel therapies with biologicals, 3D skin models can be stimulated with a disease specific pro-inflammatory cytokine cocktail prior to topical or systemic treatment.

Towards Ethical Neuroscience: Human Brain Slice Cultures as Substitutes for experiments in animals

Bak, Aniella[1]; van Loo, Karen[1]; Schmied, Katharina[1]; Peter, Sabrina[1]; Schünemann, Kerstin[1]; Schwarz, Niklas[2]; Tauber, Simone[3]; Wuttke, Thomas[2]; Kampa, Björn[4]; Weber, Yvonne[1]; Wolking, Stefan[1]; Feldemeyer, Dirk[5]; Qi, Guanxiao[5]; Yang, Danqing[5]; Ort, Jonas[6]; Höllig, Anke[6]; Delev, Daniel[6]; Koch, Henner[1]

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The investigation of the human brain at the cellular and microcircuit level remains challenging due to the fragile viability of neuronal tissue, inter- and intra-variability of the samples, and limited availability of human brain material. Here, we present an optimized work-up to use resected tissue from brain surgeries for live cell experiments in vitro, aligning with the principles of the "3 Rs" concept in animal research (Replacement, Reduction, Refinement).

We provide a reworked, detailed protocol of the production, culturing, and viral transduction of human organotypic brain slice cultures for research purposes. Our approach strongly emphasizes the "Replacement" aspect of the 3 R concept, as it completely replaces the need for animal experiments in some instances. To illustrate the potential, we offer examples of how this technique can be used to implement disease states in the well-known ischemia model, a model to study glioblastoma, and investigations of epilepsy mechanisms previously conducted in animal models into human cerebral slices.

Additionally, we highlight the critical pitfalls of the culturing process of the human brain tissue and present results on viral expression, single-cell Patch-Clamp recordings, and multi-electrode array recordings over a prolonged period of time. Furthermore, our statistics show that brain tissue from patients of any age and morbidity can be used for organotypic brain slice cultures if carefully selected.

In summary, organotypic human brain slice cultures represent a powerful and ethical tool for basic neuroscience and disease modelling, focusing on replacing animal experiments and offering a time course of three weeks for research.

Comparative analysis of human and rat neural stem-/progenitor cell functions during developmental time-dependent genotoxin exposure in vitro.

Pahl, Melanie[1]; Dittmann, Lindsay[1]; Klose, Jördis[1,2]; Koch, Katharina[1,2]; Fritsche, Ellen[1,2,3]

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Genotoxin exposure followed by unsuccessful error-prone DNA repair might alter brain development by disrupting the proliferation and differentiation capacity of neural progenitor cells (NPCs). Depending on the substance, different DNA repair pathways, molecular mechanisms and cellular responses are involved. Moreover, sensitivity of hNPCs towards genotoxic damage could change during development due to changes in DNA repair capacities. However, available data is mostly based on rodent in vivo studies or cancer in vitro cell models. Thereby, the developmental time-dependent effects of genotoxic substances on neurodevelopmental key events modelled using hNPCs are insufficiently characterized.

To address this data gap, we comparatively analyzed the effects of four model genotoxins i.e. N-Ethyl-N-Nitrosourea (ENU), Hydroxyurea (HU), 5 Azacytidine (5-Aza), and Etoposide (ETOP) using NPC-based 3D in vitro models that represent neurodevelopmental key events at different developmental stages of human and rat brain development. Our results indicate distinct effects of genotoxins on neurodevelopmental key events modelled in vitro. In both species, NPC proliferation was reduced by HU, 5-Aza, and ETOP, while all four genotoxins disturbed NPC differentiation. Interestingly, the sensitivity of the key event did not only depend on the genotoxic MoA, but also on the exposure time point and species.

We showed that NPC-based in vitro models can identify genotoxins with different modes-of-action causing characteristic neurosphere endophenotypes depending on the exposure time point. The study of more genotoxicity-related endpoints is needed to gain a deeper understanding of the developmental stage-specific susceptibility to genotoxins during human versus rat brain development and develop novel human-relevant testing strategies.

Leigh syndrome drug discovery with induced neurons and midbrain organoids unveils repositionable compounds

Menacho, Carmen[1]; Okawa, Satoshi[2]; Petersilie, Laura[1]; Rose, Christine[1]; Del Sol, Antonio[2]; Prigione, Alessandro[1]

[1]HHU, Germany; [2]Luxemburg University

In our laboratory, we employ human induced pluripotent stem cells (iPSCs) to establish alternative disease models for rare genetic pediatric neurological diseases to conduct drug discovery studies. Our work aims to replace animal testing and to develop human cell based platforms that can be used to screen drugs for conditions in which animal models are not available or do not fully recapitulate the features observed in patients.

Here, we focus on mutations in mitochondrial complex IV assembly factor SURF1 causes Leigh syndrome (LS), a rare incurable neurodevelopmental disorder typically affecting midbrain and basal ganglia structures. We previously generated a model of LS using using isogenic iPSC lines engineered with CRISPR/Cas9 to carry SURF1 mutations in either a control or patient. Here, we aimed to discover treatment strategies ameliorating the mutation-specific defects of SURF1-mutant neurons. We used single-cell transcriptomic datasets from SURF1-mutant brain organoids to feed a machine-learning algorithm that identified druggable molecular targets affected by SURF1 mutations. We validated the compounds in SURF1 mutant neurons and midbrain organoids. We identified two FDA-approved compound capable of rescuing neuronal branching in a dose-dependent manner in SURF1-mutant induced neurons. Next, we validated the drugs in midbrain organoids. Our work demonstrates the advantages of using iPSC-based screening platforms and organoid technologies to identify effective drugs to be repositioned for the treatment of incurable neurodevelopmental diseases like Leigh syndrome.

A novel, three-dimensional, human osteoblast-osteoclast co-culture assay to quantify mineral formation and resorption

Sieberath, Alexander[1,3,4,5]; Salber, Jochen[4,5]; Eglin, David[2]; Della Bella, Elena[3]; Sprecher, Christoph Martin[3]; Ferreira, Ana Marina[1]; Gentile, Piergiorgio[1]; Dalgarno, Kenneth[1]

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Osteoblasts (OBs) and osteoclasts (OCs) play key roles in the bone remodelling cycle. Co-culture models containing both cell types in the physiological ratio have immense potential to further build more complex 3D osteohomeostasis models to further decipher how the different cell types interact with each other and with the surrounding extracellular matrix. In addition, the co-culture assay presented here already offers an improvement for the bioevaluation of new biomaterials to be used as bone cement, bioink or drug release matrix and for the drug development process for diseases related to bone remodelling. The development of such a bone remodelling process model involves mimicking the environment of trabecular bone by inserting a vertical pin with the average size of a human long bone trabecule into a stereolithographically printed microwell. These microwells are on a cell culture insert that fits into conventional cell culture plates. The polymer surfaces are adapted to a bone matrix-like microenvironment with our patented coating technique of hydroxyapatite and type-1-collagen. The OBs and OCs are co-cultured in the microwells and their remodelling activity is assessed. Thereby, the co-culture shows cellular interaction and remodelling of the coated surface, which is analysed by fluorescence microscopy and quantified by an image analytical method. The resorption activity increases in co-cultures with increased osteoclast numbers, while the mineral formation activity of osteoblasts remains unaffected.
In conclusion, the developed model provides a fast and efficient method to assess the remodelling activity of osteoblasts and osteoclasts while providing a bone-like extracellular environment.

Development of an in vitro protocol for assessing developmental immunotoxicity (DIT) using human induced pluripotent stem cells

Spruck, Christiane[1]; Teschke, Lena[1]; Brockerhoff, Gabriele[1]; Fayyaz, Susann[2]; Grimm, Fabian[2]; Koch, Katharina[1,4]; Tigges, Julia[1]; Fritsche, Ellen[1,3,4]

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The developing human immune system is exposed to a variety of external substances that have the potential to interfere with its normal development. As the current regulatory developmental immunotoxicity (DIT) guidelines are performed in vivo, these studies are not suitable for chemical hazard assessment on a big scale and also bear the problem of species differences.
Therefore, we are developing in vitro new approach methods (NAMs) to study the effects of chemicals on the developing immune system, starting with an assay for primitive haematopoiesis. We differentiate hiPSCs to hematopoietic stem cells (HSCs), which form the common progenitor cell of the hematopoietic system, using a previously published protocol (Philonenko et al., 2021).
Here we show the successful differentiation of hiPSC to iHSCs at the mRNA level and at the protein level, analysing the markers CD34 and CD43, which are characteristic for HSCs. The protocol has been successfully established, but some optimizations are still necessary to increase the amount of iHSC before the actual test method can be set up for compound screening.
This work is the first step towards an in vitro battery for DIT testing, covering key events of the developing immune system.

Closing gaps of the DNT in vitro testing battery by implementing radial glia- and astrocyte-based test methods

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In due consideration of the resource-intensity, ethical concerns and uncertainties of the current in vivo test guidelines for developmental neurotoxicity (DNT) testing, new approach methods are required for a more efficient and sustainable risk assessment paradigm. Recently, international efforts have led to the development of a DNT in vitro testing battery (DNT-IVB) which was challenged with roughly 120 chemicals. Despite its substantial coverage of neurodevelopmental key events, gap analysis indicated that test methods focusing on radial glia cells (RG) and astrocytes (AC) could further improve battery performance. Therefore, we have established novel test methods for evaluating RG- and AC-related endpoints based on the human Neurosphere Assay, a high-content assay using human fetal neural progenitor cells (hNPC). In presence of EGF and bFGF, we assess how chemicals impact the proliferation, migration and morphology of RG, comparing them with carefully selected endpoint-selective controls during 24h of differentiation. Chemical effects on AC maturation are assessed after 5 days of differentiation in presence of BMP2 and CNTF, resulting in an almost pure culture of AQP4- and GFAP-positive AC with characteristic star-shaped morphology. Gene expression analysis and immunocytochemistry confirmed the presence of AC-specific markers (e.g. S100b, AQP4, GFAP) and the inflammatory competence of AC was demonstrated after exposure to TNFα by immunocytochemical staining for ICAM1. Following test method development and characterization, the novel test methods will be challenged using a training set of known in vivo DNT-positives and negatives.

Applicability of neural progenitor cell-derived oligodendrocytes for the mode-of-action-based identification of developmental neurotoxicants in vitro

Stark, Louisa Merit[1]; Klose, Jördis[1,2]; Schlüppmann, Kevin[1]; Nešporová, Tereza[3]; Masjosthusmann, Stefan[1]; Koch, Katharina[1,2]; Fritsche, Ellen[1,2,4]

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Oligodendrogenesis is crucial for the generation of myelinating cells during brain development and thus for facilitating saltatory conduction at neuronal axons. Mature oligodendrocytes synthesize large amounts of myelin, causing high metabolic rates and high oxygen and ATP consumption. This renders oligodendrocytes particularly susceptible to oxidative stress and perturbations of multiple signaling pathways, with disruption of oligodendrogenesis causing neurological disorders such as the Allan-Herndon-Dudley syndrome and periventricular leukomalacia. As part of the established developmental neurotoxicity in vitro battery (DNT-IVB), efficient and human-relevant testing of chemicals regarding their effects on developing oligodendrocytes is feasible under the 3R principle using New Approach Methodologies (NAMs).

To demonstrate the applicability of neural progenitor cell (NPC)-derived oligodendrocytes for the mechanism-based identification of neurotoxicants, stressors with different modes-of-action were applied during oligodendrocyte differentiation and maturation. Both human and rat NPC-derived differentiating oligodendrocytes (NPC5 assay) were sensitive to oxidative stress, perturbations of developmental signaling pathways such as the Notch pathway, and activation of multiple endocrine nuclear receptors. In addition, the NPC6 assay is based on the sensitivity of maturating oligodendrocytes to thyroid hormones, thus identifying endocrine disruptors.

Combined with in silico predictions, physiology-based kinetic modelling and qualitative in vitro-to-in vivo extrapolations, in vitro test methods based on NPC-derived oligodendrocytes provide a suitable model for DNT hazard assessment of chemicals with a plethora of modes-of-action. In the future, new test methods using human-induced pluripotent stem cell-derived oligodendrocytes, as an unlimited and genetically modifiable cell source, will complement the NAMs of the current DNT-IVB..

The EVEIT bioreactor system as platform for live-animal free ophthalmological scientific development and testing

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[1]ACTO e. V., Germany; [2]Kliniken der Stadt Köln gGmbh, Augenklinik Merheim

Introduction
Utilizing slaughterhouse rabbit corneas from food-industry, our Ex Vivo Eye Irritation Test (EVEIT) bioreactor enables the simulation of eye diseases such as burns, edema, toxicity, calcification, blinking and pharmacokinetic through continuous optimization by considering the 3R principles [1]. Tissue-structure and sensitivity of the rabbit cornea is very similar to young human cornea and allows good predictions about reactions to solids or liquids.
Methods
The EVEIT includes endothelial medium supply, physiological flow and pressure conditions. The outer corneal side is exposed to the environment inside the incubator (Air-Lift-culture). The cornea cultured in the EVEIT system exhibits self-healing properties and vitality and transparency can be upheld over the course of several weeks under certain conditions.
Results
The optimizing of our EVEIT system based on our long experience has led to a unique flexibility of the system to accommodate different testing conditions and requirements. Established ophthalmic disease models are involved in testing new drugs.
Conclusion
In the spirit of the 3R-principles, the EVEIT Bioreactor enables the live-animal free testing of novel treatments of ophthalmological conditions and the toxicological characterisation of substances in established ex-vivo models. We will continue to expand the application range of the EVEIT Bioreactor to enable further ophthalmological research without live animals. Validation of the system as a regulatory toxicological test is in process.
[1] Spöler, F., O. Kray, S. Kray, C. Panfil, and N. F. Schrage. 2015. 'The Ex Vivo Eye Irritation Test as an alternative test method for serious eye damage/ eye irritation', Altern Lab Anim, 43: 163-79.

Development of the NPC1_RAR_GR assay to identify putative endocrine disrupting chemicals (EDCs) disturbing glucocorticoid receptor (GR) or retinoic acid receptor (RAR) signaling in vitro

Schlüppmann, Kevin[1]; Hüsken, Saskia[1]; Stark, Louisa[1]; Dönmez, Arif[1]; Förster, Nils[2,3]; Koch, Katharina[1]; Fritsche, Ellen[1,4]

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Although prenatal exposure to endocrine disrupting chemicals (EDCs) can disturb brain development and cause developmental neurotoxicity (DNT), current EDC risk assessment does not cover systematic testing for DNT. Moreover, DNT testing relies solely on animal studies, which, aside from ethical concerns and an insufficient testing throughput, exhibit only limited predictivity for humans due to species differences thus further demanding the development of human-based in vitro methods.
Initial experiments studying hormone-dependencies of neurodevelopmental key events revealed that activation of the retinoic acid receptor (RAR) and the glucocorticoid receptor (GR) reduces proliferation in human neural progenitor cells. Thus, we developed the NPC1_RAR_GR assay based on the human 'Neurosphere Assay' to detect disruptors of RAR and GR signaling. While treatment with substances with anti-proliferative effects in combination with RAR and GR antagonists allows for the detection of EDCs activating either receptor, co-treatments of a test compound with the RAR and GR agonists can detect RAR and GR inhibitors. We established synthetic RAR and GR agonists and antagonists as positive controls and substances without endocrine activity as negative controls. Moreover, first compound testings revealed valproic acid and the DDT metabolite p,p'-DDE to disrupt RAR or GR signaling.
Since hormone regulated neurodevelopmental processes provide putative targets for EDCs and disruption adversely affects neurodevelopment in humans, screening of putative targets for their potential to cause DNT is of utmost importance for several stakeholders, including the civilian population, industry, and regulatory bodies. We therefore aim for acceptance of the NPC1_RAR_GR assay on a regulatory level.

3D-printed phantoms with optical and topological biomimicry for replacing animal experimentation during optical validation of fluorescence-labeled drug delivery systems

Motta, Alessandro; Lammers, Twan; Sofias, Alexandros Marios

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Introduction: Optical imaging is used for monitoring the accumulation and assessing the efficacy of drug delivery systems (DDS) in vivo. Early-stage optical characterization of DDS is typically done in conventional phantoms, which however cannot mimic the optical features of mouse tissues. Hence, early-stage optical validation partly takes place in vivo. This project aims to 3D-print a mouse-shaped phantom with optical and topological biomimicry that can support DDS validation studies, and replace animal experimentation.
Methods: 3D-printing materials are not well characterized in terms of optical properties. To this end, by using UV-VIS-NIR spectrophotometry we assessed the absorption, scattering, and transmission of light for 24 materials from three 3D-printing processes, namely FDM, SLA, and Polyjet™. Based on these results, we 3D-printed prototype phantoms, filled them with NIR-labelled DDS, and assessed their optical detection and linearity via fluorescence reflectance imaging (FRI) and fluorescence tomography / computed tomography (FLT/CT).
Results: 3D-printing materials displayed different optical profiles, depending on their composition, and presence of additives and colorants. When filled with NIR-labelled DDS, the 3D-printed phantoms allowed for simultaneous detection of several different DDS concentrations, and displayed higher sensitivity and low-concentration linearity. Ultimately, by utilizing the collected optical property dataset and by segmenting mouse organ topologies from an already existing CT dataset, we produced a 3D-printed mouse-shaped phantom with optical and topological biomimicry for different organs.
Conclusion: Our strategy enabled the production of 3D-printed mouse-shaped phantoms that can be used for replacing mouse experimentation during early-stage validation of fluorescence-labelled DDS.

Using a Patient-Derived Tumor Fragment Platform to Test Antibody-Drug Conjugates in Urothelial Cancer

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Ambitions to replace animals as model systems have led to the ongoing development of a variety of alternative methods. Especially for assays addressing aspects of complex tissue structures and compositions, systems with high similarity to the original environment are needed. To address this challenge, we established a patient-derived tumor fragment (PDTF) platform for histological assessment of treatment responses. Surgical specimens processed into small fragments maintain the original tissue structure and cell composition, while ensuring sufficient nutrient supply for short term culture. This technique enables the evaluation of different treatments in a human tissue context.

A relevant setting arises in terms of the treatment of metastatic urothelial carcinoma (mUC). Targeted therapies were developed utilizing antibody drug conjugates (ADCs), one of them being the approved anti-NECTIN-4 directed Enfortumab-Vedotin (EV). However, it has been shown that the expression of NECTIN-4 decreases during metastatic spread. TROP-2, on the other hand, is more robustly and almost ubiquitously expressed making it a potential alternative target in NECTIN-4-low mUC. Currently, the anti-TROP-2 ADC Sacituzumab-Govitecan (SG) is in late-stage clinical development.

Using the PDTF platform, we were able to compare the efficacy of both ADCs in a patient-specific setting of mUC with histological readout. As previously described, our cohort showed heterogenous NECTIN-4 but ubiquitous TROP-2 expression. Correlating with the varying NECTIN-4 expression, EV-treatment responses spread over a wide range. SG-treatment, however, resulted in pathological complete response in all cases, therefore suggesting SG as a suitable alternative for the treatment of NECTIN-4-low mUC.

MammaExplant: Development of a human breast cancer explant model to replace murine tumor models

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Background: Tumor-associated macrophages (TAMs) are important contributors to the tumor microenvironment, driving tumor progression and immunosuppression. In breast cancer, TAMs constitute a substantial portion of the tumor mass, necessitating a comprehensive understanding of their interaction with surrounding cancer cells. Traditional cell culture is inadequate for such studies, demanding exploration in animal models. Our 3R project aims to develop a breast tumor explant model, eliminating the need for animal experiments and facilitating cancer drug screening.
Methods: RWTH cBMB efficiently delivers freshly procured human breast tumor tissue to Navitect Bio shortly after surgery. Navitect cultivates the tissue immediately using established methods and optimized media. Syntab Therapeutics' novel synthetic antibodies, Immune System Engagers (ISErs), are then assessed for their ability to activate anti-tumor immunity in TAMs.
Results: The outlined methods for creating the breast carcinoma explant model and cultivating tumor slices for six days were successfully implemented. Navitect is currently conducting immunohistochemical analyses, exploring relevant surface markers of immune cells, and assessing proliferation and apoptosis. The Bartneck group utilizes advanced techniques such as flow cytometry and OMICS technologies to identify key factors for evaluating the anti-tumor response. Initial experiments with ISErs show promise, pending confirmation in subsequent experiments.
Outlook: The 3R project MammaExplant anticipates novel insights into immune and tumor cell interactions using explant models. Efficacy rates of immunomodulatory ISEr candidates for future clinical applications are expected. Furthermore, comparing data from tumor explants with established tumor models aims to establish the basis for replacing mouse models with patient-derived human cancer explants.

Reduction

3R by Non-invasive Magnetic Resonance Techniques

Ulrich Flögel

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Magnetic resonance imaging (MRI) is the key whole-body imaging technology for observing processes within a living object providing excellent resolution and contrast between soft tissues. MRI does not involve any harmful radiation and offers unlimited penetration depth. Beyond imaging, by dedicated techniques, e.g. magnetic resonance spectroscopy (MRS), also special proteins like myoglobin, small molecular compounds like adenosine triphosphate and glucose, respectively, or even ions in individual organs, such as the heart, can be determined. All measurements can be carried out noninvasively at the living animal in a repetitive manner.

Our research focuses on the complex interplay of function, energetics, metabolism and inflammation and their role in the development of cardiovascular disease using innovative multinuclear MRI/MRS techniques (e.g. ^{1}H, ^{2}H, ^{13}C, ^{19}F, ^{31}P). To this end, in recent years we have established a broad molecular imaging platform that can track inflammatory and thrombotic processes with high specificity in parallel to metabolic and energetic alterations together with an in-depth tissue characterization (fibrosis, edema, extracellular matrix). The talk will provide an overview of how these techniques can be applied in parallel to extract multiple pieces of information from a single experiment, as an example of refinement and reduction of animal testing. Finally, alternative models to replace mammalian models will be presented.

Pain research in a petri dish? Chances and limitations of cell culture models in pain research

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Pain is an essential sensation of organisms to protect themselves from environmental and endogenous harm. At the same time, pain is the main reason why people seek medical care and exerts an enormous physiological and psychological burden. While causes for pain are extremely diverse, the options for sufficient treatment are still limited. Therefore, it is essential to gain novel insights into mechanisms of nociception, the pathophysiology of inflammatory and neuropathic pain, and innovative treatment opportunities.

Over the last decades, animal experiments provided the basis for contemporary medical applications. Still, they are essential to identify novel targets for therapeutic interventions and to test for efficacy and toxicity.

Our workgroup focuses on the application of primary cell cultures of structures involved in nociception. Within the dorsal root ganglia, (DRG) cell bodies of sensory neurons are located. The spinal dorsal horn (SDH) is the first main site of pain modulation and integration, where information is synaptically transmitted to projection neurons and modulated by interneurons. Applying cell culture models of both structures, we assessed sensitizing effects of inflammation on neuronal responses, examined the modulatory role of resident glial and immune cells and tested cell-based therapeutic approaches.

Overall, in vitro models can provide new insights into cellular and molelular mechanisms of nociception and pain and are very valuable as preceding screening tools. However, they should rather be thought of as complementary instead of alternative approaches for in vivo experiments. Thereby, they can help to reduce the number of experimental animals used for scientific purposes.

Living up to the 3Rs – Non-invasive imaging in experimental animal research

Lydia Wachsmuth

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Imaging techniques can provide a non-invasive picture of physiological and pathological processes in the body. There is little doubt about the value of non-invasive imaging techniques such as Magnetic Resonance Imaging (MRI) in experimental animal research, given their multi-parametric capabilities and high translational value. I will give examples of how we and others are using preclinical MRI in the context of the 3Rs to reduce the number of animals used and to refine or even replace conventional, more invasive experimental techniques. In addition, I would like to address the role of non-invasive imaging in everyday experimental animal research. What are the barriers to the widespread and regular use of these technologies?

Virtual Reality and new media in Laboratory Animal Science Education

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With the implementation of the EU Directive 2010/63 on the protection of animals used for scientific purposes, the principle of the 3Rs (Refinement, Reduction, and Replacement) was for the first time incorporated into the animal welfare law. Therefore, the 3Rs and knowledge of alternative methods should be part of the education and training of staff involved in the care and use of animals.

At the RWTH Aachen University, FELASA accredited courses are provided at different levels of education. Here, a digital blended learning concept combining Virtual Reality (VR) components with classical learning contents, including e-learning modules, new media and practical skill trainings, was implemented and evaluated. Procedures, such as anesthesia induction, substance application, and blood sampling in rats, as well as the laboratory environment, were recorded in 360° videos and VR teaching/learning modules were used to better prepare participants for hands-on training (refinement) or as a complete replacement for a live demonstration (reduction).

Throughout the courses, users' experience of the VR modules has been evaluated and it was demonstrated that, despite a low previous VR experience, participants highly appreciated the VR modules showing the potential to enhance procedures and demonstrations. Interestingly, participants with previous experience in laboratory animal science agreed more strongly than less experienced participants with the VR potential to support the 3R principle and endorsed its usage for further educational purposes.

The high acceptance of this innovative training format is encouraging to develop further alternative education media in the field of laboratory animal science.

Development and evaluation of agarose-based hydrogel bioinks for human bone tissue engineering

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Human cell-based tissue engineering combined with bioprinting techniques can be used to generate more complex, 3D multicell-type constructs. These can be used in microphysiological systems for high-throughput screening of new implant materials or drugs. Such systems have great potential to avoid animal studies. Bioprinting requires biomaterials that enable adhesion, proliferation, migration and differentiation of the introduced cells. In the case of bone tissue engineering (BTE), a 3D human tissue model (hTM) must provide mechanical stability and adequate nutrient supply. Agarose, a natural polysaccharide from algae, forms a mechanically stable and porous structure during gelation. Although agarose has good biocompatible properties and has already been used in clinical applications, it lacks binding motifs that enable cell adhesion. Mesenchymal stromal cells (MSCs) represent an important cell source for BTE. Osteogenic differentiation and mineralisation of MSCs promotes remodelling of artificial scaffolds. An inadequate environment can lead to altered cellular behaviour and thus impair the remodelling process. To address this deficiency, agarose was mixed with type I collagen. Collagen is the most abundant protein in mammals, provides the necessary structural scaffold (extracellular matrix, ECM) for tissues and plays an important role in cell adhesion. The introduction of nano-hydroxyapatite (nHA) was used to more closely mimic the bony environment of MSCs. Different bioinks based on agarose-collagen I mixtures with nHA particles were prepared and evaluated to develop an ideal bioink for 3D bioprinting and to investigate the cellular behaviour of MSCs after printing support-free tubular models with a focus on viability, morphology and differentiation.

Analysis of cardiac substrate metabolism in intact cardiac tissue slices - Complementing the range of methods and implementing the 3R principle

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Investigations into cardiac metabolism contribute to unravelling disease-related mechanisms. Many of these studies are based on animal experiments, as methodological restrictions limit the applicability of cell culture-based alternatives. Therefore, we developed an extracellular flux-based method for analysing cardiac metabolism in intact tissue slices. Fresh mouse cardiac tissue pieces were prepared by high-precision vibratome slicing. Oxygen consumption rates (OCR) were measured at baseline and after mitochondrial uncoupling in palmitate and glucose enriched medium. Substrate preference was assessed by inhibition of metabolic pathways. The sensitivity of the method was tested using diabetic hearts as well as hearts after myocardial infarction.
Tissue pieces from healthy hearts used both glucose and palmitate as substrates. Unstimulated OCR was 58±8 pmol/min, and mitochondrial uncoupling by FCCP resulted in a two-fold increase in OCR. Tissue pieces of diabetic hearts showed an increase of 23% in dependency on palmitate oxidation, whereas dependency on glucose was unchanged. After myocardial infarction in non-diabetic animals, time-dependent effects on substrate metabolism were observed. Palmitate oxidation was increased early (day 3) after infarction, and reduced by 30 % at day 28.
We describe a novel method to analyse cardiac metabolism using tissue slices metabolising fatty acids as well as glucose. As multiple tissue pieces can be obtained from one mouse heart, this method will reduce the number of animal experiments required in cardiovascular research. Furthermore, the method has the potential to contribute to replacement of animal experiments, as pilot experiments show the applicability in iPSCs derived engineered heart tissue samples.
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Engineering mesoscopic 3D tumor models with a self-organizing vascularized matrix

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Introduction. Animal experimentation is essential in biomedical research, but ethical concerns motivate efforts for reducing, replacing, and refining in vivo experiments, in line with the '3R principle'. One way to implement this principle is to develop advanced, all-human in vitro models that provide biological readouts with similar predictive power to in vivo tumors. Advanced in vitro systems such as lab-on-a-chip devices have been developed, but often fall short in reproducing the tissue scale and complexity of human diseases. In this study, we established a bio-printed artificial tumor model with endothelial and stromal cells self-organizing into perfusable and functional vascular structures.
Methods. Vascular bioink of gelatin mixed with endothelial cells (EC) is bioprinted inside imaging-compatible bioreactors. Tumor spheroids are casted in a collagen-fibrinogen hydrogel mix comprising EC and stromal cells. After hydrogel solidification, bioreactors are connected to a peristaltic pump in a recirculating fluid loop and dynamically cultivated up to three weeks.
Results. Vascularized tumors grew at mesoscopic size-scales in perfusable bioreactors. Anastomoses between self-evolved vascular networks and bioprinted endothelium facilitated the movement of spontaneously migrating cancer cells from tumor spheroids (in)to flowing fluid medium. This sequence of events mimics the initial steps of the metastatic cascade. Importantly, we demonstrated that patient-derived TNBC tumoroids grown in these perfused bioreactors, closely resembled the genetic and phenotypic signature of the original patient tumors.
Conclusion. Our modular approach opens up new avenues for exploring tumor biology and drug testing at clinically-relevant spatial and temporal scales, while reducing the need for in vivo experimentation.

Investigating the emergence and effect of fibroblast phenotypic plasticity on advanced in vitro tumoroids

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Introduction. Cancer-associated fibroblasts (CAF) can influence the tumor microenvironment (TME) and foster cancer progression. Depending on the TME, CAF can obtain different phenotypes, including myofibroblasts, inflammatory fibroblasts and vascular fibroblasts. Due to this phenotypic complexity, it would require extensive in vivo experimentation to thoroughly investigate the contribution of each CAF population in tumor progression. Hence, in this project we developed an advanced breast cancer in vitro system aiming to the systematic analysis of CAF phenotypes and behavior.

Methods. To induce different CAF phenotypes, human dermal fibroblasts were treated with specific cytokines (TGF-β, IL-1β, PDGF-CC), and then co-cultured with human breast cancer cells in 2D cultures or 3D tumoroids. The effect of cytokines on CAF phenotype and behavior was evaluated using bright-field microscopy, immunofluorescence, and gene-expression analysis via PCR.

Results. Both in 2D and 3D, CAF were successfully polarized towards myofibroblasts (FAP-high, αSMA-high, IL6-low), inflammatory fibroblasts (FAP-high, αSMA-low, IL6-high), and vascular fibroblasts (FAP-high, PDGFRβ-high, Angiopoietin-high), upon continuous stimulation with TGF-β, IL-1β, and PDGF-CC. Discontinuation of cytokine stimulation resulted in loss of acquired CAF features. Most importantly, cytokine cocktails resulted in the simultaneous overexpression of multiple CAF markers and the acquisition of diverse phenotypes, resembling the complexity of CAF in vivo.

Conclusion. Dissecting CAF polarization in advanced in vitro models will facilitate a better understanding on their contribution to tumor growth and will support the design of CAF-specific therapies for cancer resolution. Ultimately, the ability to investigate these complex mechanisms in vitro, will contribute to a strong reduction of animal experimentation.

Refinement

Refinement Research – State of Affairs?!

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Refinement, as defined by Russell and Burch (1959), aims at eliminating all potential negative variables that can lead to suffering in laboratory animals. The authors refer to the term "distress", which they equate with "inhumanity". Distress goes far beyond the concepts of pain or fear and describes a wide variety of mental states that can contribute to the deterioration of an animal. These states can be caused by pain and fear, but also by behavioural conflicts, hunger or physical discomfort. Against this background, the diversity of refinement research becomes apparent. The development of suitable anaesthetic and analgesic regimes can be seen as a milestone in refinement. Furthermore, the improvement of husbandry systems or sophisticated training of people working in animal experiments in the sense of 3R education has contributed significantly to increasing the welfare of laboratory animals. Consequently, various levels of reflection are being addressed by refinement research, which, apart from animal welfare in the broader sense, tackles with moral issues about the use of laboratory animals. The lecture will provide a brief historical review, insights and outlooks into this important field of science.

Components for Establishing Refinement Strategies in Large Animal Models: A practical example

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Establishing refinement in animal experiments involves a comprehensive assessment of distress, encompassing pain, suffering, and harm. To effectively evaluate severity in a model, various factors must be considered, such as animal species, type of intervention, duration of distress, and categorisation. Profound knowledge of species-specific ethological behavior patterns is essential. This discussion illustrates the severity categorization process and potential refinement strategies using a sheep tibia defect model as an example:

In the context of fracture treatment for limbs, casting is a standard practice in both human and veterinary medicine. Sheep are a significant species in testing new osteosynthesis materials using tibia defect or fracture models. However, the conventional method involves suspending sheep for 4-6 weeks post-surgery, severely limiting their movement and natural behavior.

To refine this approach, a walking cast for sheep (SWC) was developed, allowing immediate species-typical movement after surgery. Nine Rhone-race sheep were observed for four weeks following tibia defect surgery and cast attachment. All animals began weight-bearing on the operated leg from the first postoperative day. Evaluation of the walking process and individual step length, along with a lameness scale, yielded promising results for enhancing animal welfare. Group housing was possible from the third day after surgery, and regular radiographic checks and cast changes ensured proper cast fitting and healing. This study demonstrates a system for cast stabilization, ensuring anatomically correct positioning and function of the hindlimb during the healing process, providing a refinement option for animals weighing up to 70kg.

Refinement and Validity of a Cardiological Surgical Mouse Model

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Refinements and validation of mouse models used for cardiovascular system research are main goals of our animal core facility. The discussed model involves surgically induced aortic valve stenosis (AVS) and longitudinal ultrasound examinations. The adjustments made aim to enhance animal welfare (Refinement) and reduce the number of animals needed (Reduction).

Out of 2768 operated mice, a significant reduction in mortality rates was achieved, from 22% to 7.21%, through the implementation of changes in postoperative injection timing, reducing buprenorphine application intervals, adjusting the age at surgery, and using both genders. Postoperative stress criteria, especially general well-being and spontaneous behavior, showed significant improvement. Comparison between verum and sham operated mice indicate a subclinical course of AVS with minimal animal distress.

The study also confirmed the model's internal validity by continuously monitoring blood flow velocity over the aortic valve as a success control for AVS. While there were some sex differences, both male and female animals proved suitable for the model, reducing the number of unusable animals.

The findings emphasize the importance of continuous quality management in animal experiments to meet high ethical standards, reduce animal stress, and enhance the validity and ethical acceptability of animal experiments in the field of cardiovascular research.

Rehabilitation after mild Traumatic Brain Injury - An essential translational approach in a mouse model.

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Mild traumatic brain injury (mTBI) may be associated with diffuse neuropsychiatric symptoms such as headache, anxiety, dizziness, or gait disturbances. In particular, repetitive mTBI, as occurs in contact sports (e.g. boxing), can cause secondary neurodegeneration or chronic encephalopathy for which adequate therapeutic options are lacking. In the absence of animal models for neuropathological characterization of structural consequences of mTBI, we investigated neuroinflammation and integrity of the blood-brain barrier (BBB) in a mouse model newly developed for this purpose.

The mTBI was induced in male C56Bl/6j mice (n=22). An electromagnet-controlled impactor triggered a mTBI laterally on the skull over the primary motor cortex either once or repetitively, resulting in a rotation of the head by 30°. The repetitive mTBI's were triggered either every other day or weekly. In parallel, trauma animals were compared with control animals for cognitive impairment, histologic neuroinflammation, and BBB disruption.

Early structural MRI measurement revealed that even repetitive mTBI's did not result in brain contusions or microhemorrhages in this model. Extravascular accumulation of immunoglobulin G and fluorescent dextran (40kDA) was detected. Long-term monitoring of cognitive abilities showed significant impairment of spatial cognition in the repetitive SHT. This impairment correlated with BBB disruption in the hippocampal region (R^2=0.29, p<0.01).

This new mTBI mouse model represents a significant advancement and refinement in the field of rodent neurotrauma research. The skull remains intact, neither hemorrhages nor brain contusions occur. In summary, this model is suitable to make a translational contribution to the research of new treatment options for mTBI.

Broad human hematopoietic engraftment and persistent T cell development in mice lacking MHC I/ II and supplemented with lentiviral vectors expressing HLAs

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Fully humanized mice became a benchmark model for immuno-oncology. A major limitation is the mismatch between the major histocompatibility complexes (MHCs). We evaluated if NOD/SCID/GAMMA (NSG) mice with MHC class I and II knock-out could be humanized with CD34+ cord blood cells. We tested lentiviral vector (LV)-mediated delivery of HLAs, human cytokines and a viral antigen to boost immune reconstitution. Three cohorts were compared: (i) NOD.Cg-Rag1tm1Mom Il2rgtm1Wjl/SzJ (NRG control, n=11), (ii) NOD.Cg-Prkdcscid H2-K1tm1Bpe H2-Ab1em1Mvw H2-D1tm1Bpe Il2rgtm1Wjl/Sz mice lacking MHC class I and II molecules (NSG-DKO, n=8), and (iii) NSG-DKO injected with LVs expressing HLA-DR4/fLuc, HLA-A2.1/fLuc and LV-hGM-CSF/ hIFN-alfa/ HCMV-gB (NSG-DKO/LV, n=9). Lentiviral expression of the luciferase reporter was persistent for twelve weeks in spleen and liver. Frequencies of human CD45+CD3+ T cells in peripheral blood analyzed longitudinally by flow cytometry were significantly higher for NSG-DKO/LV. Analyses of thymus showed lower total counts of human T cells in NSG-DKO than in NSG-DKO/ LV. Detection of CD4+ and CD8+ T-cells by flow cytometry were highest in the NSG-DKO/LV cohort in spleen and bone marrow. Expression of memory and activation markers were higher for CD4+ and CD8+ T cells from NSG-DKO/LV mice. Analyses of bone marrow samples by mass cytometry (CyTOF) with barcoded human CD45 revealed increased frequencies of CD4+ T, CD8+ T, gamma/delta T and NK cells in NSG-DKO/LV. Concurrently, NSG-DKO/LV mice showed the highest frequencies of human neutrophils, monocytes and myeloid dendritic cells. In summary, fully humanized NSG-DKO mice supplemented with lentiviral vectors showed high reconstitution of human hematopoietic and T cells.

Moral distress measurement in animal care workers: a systematic review

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The study addresses the significant psychological stressors veterinarians face, such as performing euthanasia and witnessing animal suffering, which can lead to various mental health problems including compassion fatigue, anxiety, burnout, depression, and suicidal thoughts. Moral distress, initially defined in nursing, is the inner conflict experienced when one knows the right action but is hindered by institutional constraints.

This review aimed to identify and evaluate the measures used to assess moral distress in this group, focusing on their psychometric validity. The review adhered to a two-step protocol based on the PRISMA guidelines. The first step involved identifying moral distress measures from eligible studies on animal care workers, and the second step assessed their psychometric validity, emphasizing content validity, a key aspect of patient-reported outcome measures (PROMs). The Consensus-based standards for the selection of health measurement instruments (COSMIN) were followed for this assessment.

The results showed that only one PROM, specifically adapted for veterinary contexts, was identified: the Measure of Moral Distress for Animal Professionals (MMD-AP), a revised version of the Measure of Moral Distress for Healthcare Professionals (MMD-HP). Both PROMs were evaluated for development quality and content validity, with the MMD-HP deemed to have sufficient content validity, while the MMD-AP's validity was inconsistent. However, the quality of evidence for both was considered low.

In conclusion, this review highlights the scarcity of standardized, evidence-based methods for measuring moral distress in animal care workers. It emphasizes the need for developing and validating appropriate methods tailored to this specific context.

Assessment of postoperative pain in rats under analgesia using PET imaging of the σ1 receptor

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An important aspect of animal welfare assessment is the quantification and correct localization of pain. However, severity tests often lack the sensitivity to accurately detect pain and it can be challenging to determine whether an animal receiving analgesia would experience pain without the medication. Following the need to sensitively assess pain, the aim of this study was to objectively quantify and localize postoperative σ1-receptor-mediated pain during and after analgesia.
Eighteen rats underwent surgical interventions (skin-incision or partial hepatectomy), while nine rats served as control. Analgesia was administered for three consecutive days. PET/CT imaging with [18F]Fluspidine was performed on days 1, 4, and 7 after surgery. At each time point, three animals were euthanized for histologic examination of the incision sites and organs. Postoperative pain was also monitored by score-sheet, Open-Field-Test, and Von-Frey-Test.
Despite analgesic treatment, image analysis and immunohistochemistry revealed significantly higher σ1-receptor expression on postoperative day 1 at the incision site of rats that underwent partial hepatectomy compared to the other groups, which might be caused by the additional opening of the peritoneum. However, behavioral and pain tests showed no impairment of animal welfare due to efficient analgesia. No σ1-receptor-mediated pain could be detected by image analysis and histology on days 4 and 7, which is in line with the Open-Field-Test and score-sheets.
Imaging was able to sensitively detect postoperative σ1-receptor-mediated pain independent of analgesia administration. Therefore, [18F]Fluspidine PET/CT imaging may remarkably refine pain monitoring in future preclinical studies and may be used as reference to other severity tests.

Non-invasive Monitoring of Melanoma Heterogeneity Dynamics using Substrate-specific NanoLuciferase Variants

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Malignant melanoma is a rare but very aggressive form of skin cancer. Despite tremendous successes of immunotherapeutic approaches treatment of melanoma patients challenging. Tumor plasticity such as shifting between differentiated and undifferentiated cellular states altering the expression of tumor-antigens contributes to the development of therapy resistance. The cellular and molecular mechanisms of tumor plasticity and allowing certain melanoma subpopulations to escape from therapy are currently not well understood. To elucidate molecular mechanisms and cellular interplay within heterogenic melanoma models over time, we establish a NanoLuciferase (NanoLuc) based assay that allows quantification of the cellular composition of heterogenic mouse melanoma models non-invasively by measuring substrate-specific NanoLuc activity in mouse urine. NanoLuc is a small enzyme that can undergo renal excretion in mice and can act promiscuously with a variety of substrates. We identified a novel point mutation in NanoLuc which reduces luciferase activity with its substrate coelenterazine ca. 1000-fold compared to its activity using furimazine as a substrate. In combination with the reverse mutant NanoLuc18Q27L variant which has enhanced activity with coelenterazine, we can tag two distinct tumor subpopulations and quantify their frequency within a heterogenous mixture of tumor cells. This method will allow highly time-resolved longitudinal non-invasive monitoring of the kinetics of frequencies of tumor subpopulations in mouse models of melanoma. It is a novel approach to refine animal experiments and reduce the number of animals needed for studying the kinetics of tumor subpopulation growth in vivo.

The Chorioallantoic Membrane Assay: A 3D-In-Ovo-Model for Evaluating Antibody-Drug Conjugates

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Antibody-Drug Conjugates (ADCs) represent an emerging treatment class in cancer therapy since they combine the cytotoxicity of conventional chemotherapy with the selectivity of antibody-based therapy. As demand is growing for the invention of new ADCs, as is the need for an effective in vivo testing system.

The Chorioallantoic Membrane Assay is a 3D in-ovo tumor model that represents a promising alternative to widely used mice model, especially regarding testing drug-based cancer therapies. In the CAM-Assay, a tumor is grown from e.g., established cell lines, placed on the highly vascularized chorioallantoic membrane (CAM) of the 8-day-old chicken embryo. The use of CAM-Assay for testing ADCs has been established in our group and optimized to achieve a much-reduced hands-on time. Despite the short observation period, the CAM-grown tumor is histologically like the primary carcinoma and has morphological characteristics of the original tumors, which is a major advantage over tumor cells in culture. In addition, the cost of the basic equipment and the eggs themselves are low compared to mice experiments. In summary, the CAM model represents an optimal testing system regarding the 3R guidelines and for testing new treatment options, e.g., Antibody-drug conjugates, in a 3D model. Therefore, the CAM model could be a model bridging the gap between cell-based and animal-based assays.

Multifactorial analysis for the refinement of Vitamin K2 administration in mice

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Background:
Vitamin K2 (VK2) is used in mouse models by various routes, most prominently oral gavage (o.g.), intraperitoneal injection (i.p.) and subcutaneous injection (s.c.). These application strategies are used interchangeably and spanning different age groups, while the least burdening strategy remains ill-defined.
Methods:
Female C57BL/6J mice in early adolescence or on the verge of adulthood underwent posterolateral spinal fusion surgery followed by thrice-weekly i.p., s.c. or o.g. administration of VK2 (30mg/kg or 60mg/kg) or corn oil control. Additional once-weekly i.p. treatment with vitamin D3 (280IE/kg) or isotonic saline control was administered as per study protocol.
After 10 weeks, mouse behaviour in the open field test was recorded and assessed with the open-source video analysis pipeline ezTrack.
Results:
While total distance did not differ between age groups overall, i.p.-treated animals travelled significantly more than o.g.-treated mice.
The i.p.-group further showed increased distance compared to s.c.- and o.g.-treated adults as well as all early-adolescent counterparts.
Combining both age groups, sub-analysis of treatment groups revealed significantly elevated distance travelled in i.p.- vs. o.g.-mice for low-dose VK2/D3, VK2/saline as well as corn oil/saline control regimens.
Distance in the low-dose VK2/saline was also significantly higher when compared to high-dose VK2/saline and high-dose VK2/D3.
Moreover, adult mice tended to spend more time along the edges and less in the corners.
Conclusion:
Collectively, these data suggest that VK2 administration should preferably be administered i.p. at low-dose for weeks-long, repetitive treatment in adult female mice when therapeutically equivalent.

Refinement of stem cell-based in vitro assays towards a regulatory use for developmental and adult neurotoxicity testing of chemicals in the Horizon Europe PARC Project

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As an alternative for regulatory animal-based assessment for developmental neurotoxicity (DNT), a DNT in vitro testing battery (DNT IVB) covering several key events (KEs) of human brain development has been developed recently.

DNT IVB data gaps concerning complex processes like neural network formation and function assessed in human-based systems have been recently addressed by the establishment of the human neural network formation (hNNF) assay. Co-cultures comprised of human induced pluripotent stem cell (hiPSC)-derived excitatory and inhibitory neurons and human astrocytes (NeuCyte, USA) differentiate on micro-electrode arrays (MEAs) and form functional networks. Challenging the assay with 27 pesticides confirmed the suitability for DNT compound screening.

As opposed to this, an IVB for adult neurotoxicity (ANT) is currently not available. We aim to optimize the human multi-neurotransmitter receptor (hMNR) assay, that has been set up for acute and chronic exposure of hiPSC-derived 3D BrainSpheres on MEAs, followed by neuronal subtype-specific evaluation, identifying glutamatergic, GABAergic, dopaminergic, cholinergic, glycinergic and serotonergic neurons using spike sorting.

Refinement of both assays is currently in progress as part of PARC 5.2.1e, aiming at cost reduction and improvement of their suitability for regulatory application by testing positive and negative compounds to further determine their applicability domains.

Swollen Ampulla as an Indicator of Successful Pregnancy in B6C3F1 Recipient Mice used for Assisted Reproduction

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In vitro fertilization, embryo cryopreservation, and embryo transfer (ET) are assisted reproductive technologies that are used extensively for the maintenance of mouse models in animal research. Inbred mouse strains with different genetic backgrounds vary in their reproductive performance. Cryopreservation can affect embryo viability, and the genetic background of ET recipients can influence the ET result. In this retrospective study, we analyzed the outcomes of ETs performed in our facility during the last 6 y. We found that B6C3F1 mice with swollen ampullae show almost 3-fold higher pregnancy rates than mice with nonswollen ampullae when either freshly isolated or frozen-thawed embryos are implanted. Implantation of freshly collected embryos in recipients with swollen ampullae led to significantly higher pregnancy rates in comparison to implantation of frozen-thawed embryos, regardless of whether the latter were fertilized in vivo or in vitro. Moreover, we found a significant effect of genetic background on the birth rate; C57BL/6J mice and mice with a mixed genetic background had 34% higher birth rates than did C57BL/6N mice. Within the C57BL/6J group, the birth rates were significantly higher when using fresh in vivo-fertilized embryos, and cryopreservation negatively affected both in vivo- and in vitro-fertilized embryos. The success rate of obtaining one living pup was not significantly different between frozen-thawed and fresh embryos. Overall, a swollen ampulla is a strong indicator for successful pregnancy. A better understanding of the factors that affect the reproductive outcome might lead to optimization and reduction in the number of mice used for these procedures.

New Thinking in 3Rs

On the way to 3Rs with the EQIPD Quality System

Björn Gerlach

Central Institute for Mental Health, Mannheim, Germany

Biomedical research serves to address unmet medical needs, however, its success rates have been declining over the past decades. Recent evidence shows that many studies have questionable research practices and lack sufficient protection against bias. Adherence to appropriate research rigor is important to ensure trustworthy and robust research data. Strategies to increase robustness of preclinical data will contribute to the 3Rs and most likely accelerate the innovation for the benefit of patients.

Today's research environment has multiple stakeholders and needs to accommodate their diverse requirements, such as guidelines and best practices. A unified approach was established with EQIPD QS (Enhancing Quality in Preclinical Data quality system) to increase trustworthiness and robustness of research data. This system provides a flexible, easy to apply and fit-for-purpose approach to implement and maintain best practices. This holistic solution can be applied to any research lab by setting basic requirements and expanding to specific needs. The system is published, free to use and the support structures are in place to provide feedback for labs during implementation. Several groups have successfully implemented the system and are already certified.

This presentation will provide an overview of the EQIPD QS and present specific examples about its application. It will be shown how research labs can profit from its implementation and how it directly contributes to the refinement and reduction of animal research in the context of the 3Rs.

CIRS-LAS – Redefining animal welfare: the impact of critical incident reflection

Sabine Bischoff

Animal Welfare Office, Jena University Hospital, Jena, Germany

To learn from critical events in laboratory animal science, open and transparent communication is necessary. To ensure a good error culture and a motivated team in the sense of an institutional culture of care, it is important to give every team member the opportunity to deal openly with unexpected events or even mistakes. Raising awareness promotes the well-being of all animal care and research staff. In a facility with a well-established culture of care, it is possible to learn from each other. Constructive handling of mistakes and open discussions about possible refinement measures improve animal welfare and science.

The presentation will outline the importance of a constructive error culture and provide insight into the use of the Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS) database, which is recognized worldwide for reporting critical incidents in laboratory animal research. CIRS-LAS is open to anyone who works with laboratory animals. A short form is used to enter information about the animal involved, the incident and its background, possible reasons for the incident, and opportunities for improvement. Searching the online database, which is also available via an app, provides effective learning opportunities for technical and scientific staff to improve animal welfare or avoid repeating failed experiments. CIRS-LAS is an essential component for open and constructive error management in any scientific institution.

3R in kidney research

Bernhard Schermer

CECAD Research Center, Cologne, Germany

Diseases of the kidney are common and often lack specific therapies. In basic kidney research, mouse models are frequently used, which are becoming increasingly specific and accurate through the use of genome editing.

In addition, kidney organoids are playing an ever greater role. This talk aims to provide insight into kidney research and how the 3R principles are considered in this context. It is less a systematic scientific talk and more of an application note with the rather personal views of a principal investigator who has been working with animal experiments for years, but at the same time only conducts this line of work because it is still essential for translational projects.

“Initiative Transparente Tierversuche” – the German initiative to promote transparent communication on animal research

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Trust in science is a valuable asset that can contribute significantly to social cohesion as well as to rational, democratic decision-making. Since trust is not a given good, but a joint effort based on openness, truthfulness and dialogue, it gives rise to a responsibility that radiates to science as a whole. In a controversial subject area such as animal experimental research, transparent information about scientific work and an open exchange of views on the choice of research methods that is open to dialogue are particularly important, as are ethical responsibilities. In this societal dialogue, the scientific community is called upon to continuously fulfill its central role and special responsibility. To promote open dialogue and transparency in animal research in Germany, the Permanent Senate Commission on Animal Protection and Experimentation of the German Research Foundation and the information initiative "Tierversuche Verstehen" of the Alliance of Scientific Organizations founded the “Initiative Transparente Tierversuche” (“Initiative on transparent animal research”). Comparably to other similar activities, the initiative formulates 4 goals which aim on fostering a culture of open dialogue and proactive information sharing. Universities, research institutions, industries and zoos are invited to join and support the initiative and implement their goals. The initiative was founded in summer 2021 and over 100 institutions have joined until late 2023. The initiative offers an annual workshop, a symposium, direct consultations and other resources to supporters of the initiative to help improve their own communication, outreach and transparency efforts.

Bundesnetzwerk 3R - transforming the dialogue together

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To date, animals continue to be an indispensable model for research. Animal testing delivers important data about how effective novel drugs are and whether certain substances are toxic for humans. An ethical dilemma emerges – the striving for knowledge and the safety of humans on the one hand and animal welfare on the other. In this regard, the principle of the 3Rs (replace, reduce, refine) provide the current framework for doing research that is as best in line with animal welfare requirements as possible.

In 2022 the German Federal Ministry of Education and Research (BMBF) thus launched the "Bundesnetzwerk 3R" as a national platform for exchange about the 3R principles and possibilities for their implementation. The mission of the network is to facilitate an inter- and transdisciplinary dialogue between science, industry, politics, regulatory authorities and other stakeholders, thus building a strong community that jointly advances 3R research and its transfer into practical application.

The new digital platform www.bundesnetzwerk-3R.de, which launched in September 2023, forms a key element of the networks activities. All stakeholders of the German 3R landscape, each with their own research focuses and interests, are invited to become part of the interactive map and the network. Once registered, members are invited to browse through peoples expertise's and use the provided contact information to connect. Furthermore, members are invited to several events and frequently get informed through the Newsletter. The participatory approach allows all interested stakeholders to get involved in the discourse and help shape the Bundesnetzwerk 3R.

“Tierversuche verstehen” – A German initiative to inform about animals in research

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"Tierversuche verstehen" (TVV, engl.: understanding animal research) is an initiative of the German scientific community, coordinated by the Alliance of Science Organisations. Since 2016, it provides comprehensive, up-to-date and fact-based information about animal experiments at publicly funded research institutions. TVV provides insights into the necessity of responsible animal use in research but also into available alternatives. Acting responsibly means always balancing the protection and welfare of animals with the importance of scientific knowledge for humans. However, acting responsibly also means developing and using alternative and complementary methods. In this way, TVV contributes to an objective discussion about animal research and the 3Rs. TVV promotes dialog between science and the public. The communication supported by scientific organisations and professional associations provides reliable data and facts on animal experiments together with important background information.

By spearheading a cultural change towards more openness and transparency, TVV aims to not only increase understanding for responsible animal use in the general public but also to increase the value and quality of research in the life sciences.

3R-SMART: Information and training platform for methods to replace and supplement animal experiments

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

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

In addition to providing educational content on alternative methods, 3R-SMART also addresses the legal and ethical aspects of working with laboratory animals. The information is tailored to different needs, providing either a quick overview or more in-depth information in the form of video or text content.

The website also provides news and updates, a calendar of upcoming events and a forum for exchanging ideas in the field of the 3Rs.

Interactive maps of the 3R Centres in Germany and Europe provide an overview of the 3R Centres and information on the activities and priorities of each 3R Centre.

Furthermore, 3R-SMART supports the 3R research activities of various stakeholders by enabling them to present their latest 3R findings on 3R-SMART in order to increase the reach of their research results.

In this way, 3R-SMART is constantly being expanded and developed.

In order to disseminate and transfer knowledge about the 3Rs, 3R-SMART will provide open educational resources (OER) and will also offer 3R seminars and other learning opportunities.

In this context, work is being done in cooperation with LAS interactive (https://las-interactive.de) on a combined continuing education portal on laboratory animal science and alternatives to animal experimentation (fee-based) for continuing professional development.

Zebrafish Core Facility – University Bonn

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After mice – fish are the second most commonly used laboratory animals in Germany. Especially zebrafish (Danio rerio) are increasingly chosen as a vertebrate model for biomedical research. The genetic similarities to mice/human and the transparent embryonic life phase make zebrafish an excellent animal model for genetics, developmental biology or cancer research. The high fecundity rate and short life cycle of zebrafish, enables fast generational turnover and makes it appealing for genetic manipulations – a growing field with already > 50% of laboratory animals being genetically modified.

The Zebrafish Core Facility of the Uni Bonn has husbandry capacity up to 10.000 zebrafish. With cutting-edge imaging techniques (fluorescence, confocal, two photon, electron microscopy), the Core Facility is open to external research groups willing to use the in-house expertise or custom-tailored fish lines. Experiments of gene expression/function may be facilitated by the injection of cDNA, mRNAs and morpholino antisense oligos, as well as CRISPR/Cas9 gene editing technology for mutant lines.

The Zebrafish Core Facility serves as a hub for researchers interested in using the zebrafish as their model species – which in some cases can be seen as a replacement method, too. Studies traditionally performed in rodents may now be carried out in fish – a vertebrate species that is considered less sentient than mammals. Next to the fast and transparent ex-vivo embryonic phase and genetic similarity to the human, the perception as a rodent-replacement-vertebrate-model has facilitated the huge increase of zebrafish use in biomedical research in the last years and ongoing.

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

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Pigeons have a long-standing tradition as laboratory animals in the behavioral sciences, used in experiments on perception, learning, and categorization. Although birds and mammals show comparable behavioral output, both groups are characterized by different gross anatomical organization of their brains. We try to understand how this is possible. To elucidate this riddle, the Department of Biopsychology at Ruhr University, and especially subproject A01 of the DFG-funded SFB 1280, have established technical breakthroughs that enable neuroscience-based cognitive behavioral research while consequently implementing the 3R principles: The first breakthrough is the establishment of small animal magnetic resonance imaging allows non-invasive repeated experiments and within-subject designs. This technique leads to a reduction of animals required in the experiments while simultaneously improving data quality. The second breakthrough is optogenetics, a method unsurpassed in terms of temporal resolution. It allows direct intervention in neural systems in within-subject experimental designs. Resulting causal explanations go far beyond correlational results achieved with conventional methods and tremendously reduce the number of animals needed for experimentation. 3. The third breakthrough is high-density electrophysiology. Silicon probes equipped with a high number of recording sites in combination with improved electrode placement using computer-based motorized stereotactic surgeries result in a higher yield of neurons and a reduced probability of placement errors ultimately leading to a reduction of animals. In addition, the duration of surgeries is reduced, and the implementation of balanced anesthesia has become possible, a protocol recently established in our lab strictly following the principle of refinement.

3R Competence Network North Rhine-Westphalia – Medical progress in line with the best pos-sible animal welfare

To Vinh, Michael[1]; Bali, Burak[1]; Henderson, Antonia[1], Kistermann, Lena[2]

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Animal research is still a major impetus to advance medicine for human and animal health. Nonetheless, from the perspective of animal welfare and research ethics, medical progress cannot be the sole benefit of animal experiments. Here, the principle of 3Rs (replacement, reduction, refinement; Russell & Burch, 1959) provide a framework for researchers to continuously impro-ve animal welfare.

In 2022, the eight faculties of medicine in North Rhine-Westphalia formed the 3R Competence Network NRW to promote medical progress in line with the best possible animal welfare.

Forming a platform for inter- and transdisciplinary exchange as well as expanding the range of training and further education, the 3R Competence Network NRW creates new synergies between researchers from different scientific disciplines.

The 3R network facilitate the dialogue between science, industry, politics, regulatory authorities and other stakeholders and promote the development of new 3R methods. For this, the network organizes scientific events such as symposia, workshops and an annual network conference on 3R related topics. A monthly online colloquium is also offered for further education and training. There is a high demand for the 3R-related activities and events where they are very well received in and out of the network. The network also engages in public relations science communication and build bridges between science and society. This includes dialogue events and cooperation with schools.

The 3R Competence Network North Rhine-Westphalia creates an environment for a more sustainable, future-oriented (bio)medical science in line with the best possible animal welfare and promotes 3R principle as a catalyst for innovation.

Forum 3R: animal models and alternative methods as complementary measures to strengthen the 3R

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There is currently no superordinate regional 3R center in Thuringia. With the internal 3R Forum, we created a local opportunity to strengthen preclinical methods in the research profile of the University Hospital Jena (UKJ) by promoting alternative models and highest animal welfare standards. In the consistent implementation of the 3R principle, Forum 3R bundles resources by creating a research-map of animal models and alternative methods for networking of both complementary research approaches. The development of a teaching concept helps to define suitable animal or alternative methods for individual scientific questions. Networking with regional 3R centers and scientific institutions helps to provide new methods for implementing 3R at UKJ. We increase the use of digital platforms for inter-laboratory. Conducting animal experiments also requires scientists to communicate well with the public. Therefore, the training of UKJ`s staff who are involved in animal experiments at all levels of education in transparent communication is also a mission of Forum 3R. Forum 3R means interlocking existing and new in-vitro and in-vivo models for sustainable biomedical research and the anchoring of the 3R concept at the UKJ. The interdisciplinary work of the UKJ`s research institutions is possible by use of modern digital instruments for reducing animal experiments and minimizing the burden on laboratory animals. The positive development of Forum 3R shows that it`s possible to establish the mission of the 3R principle within the institution. This formation of an internal network is particularly interesting for institutions that do not belong to a regional 3R center.

Emerging networks in Berlin: Charité 3R, Der Simulierte Mensch and Einstein Center 3R

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With its large number of research institutes, Berlin is a center of biomedical research with an ever-growing and vibrant scientific community.

Charité - Universitätsmedizin Berlin established Charité 3R to anchor the 3Rs in its biomedical science community. Together with Technische Universität Berlin, both institutions acquired funding for a new research building, "Der Simulierte Mensch," opening in 2025, focusing on alternative methods to animal experiments. Furthermore, in 2021, universities and non-university partners established the Berlin-wide Einstein Center 3R (EC3R).

Charité 3R is active in three pillars: scientific and political communication on animal experiments and alternatives; education and support to promote the 3Rs; direct research funding in all 3Rs aspects.

Der Simulierte Mensch will be a joint research center of TU Berlin and Charité. It houses interdisciplinary scientific projects of researchers and engineers aiming to simulate human physiology. Two publicly open floors for science communication and outreach to and from the scientific community are a special feature.

The Einstein Center 3R (EC3R) aims to strengthen Berlin's 3Rs activities by networking, including measures in communication, training, and research. The latter focuses entirely on developing robust 3D cell culture models.

All these initiatives work highly intertwined with developing high-quality and forward-looking NAMs (new approach methodologies) for biomedicine. Still, animal models are relevant to investigate various biomedical questions, motivating especially Charité 3R to support Refinement efforts.

The presented 3Rs community can significantly strengthen Berlin as a science location by opening scientific perspectives, promoting spin-offs, and seeking inter-/national cooperation.

Interdisciplinary Centre for 3Rs in Animal Research (ICAR3R)

Pabst, Celina; Krämer, Stephanie

Interdisciplinary Centre for 3Rs in Animal Research (ICAR3R)

ICAR3R is an interdisciplinary cooperation with a 3R professorship for Refinement at the Department of Veterinary Medicine and a 3R professorship for Replacement at the Department of Human Medicine of Justus Liebig University. It aims to promote animal protection in biomedical research and drive forward concepts for the development of alternative methods and husbandry conditions in order to make a sustained contribution to the reduction of laboratory animals.

Here, the expertise of the departments complements each other perfectly. The 3R professorship for Replacement, led by Prof. Jedlička, focusses on computer-based modelling, while the 3R professorship for Refinement, led by Prof. Krämer, specializes in animal welfare. As a fundamental in the Concept of the 3R, animal welfare in the field of Refinement is not only about the improvement of husbandry, but also in implementing a Culture of Care and 3R-Education.

As ICAR3R considers interdisciplinarity to be crucial for the implementation of the 3R, not only veterinary medicine and human medicine are represented, but also an exchange is sought with research disciplines that are not initially associated with the topic of animal testing. Being an active contributor to the discourse on the subject of animal experiments ICAR3R for example cooperates with the humanities at the Justus Liebig University to approach the discussion from an ethical and moral perspective. The highly interconnected structures of the Research Campus of Central Hessen form suitable conditions, so that ICAR3R covers a sustainable contribution to the implementation of the 3R concept of Russell & Burch.