Meeting Report

R2N Science Camp

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The science camp of the R2N consortium (Replace and Reduce in Niedersachsen/Lower-Saxony) took place from January 20 to 22, 2020 in Braunlage, Germany. The aim was to discuss the state of the art in replacing and reducing animal models, as well as to facilitate exchange of knowledge among industry, academia and students. Prof. André Bleich (Director of the Institute for Laboratory Animal Sciences, Hannover Medical School/MHH) opened the meeting by highlighting some of the challenges but also the opportunities for replacing and reducing animal models in the near future.

The first presentation was given by Dr **Helena Kandarova** (Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia). She started with a historical overview of the origins of the 3R principles (Replace, Reduce, Refine) and their integration into legislation. She gave examples of alternatives that have been successfully validated so far and named some of the challenges for replacing animal models with alternatives: (i) changing the mindset about using animal models, (ii) regulation usually changes slowly, (iii) lack of finances to conduct validation trials, and (iv) lack of communication between method developers and regulators. Fortunately, there are also great opportunities; we have multiple tools today that may help to replace animal models, and in some cases, an alternative method combined with the knowledge of a compound may actually be more appropriate than an animal test.

Next, four PhD-students from the R2N consortium presented their projects for replacing or reducing animal models. **Carina Mikolai** (group of Prof. Stiesch / Dr Winkel, MHH) presented on interaction between oral multispecies biofilm and peri-implant mucosa in a three-dimensional *in vitro* model. 26% of patients with a dental implant develop a peri-implant infection. One reason is the microbial shift to pathogenic biofilms, which disrupts the host-microbe homeostasis and induces inflammation. A 3D model composed of oral mucosa, implant material, and an oral biofilm was developed in this project. The relationship of the tissue (gene expression, cytokine secretion) with the biofilm (bacterial species distribution) shows similar patterns to the *in vivo* peri-implant situation, making this a promising alternative to *in vivo* models.

Talke zur Brügge's presentation (group of Prof. Bleich/Dr Büttner, MHH) was titled "*CD14* and *ALPK1* affect expression of tight junction components and proinflammatory mediators upon bacterial stimulation in a colonic 3D organoid model". The etiology of inflammatory bowel disease (IBD) is still unknown. Murine intestinal epithelial organoids may be an alternative to animal experiments because they display *in vivo*-like crypt structures, are easy to cultivate from different colitogenic mouse genotypes, and can be manipulated via bacterial stimulation. Two candidate genes involved in IBD onset are *Cd14* and *Alpk1*. Bacterial stimulation of colonic WT, CD14-/- and ALPK1-/- organoids led to changes in gene and protein expression of tight junction markers and proinflammatory mediators comparable to the *in vivo* situation, making the organoids a promising tool to investigate mechanisms of IBD development *in vitro*.

Retroviral vector gene therapy is an exciting and effective treatment for patients with genetic disorders. But, as **Antonella Bastone** (group of Prof. Schambach/Dr Rothe, MHH) stated during her presentation "Development of a new all-in-one *in vitro* safety assay for gene therapy", this therapy carries the risk of insertional mutagenesis. Animal models are not always informative or sensitive enough. Ms Bastone further developed the SAGA (Surrogate Assay for Genotoxicity Assessment), an *in vitro* method to assess insertional mutagenesis. The test was advanced into an all-in-one assay for lymphoid and myeloid cells. Future plans comprise identifying the core genes for lymphoid immortalization after vector integration and developing SAGA in human cells.

Alina Schadenhofer's presentation (group of Prof. Osterhaus, University of Veterinary Medicine Hannover) "Investigating the influence of selected viral mutations on viral fitness and antiviral evasion mechanisms of human respiratory syncytial virus (HRSV) in *in vitro* and *ex vivo* systems" showed the necessity to develop alternative model systems for respiratory viral infections. In a collaboration, wild-type HRSV reverse genetics systems based on the complete viral genome of HRSV strains isolated from infected patients were established and specific mutations can now be rapidly introduced into the viral genome. The aim of the project is to replicate recently published data of experiments performed in ferrets using *in vitro*/*ex vivo* model systems. Prof. Osterhaus' group utilizes advanced *in vitro* and *ex vivo* models such as lung organoids, air-liquid-interface (ALI) cultures, and precision cut lung slices (PCLS), and has already shown that HRSV readily infects ferret respiratory organoid and human ALI cultures. Additional research is focused on investigating the influence of selected viral mutations on viral fitness and antiviral evasion mechanisms, and using alternative *in vitro*/*ex vivo* systems to characterize newly discovered respiratory viruses.

The second day started with the presentation of **Prof. Dr Lorenzo Moroni** (Maastricht University, MERLIN Institute for Technology-Inspired Regenerative Medicine, Chair of Complex Tissue Regeneration Department) on tools for 3D *in vitro* models and regenerative medicine applications. He presented applications of additive manufacturing (also known as 3D printing) and showed how 3D printing can create anatomical scaffolds where the specifics of the pores determine how cells populate the scaffold. Another technique is electrospinning, which is used to deposit very small fibers. Mesh size, porosity and stiffness can be manipulated, e.g., to create a mesh with the same dynamic stiffness as cartilage. Prof. Moroni also presented his approach to use stem cells and micro-engineered objects to create full tissues and showed an *in vivo* high throughput screening method, where small 3D-printed wells can be

implanted into an animal, enabling testing of many compounds in one animal, which could potentially reduce the number of animals needed for tests.

Prof. Dr-Ing. **Theodor Doll** (Hannover Medical School, Department of Otorhinolaryngology, Excellence Cluster Hearing4all) presented on techniques and regulatory aspects of additive fabrication in medicine. The future of medicine is personalized medicine and an example of this is the 3D printing of electro-cortical grid arrays. Prof. Doll gave examples of challenges that need to be overcome for 3D printing implants, e.g., biocompatibility issues of silicon rubber can now be solved by adapting the printing method. Approval of customized and custom-made devices is more complicated than of mass-produced ones, posing regulatory challenges. Prof. Doll envisions Hannover to be a leading partner in the validation of personalized fabricated medicine meeting regulatory requirements. He outlined important roles for groups like the R2N consortium in improving the quality and speed of validation and implementation of new personalized medicines.

Dr **Christina Hesse** (Fraunhofer ITEM, Group Leader Respiratory Pharmacology) presented on the use of human lung tissue. She gave an overview of how human lung tissue is prepared and used for studies. As the tissue contains all the different cell types of the lung, such as epithelial and endothelial cells, fibroblasts and nerve fiber endings, effects of different chemicals or mediators, infections with virus or bacteria, but also interactions in co-culture with, e.g., immune or tumor cells can be investigated. Several models for lung diseases with high translational value have been established, e.g., for pulmonary fibrosis. Usually, research starts *in vitro* and then moves *in vivo*, but animal models do not fully reflect the pathogenesis, e.g., fibrosis is reversible in animal models. The *ex vivo* human lung tissue could help to avoid some of the *in vivo* experiments.

Julia Grabow, MSc. (ProBioGen AG, Deputy Head of Department for Bioassays) gave a presentation on the human artificial lymph node model (HuALN) for biopharmaceutical testing and disease modelling *in vitro*. The HuALN model is a dynamic perfusion bioreactor-based model for long-term treatment of tissue-engineered lymph nodes (lymph node microorganoids) in hydrogel-based 3D cell matrices. It allows monitoring cellular and humoral immune responses, e.g., by FACS analyses, measurement of cytokines, or drug specific antibodies. The system can be used to test drug-induced immune stimulation and modulation, immunotoxicity and to benefit vaccine efficacy evaluation.

Biological barriers protect vulnerable organs from physical and chemical damage and infections, maintain homeostasis, and act as selective filters for regulated molecule transport. Dr **Winfried Neuhaus** (Austrian Institute of Technology GmbH, Centre for Health & Bioresources, Competence Unit for Molecular Diagnostics, President of European Society for Alternatives to Animal Testing – EUSAAT) used the blood-brain barrier (BBB) as an example to explain the requirements for an *in vitro* model to be accepted as a replacement method. *In vitro* BBBs can be used for toxicity screening, drug screening, or disease modelling. Currently, different *in vitro* models are available, including static transwells, dynamic hollow fibres, spheroids, and complex microphysiological systems. Each has advantages and disadvantages, and the optimal model depends on the context and research question. Any model should be as simple as possible and only as complex as necessary.

Dr **Robert Zweigerdt** (Hannover Medical School, Department of Cardiothoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hans Borst Centre) presentated on generating human induced pluripotent stem cells (hiPSC) in high quantities and of high quality. hiPSCs can be derived from patient-specific cells and can differentiate into any functional cell type. In Zweigerdt's group, 3D culture methods have been developed and optimized for hiPSC cultivation in industry-compliant bioreactors, increasing the cell yield at decreased production costs, making hiPSCs more accessible for research and industry-compliant large-scale screening purposes. Dr Zweigerdt presented the production of heart forming organoids (HFOs) from iPSCs by an approach resembling early steps of native heart development with proper 3D organization of native heart tissue. This approach also represents heart development at the interplay with foregut endoderm, including liver and lung, thereby providing a valuable, versatile new tool for R2N research of multiple cell lineages.

The last presentation was given by Dr **Thomas Steger-Hartmann** (Bayer AG, Research & Development, Pharmaceuticals, Head of Investigational Toxicology), who presented on the 3Rs in preclinical drug safety. In drug development, fewer than 1% of substances successfully transfer from research to market approval. Around 80% of compounds fail before the first human administration due to safety issues in animals and in Phase I trials another 62% fail because of human safety issues. While R&D costs keep increasing, the number of approved molecular and biological entities remains stable. Animal studies are still required for safety studies. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) aims to harmonize regulations between different regions, and advises regulatory entities, industry, and researchers, also on alternatives. Thanks to the ICH, acute toxicity testing for drugs has been eliminated. Most local and topical endpoints are well-covered by alternatives, but alternatives for certain endpoints (e.g., behavior) are not realistic. *In silico* methods may improve safety assessment, e.g., big data concordance analysis of preclinical and clinical data as, e.g., provided in PharmaPendium, will provide a better insight into which animal studies predict human outcome well and which are obsolete.

The meeting continued with an internal strategic R2N meeting and two workshops: one by Dr **Thomas Koch** (Expert Training and Consultancy Services in Research Management, Munich) on research funding in Germany, and another by Prof. Dr **Alexander Grossmann** (Leipzig University of Applied Sciences) on new perspectives in scientific publishing. We thank the organizers, especially Ms. Dorothea Mühe, the speakers and participants, as well as the members of the R2N advisory board (Prof. v. Messling, Federal Ministry of Education and Research, and Prof. Hartung, John Hopkins University) for their valuable input and the enriching scientific exchange.

Stevie van der Mierden, Cathalijn Leenaars and André Bleich

Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany