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Swiss 3R
Competence
Centre

Swiss 3Rs Day

11th October 2022
Hotel Kreuz Bern

Raphaël Doenlen (CH)
Lynne Sneddon (Swe)
Julika Fitzi-Rathgen (CH)
Paulin Jirkof (CH)
Guillaume Andrey (CH)
Volker Lauschke (Swe)
Gregory Segala (CH)

Remi Villenave (CH)
Johannes Bohacek (CH)
Julie Vériepe (CH)
Urs Meyer (CH)
Pauline Zamprogno (CH)
Bernard Voelkl (CH)
Roland Dijkman (CH)

Scientific and Organising Committee

Jenny Sandström
3RCC, Bern

Armand Mensen
3RCC, Bern

Chris R. Cederroth
3RCC, Bern

Kathryn Sadowski
3RCC, Bern

Mathias Yamahachi
UniBe, Bern

Stéphanie Claudinot
UniL, Lausanne

Danielle Roppolo
UniGe, Geneva

Alexandre Widmer
EPFL, Lausanne

Certificate of Attendance

Participants will receive their certificate of attendance after the conference. This certificate of attendance is not the certificate for continuing education mentioned below and therefore should not be presented to the Swiss Veterinarian authorities as such.

Continuing Education Certificate

The Swiss 3Rs Day has been accredited as one day of continuing education for stuy directors and experimenters by the Federation of Swiss Cantonal Veterinary Officers (VSKT).

Study directors and experimenters working in Switzerland can obtain the certificate ONLY if they have signed the list of attendance at the registration desk at the beginning and at the end of the meeting.

Participants can request their Continuing Education Certificate to the 3RCC secretariat email: secretariat@swiss3rcc.org

3RCC Poster Award

The 3RCC will select one best poster presentation award that represents CHF 500. The award will be announced during the closing ceremony of the Swiss 3Rs Day.

The awardee will be selected based on the quality of their reseach, its impact on 3Rs, its benefits compared to existing methodologies and the clarity of their presentation. The authors should be present at the closing ceremony to be eligible for the award.

Scientific Program Overview

08:30 - 09:15	Registration
09:15 - 09:30	Welcome message **Jenny Sandström**, Director, Swiss 3RCC **Armand Mensen**, Scientific Officer, Swiss 3RCC

3Rs implementation in practice - Chair: Hanno Würbel (UniBe)

09:30 - 10:00	Digital Ventilated Cage (DVC®) – A true home-cage monitoring technology **Raphaël Doenlen**, Head of Phenotyping Unit, EPFL, Switzerland
10:00 - 10:30	Refining zebrafish experiments: Importance of behavioural mon itoring in welfare assessment **Lynne Sneddon**, University of Gothenburg, Sweden
10:30 - 11:00	*Coffee break*
11:00 - 11:30	Re-homing of small laboratory species at Swiss Universities **Julika Fitzi-Rathgen,** Schweizer Tierschutz, Switzerland **Paulin Jirkof**, UZH, Switzerland
11:30 - 12:00	Embryos derived from tetraploid complementation to study Pitx1 mis-activation in vivo **Guillaume Andrey**, University of Geneva, Switzerland
12:00 - 13:00	*Lunch break*

Keynote lecture lecture (13:00 - 14:00)

Chair: Chris R. Cederroth (3RCC)

Organotypic and microphysiological human tissue models for translational pharmacology

Volker Lauschke, Karolinska Institutet, Sweden

3Rs innovation and research - Chair: Laura Suter-Dick (FHNW)

14:00 - 14:30	3D Multi-Organoid Systems for Drug Discovery **Gregory Segala,** Fluosphera SA, Switzerland
14:30 - 15:00	Application of Microphysiological Systems in the pharmaceutical industry: Lessons learned and perspectives **Remi Villenave**, F. Hoffmann-La Roche Ltd, Switzerland
15:00 - 16:00	*Poster Session*
16:00 - 16:30	Frontiers in behavioral analysis: More dots, more data, fewermice. **Johannes Bohacek,** ETH Zürich, Switzerland
16:30 - 17:00	Using C. elegans to diversify study models **Julie Vériène**, University of Lausanne, Switzerland

Awardees presentations - Chair: Armand Mensen (3RCC)

17:00 - 17:15	Micropipette-guided drug administration in mice **Urs Meyer,** UZH, Switzerland
17:15 - 17:30	Second-generation lung-on-a-chip **Pauline Zamprogno**, ARTORG Center, Uni Bern, Switzerland
17:30 - 17:45	The standardization fallacy **Bernard Voekl,** Vetsuisse, University of Bern, Switzerland
17:45 - 18:00	SARS-CoV2 in the human respiratory epithelium **Ronald Dijkman**, University of Bern, Switzerland
18:00 - 18:15	Poster prize and closure **Chris R. Cederroth,** Scientific Officer, Swiss 3RCC, Switzerland

Session 1

3Rs implementation in practice - Chair: Hanno Würbel (UniBe)

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Oral presentations - Session 1

09:30 - 10:00

Digital Ventilated Cage (DVC®)
A true home-cage monitoring technology

Raphaël Doelen, Head of Phenotyping Unit, EPFL, Switzerland

The Digital Ventilated Cage (DVC®) technology is a new generation of housing racks helping the animal facility and the scientists to monitor the well-being of experimental mice and at the same time collect scientific data of mice activity in its true home-cage. This monitoring does not require any manipulations of the mice and is therefore totally non-invasive and non-stressful. This technology mixes normal housing, well-being monitoring and collection of scientific data. It also does not require to single cage the animal to monitor the activity. Rodents stay together in the cage and the system automatically scores how they move and interact to each other. It offers for the first time a true home-cage monitoring and give access to how mice behave in its housing cage without modifying the environmental conditions.

10:00 - 10:30

Refining zebrafish experiments: Importance of behavioural monitoring in welfare assessment.

Lynne Sneddon, University of Gothenburg, Sweden

Zebrafish are an important model species and their use is increasing globally. To ensure good welfare in laboratory experiments we need a method of assessing any pain caused by invasive procedures. Behaviour provides a relatively easy and non-obtrusive means of understanding the welfare status of animals and the development of monitoring software tools can allow assessment of pain and further

when to intervene to alleviate pain. This presentation will highlight behavioural changes observed in individual and group held zebrafish as well as discuss the most effective drugs that provide analgesia.

11:00 - 11:30

Re-homing of small laboratory species at Swiss Universities

Julika Fitzi-Rathgen, Schweizer Tierschutz STS, Switzerland
Paulin Jirkof, University of Zurich, Switzerland

While there are some well-known re-homing programs for cats and dogs, the re-homing of smaller laboratory animals such as rodents or rabbits, on the other hand, is less well known. In collaboration with the Swiss Animal Protection (SAP / STS), the University of Zurich and the EPFL established a re-homing project with the aim of giving rodents and rabbits from animal experiments a new life in private homes. For experimental and legal reasons not all laboratory animals can be re-homed after the experiments. However, until now several hundreds of rabbits, rats and mice have already been successfully re-homed. The re-homing project receives great support from the experimental animal husbandries and the research groups involved as well as from the participating animal welfare organizations and the private persons who are willing to offer the animals a good place to live. In this talk, we will present the prerequisites, challenges and potential of the re-homing program from the perspective of an university and an animal protection organization.

11:30 - 12:00

Embryos derived from tetraploid complementation to study Pitx1 mis-activation in vivo

Guillaume Andrey, University of Geneva, Switzerland

Developmental genes are frequently controlled by multiple enhancers sharing similar specificities. As a result, deletions of such regulatory elements have often failed to reveal their full function. Here, we use the Pitx1 testbed locus to characterize in detail the regulatory and cellular identity alterations following the deletion of one of its enhancers (Pen). By combining tetraploid complementation to derive embryos from Embryonic Stem Cells, single cell transcriptomics and a novel cell tracing approach, we observe an increased fraction of Pitx1 non/low-expressing cells and a decreased fraction of Pitx1 high-expressing cells. We find that the over-representation of Pitx1 non/low-expressing cells originates from a failure of the Pitx1 locus to coordinate enhancer activities and 3D chromatin changes. This locus mis-activation induces a localized heterochrony and a concurrent loss of irregular connective tissue, eventually leading to a clubfoot phenotype. This data suggests that, in some cases, redundant enhancers may be used to locally enforce a robust activation of their host regulatory landscapes.

Keynote Lecture

13:00 - 14:00

Chair: Chris R. Cederroth (3RCC)

Organotypic and microphysiological human tissue models for translational pharmacology

Volker Lauschke, Karolinska Institutet, Sweden

Abstract

The number of successful drug development projects has been stagnant for decades despite major breakthroughs in chemistry, molecular biology and genomics. Unreliable translatability of preclinical in vitro and in vivo models has been identified as the cause of most failure. Organotypic and microphysiological culture of primary human cells has emerged as a set of promising tools for preclinical drug development to narrow this translation gap. In this talk I will provide an overview of our recent efforts in developing 3D human tissue cultures and microfluidic models for both efficacy and safety assessments using phenotypic screening. In addition, the talk will present recent use cases where the use of such organotypic cultures has had direct impacts on market authorizations.

Volker Lauschke
Karolinska Institutet, Sweden

Volker M. Lauschke (V.M.L.) is Associate Professor and group leader in Personalized Medicine and Drug Development (since 2017) at Karolinska Institutet (KI), Stockholm, Sweden and Deputy Head of the Institute of Clinical Pharmacology (IKP) in Stuttgart, Germany (since 2021). His research group engineers organotypic and microphysiological human tissue models to develop novel therapeutic strategies for inflammatory conditions, infectious diseases and complex metabolic diseases.

V.M.L. has authored over 120 papers and is the recipient of multiple awards in the area of genetics, pharmacology and drug discovery, including the Malin and Lennart Philipson Prize 2016 and the AAPS High Impact Award 2020. Besides his academic work, he is co-founder and CEO of HepaPredict AB, a biotech company offering 3D human liver models for drug discovery and development, as well as co-founder and CSO of PersoMedix AB, offering services for personalized drug response predictions.

Session 2

3Rs innovation and research - Chair: Laura Suter-Dick (FHNW)

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Oral presentations - Session 2

14:00 - 14:30

3D Multi-Organoid Systems for Drug Discovery

Gregory Segala, Fluosphera SA, Switzerland

Drug development costs $2.6 billion with a failure rate of 90% because in vitromethods do not mimic the systemic organization of the human body and animal models are too different from Humans. The lack of relevance of current in vitrotesting and animal experimentation dramatically increases drug development failure in clinic due to unforeseen toxicity (40% of failure), and unpredicted lack of efficiency (50% of failure). As a result, the limitations of in vitro testing reduce the probability to develop safe and efficient drugs for patients. To solve this technological limitation, we have invented a patent-pending technology to generate in vitro human biosystems that recapitulate essential physiological communications normally happening into the human body to reliably identify the most promising lead compounds during pharmaceutical drug discovery. By recapitulating systemic effects, our technology has the potential to become a concrete alternative to animal experimentation.

14:30 - 15:00

Application of Microphysiological Systems in the pharmaceutical industry: Lessons learned and perspectives

Remi Villenave, F. Hoffmann-La Roche Ltd, Switzerland

Scientific challenges and societal changes have compelled the pharmaceutical industry to explore the application of MPS throughout the entire drug development pipeline. New molecules and modalities have highlighted the limits of animal models translatability, while ethical considerations on animal testing have evolved

for the regulators and the general public. While MPS have been used in the industry at each stage of the preclinical process for almost a decade now, there remain numerous challenges and their impact is still hard to measure. Here, we will present a partial overview of MPS use at Roche, with a focus on preclinical safety assessment, discuss the lessons learned from recent years of MPS application and give a perspective on successes, current and foreseen challenges, as well as future developments.

16:00 - 16:30

Frontiers in behavioral analysis: More dots, more data, fewer mice.

Johannes Bohacek, ETH Zürich, Switzerland

The assessment of rodent behavior forms a cornerstone of preclinical assessment in neuroscience research. Nonetheless, the true and almost limitless potential of behavioral analysis has been inaccessible to scientists until very recently. Now, in the age of machine vision and deep learning, it is possible to extract and quantify almost infinite numbers of behavioral variables, to break behaviors down into subcategories and even into small behavioral units, syllables or motifs. In this talk we will demonstrate how pose estimation algorithms can be applied to raw videos to gather a wealth of information that was previously inaccessible. These data can then be combined with unsupervised machine learning methods to analyze large datasets from multiple experiments. This allows detailed behavioral analyses to identify novel phenotypes. We further demonstrate the transitions between the identified behavioral clusters can boost the statistical power and resolve phenotypes using smaller groups of animals. This will allow smaller cohort sizes, positively impacting animal welfare.

16:30 - 17:00

Using C. elegans to diversify study models

Julie Vériène, University of Lausanne, Switzerland

Caenorhabditis elegans is a 1mm long nematode whose genome has been completely sequenced. The ease of use, the rapidity of its life cycle and its low cost make *C. elegans* a study model of choice. 1536 laboratories around the world use *C. elegans* and more than 34,000 publications are listed on Pubmed. *C. elegans* is a simple organism, with a highly conserved intracellular system compared to humans and similar nervous and intestinal systems. *C. elegans* has been instrumental in the discovery of apoptotic genes, RNA interference, microRNAs, etc. If *C. elegans* cannot replace mice experiments, it serves to drastically reduce their number. Concrete examples will be given on personal studies on *C. elegans* alone or in addition to other models.

Poster abstracts

Poster 1

A Human Bone/Bone-Marrow-on-a-Chip Approach for in vitro culture of human bone marrow and benchmark against clinical reality

Melanie-Jasmin Ort [1,2], Ioanna Maria Dimitriou[1,2], Martin Textor[1], Marcel Niemann[1,3], Nina Stelzer[1]

[1] Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Julius Wolff Institute, Augustenburger Platz 1, 13353 Berlin, Germany
[2] Institute of Chemistry and Biochemistry, Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, 14195 Berlin, Germany
[3] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Center for Musculoskeletal Surgery, Augustenburger Platz 1, 13353 Berlin, Germany

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3D microfluidic cell culture systems (organ-on-a-chip) allow for studying human physiology at organ level. Trabecular microarchitecture of cancellous bone provides not only mechanical stability, but also a microenvironment for reticular connective tissue and vital hematopoiesis in the bone marrow (BM). In joint arthroplasty, one major clinical problem is aseptic peri-implant osteolysis due to release of metallic particles and ions. Previously, we found that the in vitro osteogenic capacity of bone marrow mesenchymal stromal cells (BMSCs) is impaired by in vivo exposure to cobalt and chromium ions in the BM. For further studies we aim to establish a human bone/BM organ-on-a-chip system to (i) benchmark it to human BM specimens in regard to bone tissue homeostasis and cellular composition and (ii) to validate the system for predicting metal-ion-induced adverse biological effects. Primary cells (MSCs, osteoblasts and mononuclear cells) from healthy BM were isolated. Human cancellous bone was decellularized, cylindrical scaffolds were prepared, MSCs and osteoblasts were seeded and matrix mineralization was induced for five

days under static conditions. Subsequently, the scaffolds were transferred to the 2-Organ-Chip (TissUse GmbH, Berlin, Germany) and BM mononuclear cells were seeded before osteoclastogenesis was induced by growth factor supplementation under dynamic culture conditions (70 µl/min) for eight days. Subsequently, the scaffold was dynamically cultivated for 21 days without further growth factor supplementation. Lactatdehydrogenase (LDH) quantification revealed cellular integrity over the whole experimental period. Quantification of soluble bone turnover factors indicated active bone metabolism. Two-photon excitation microscopy confirmed the presence of active osteoblasts, osteoclasts and the formation of reticular fibers. Flow cytometry indicated stable T-cell and monocyte populations as well as detectable levels of hematopoietic precursor cells and B-cells. For toxicity testing, treatment with clinically relevant metal ion concentrations revealed a significantly higher LDH release compared to untreated controls. Interestingly, for the same concentrations, this effect could not be observed in a 2D MSC monoculture assay. Developing a reliable in vitro system for predicting adverse effects induced by implant wear in the BM is our main focus for improving preclinical routines to keep patient safety on the highest level.

Poster 2

Handling methods affects anxiety-like behaviour but not measures of chronic stress

Janja Novak[1], Ivana Jaric[1], Marianna Rosso[1], Reto Rufener[2], Chadi Touma[3], Hanno Würbel[1]

[1] Animal Welfare Division, Vetsuisse Faculty, University of Bern, Bern, Switzerland.
[2] Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, Institute of Parasitology, University of Bern, Bern, Switzerland.
[3] Department of Behavioural Biology, University of Osnabrück, Osnabrück, Germany.

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Despite evidence that tunnel handling can reduce measures of anxiety and stress in laboratory mice, tail handling continues to be used routinely. This is mostly because

current evidence in support of tunnel handling is restricted to behavioural outcomes, found after extensive daily handling. While daily handling may be relevant for habituating the animals to the experimenter, the results may not apply to routine husbandry practices. The aim of our study was to assess whether different handling methods during routine husbandry induce different behavioural and physiological stress responses in laboratory mice. We compared these effects to a validated paradigm of unpredictable chronic mild stress (UCMS). We housed mice of two strains (Balb/c and C57BL/6) and both sexes either under standard laboratory conditions (CTRL) or under UCMS. Half of the animals from each housing condition were tail handled and half were tunnel handled twice per week. We found strain dependent effects of handling method on measures of anxiety. UCMS housed mice showed increased plasma corticosterone levels and reduced sucrose preference, however, we found no effect of handling method on these measures. Our results indicate that routine tail handling may affect anxiety, but may not be a significant source of chronic husbandry stress.

Poster 3

Next-generation inner ear organoids to tackle hearing loss

Sara Alonso Jimenez[1], Daniela Doda[1], Victoria Valsamides[1], Hans Ruedi Widmer[2] and Marta Roccio[1]

[1] Inner Ear Stem Cell Lab. Dep Otorhinolaryngology Head and Neck Surgery, University Hospital Zurich and University of Zurich
[2] Experimental Neurosurgery Laboratory, Dep. Neurosurgery, University Hospital Bern and University of Bern

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Inner ear hair cells and auditory neurons are essential for sound detection. Their damage or loss is irreversible in humans and is a major cause of permanent hearing deficit. A major bottleneck for the development and clinical translation of novel therapies for sensorineural hearing loss is represented by the lack of suitable

cellular assays, based on human sensory cells, for the validation of compounds or gene therapies in pre-clinical phase. Directed differentiation of human induced pluripotent stem cells (hiPSC) towards inner ear cell types offers a potential solution to this problem. Moreover, such an approach enables to avoid primary culture from rodents' sensory organs, which have proven in many instances not predictive of the human response. We show how growth factors and morphogens guidance in 3D aggregates of hiPSC faithfully recapitulates inner ear morphogenesis and otic development in vitro, generating sensory epithelia and neurons. These sensory units are however embedded in large 3D constructs containing additional cranial tissues, not ideal for compound testing. Current focus of our research is to establish novel culture methods for iPSC-derived sensory cells on multi-well plates and/or microfluidic chips to establish a first platform to validate protective and regenerative compounds for hearing restoration

Poster 4

Unlimited generation of functional auditory neurons for high throughput in vitro assays

Francis Rousset[1], Giulia Schilardi[2], Dominik Schmidbauer[3], Stefan Fink[4], Rebecca Sipione[1], Stéphanie Sgroi[1], German Nacher-Soler[1], Sonja Kleinlogel[2], Marcus Müller[4], Rudolf Glückert[3], Pascal Senn[1, 5]

[1] The Inner Ear and Olfaction Lab, Department of Pathology and Immunology, University of Geneva, Switzerland.
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[3] Inner Ear Laboratory, Department of Otolaryngology, Medical University of Innsbruck, Innsbruck, Austria.
[4] Tübingen Hearing Research Center, Department of Otolaryngology, Head and Neck Surgery, University of Tübingen, Germany.
[5] Department of Clinical Neurosciences, Service of ORL & Head and Neck Surgery, University Hospital of Geneva, Switzerland.

Contact: Francis.Rousset@unige.ch

Hearing loss affects over 460 million people worldwide and is a major socioeconomic burden. Both genetic and environmental factors (i.e. noise overexposure, ototoxic drug treatment or ageing), promote irreversible degeneration of cochlear hair cells and associated auditory neurons, known as sensorineural hearing loss. In contrast to birds, fish or amphibians, the mammalian inner ear is virtually unable to regenerate due to the limited stemness of auditory progenitors and no causal treatment is able to prevent or reverse hearing loss. As of today, a main limitation for the development of otoprotective or otoregenerative therapies is the lack of a robust and high-throughput compatible, preclinical model for drug development. As a consequence, research in the field mostly relies on high numbers of experimental animals, resulting in high variability of screening results, significant costs and ethical concerns. We have previously identified and characterized the phoenix auditory neuroprogenitors (ANPGs) as highly proliferative progenitor cells isolated from the A/J mouse cochlea. In the present study, we aimed at identifying signaling pathways responsible for the intrinsic high stemness of phoenix ANPGs. We therefore compared the transcriptome of phoenix cells to traditionally low stemness ANPGs, isolated from C57BL/6 mouse cochleae. Based on the differentially expressed pathways, we developed a reprogramming protocol with the aim to reactivate dormant stemness pathways in presenescent ANPGs (i.e. from C57BL/6 mice). Pharmacological treatment with a WNT agonist and dual smad inhibitors resulted in a dramatic increase in neurosphere growth and virtually unlimited passage number in stemness-induced ANPGs. Stemness-induced ANPGs could be frozen and thawed multiple times, allowing distribution to other laboratories. Importantly, even after 20 passages at an exponential expansion rate, stemness-induced ANPGs retained their ability to generate mature neurons that resembled anatomically and electrophysiologically type I and type II auditory spiral ganglion neurons. Our findings are relevant to reduce the numbers of experimental animals, to reduce variability in high-throughput screens, to reduce costs and to meet 3R principles. Finally, this study may also aid to overcome significant roadblocks in the field of inner ear regeneration.

Poster 5

In vitro model to replace in vivo traumatic brain injury rodent model

C. Loussert-Fonta[1], L. Stoppini[1], M. O. Heuschkel[1], O.Righini[2], L. Gomez Baisac[1], Y. Neuenschwander[3], D. Prim[2], C. Schmidt[3], M. E. Pfeifer[2], J. Extermann[3], A. Roux[1]

[1] Tissue Engineering Laboratory, HEPIA HES-SO University of Applied Sciences and Arts Western Switzerland, Geneva, Switzerland.
[2] Diagnostic Systems Research Group, Institute of Life Technologies, School of Engineering, University of Applied Sciences and Arts Western Switzerland (HES-SO Valais-Wallis), Sion, Switzerland.
[3] Micro-Nanotechnology group, HEPIA HES-SO University of Applied Sciences and Arts Western Switzerland, Geneva, Switzerland.

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Traumatic brain injury (TBI) is caused by an extensive range of physical events and can induce an even larger spectrum of short and long-term physiopathology. Neuroscientists have relied on animal models to understand the relationship between mechanical damage and functional alterations of neural cells. However, these in vivo models raise ethical issues as nervous system disorders are extremely painful. To overcome these concerns and bring a more accurate model of human TBI, we engineered an in vitro platform mimicking injury via a controlled expulsion of liquid onto a 3D neural tissue from human iPS cells. With this platform, biological mechanisms involved in neural cellular injury are recorded through electrophysiology measurements, quantification of biomarkers release and various imaging methods (immunoCLSM, OPT, SEM). With this platform, we 3D-spatially reconstruct the injured area after staining with specific nuclear dyes. Furthermore, the preliminary results enlighten that TBI is inducing a drastic change in the electrophysiological activity of the tissue and the release of glial and neural biomarkers. As a perspective, we hope to monitor TBI-induced injury impact over time and better understand the kinetic of biomarker release and cell recovery.

Poster 6

Collective organisation of circadian rhythm and cell cycle in murine intestinal organoids

Elena Tonin[1], Felix Naef[1]

[1] Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

The circadian clock orchestrates physiological and behavioural rhythms in living organisms with a period of approximately 24 hours. This vital adaptation on Earth allows mammals to compartmentalise biological processes at different times of the day. Disruption of these rhythms has deleterious consequences, for example on tissue regeneration and cancer progression. However, there is a lack of understanding of how the molecular clock, cell differentiation and cell cycle integrate during homeostasis and disease. Work in animals is controversial: some suggest that the clock may 'gate' the cell cycle to certain time windows, others have shown that cell cycle progression influences the clock. Overall, research on the topic is mainly conducted in vivo or in homogeneous populations of cells. We now aim to implement a more physiological and multicellular system like 3D mouse intestinal organoids to study the collective behaviour of circadian rhythm during proliferation and differentiation, with quantitative and mathematical analysis. With the help of in vitro editing techniques, we will replace and reduce animal work to disentangle clock dynamics during homeostasis and disease, setting the basis for in vitro multicellular circadian studies that will shed new light onto current difficulties of improving the treatment of neoplasms and regeneration.

Poster 7

Establishing a colorectal cancer (CRC) model in Drosophila to identify novel regulators in CRC metastasis

Alexandra Traistaru[1], Jamie Little[1], Konrad Basler[1], Erich Brunner[1], George Hausmann[1]

[1] Institute of Molecular Life Science, University of Zurich, Zurich, Switzerland".

Colorectal cancer (CRC) is one of the most prevalent cancers and common cause of cancer related deaths. Hyperactivation of Wnt and Ras signalling were often observed in CRC leading to loss of cellular repair mechanism and distrupting homeostasis. Investigating molecular factors that are involved would be ehtical challenging due to the overload use of mice tumor models. As an alternative, a Drosophila model was established in previous attempts by clonally inducing loss of APC combined with Ras acitvation and overexpression of Snail to mimic epithelial-mesenchymal transition in CRC. The aim of my master thesis is to improve and develop the CRC model in flies and to screen for candidate genes involved in metastasis in the context of CRC.

Poster 8

3Rs: Tailor-made in vitro assays for replacement

Joachim Köser[1], Carine Gaiser[1], Floriana Burgio[1], Fabrice Müller[1], Laura Suter-Dick[1]

[1] School of Life Science, FHNW, Hofackerstrasse 30, CH 4132 Muttenz

In recent years the principles of 3R have gained new momentum due to technological advances such as the generation of self-assembled spheroids (microtissues) and organoids, the availability of microphysiological systems, and developments in the field of material sciences. Applying these new technological approaches to cell cultures of immortalized and primary cells, in combi-nation with suitable biomarkers and assays opens the opportunity to generate optimally suited cell models. The

premise as simple as possible, but as complex as necessary remains a key guiding principle. This poster focuses on three specific examples, where complex in vitro systems are implement-ed as potential replacements for animal studies, some of which are of high severity (e.g. liver fibrosis model, spinal cord injury model). Thus, the research actively supports the 3R principles (reduce, refine and replace animal testing). We implement microfluidics, 3D spheroids and com-plex extracellular matrix as required to address the specific research question. The results show that long-term, multicellular co-cultures of liver cells are required for the development of fibrosis, that microfluidic-based systems are able to promote endothelial barrier functions mimicking BBB, and that well-designed topological and biochemical cues support the regeneration of neu-rons upon an inflicted injury. From a 3R-perspec-tive, these assays are very relevant and should be further validated and broadly implemented.

Poster 9

Human Cerebral Organoids as Model for Neurotropic Virus Infection

Isabel Schultz-Pernice[1,2,3], Amal Fahmi[1,2,3], Beatrice Zumkehr[1,2], Antoinette Golomingi[1,2], and Marco P. Alves[1,2,4]

[1] Institute of Virology and Immunology, Bern, Switzerland
[2] Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[3] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland
[4] Multidisciplinary Center for Infectious Diseases (MCID), University of Bern, Bern, Switzerland

Driven by factors influencing vector distribution, as changing climatic conditions, evolving human habits and socioeconomic factors, emerging infectious diseases (EID) have risen to represent a significant and increasing concern for global economics and public health. More than 60% of EIDs and most of the recent epidemics are of zoonotic origin. In this regard, vector-borne flaviviruses have demonstrated

a remarkable epidemic potential. Neurovirulent flaviviruses, as tick-borne encephalitis virus (TBEV), represent a major threat to European countries. To date, our knowledge regarding pathological mechanisms driving acute neurological manifestations in TBEV patients remain poorly understood. Existing knowledge mainly relies on rodent models and post-mortem studies, allowing limited insight on pathways driving neuropathogenesis in the human host. In the past years, advanced 3D cell culture methods have demonstrated their capability to recapitulate processes shaping human organs in health and disease in vitro. Human cerebral organoids (hCOs) are at present the most advanced system to study human brain development and neuropathology and provide unprecedented opportunities to study host-pathogen interactions in an in vivo-like environment accessible to experimental manipulation. In this project, we aim to employ hCOs to shed light on mechanisms driving TBEV replication, cellular tropism, host response and neural injury. In addition, via a high-throughput screening approach, we aim to identify antiviral compounds with therapeutic potential against TBEV.

Poster 10

On the use of tunnel handling in Swiss animal facilities

Christopher R. Cederroth[1], Paulin Jirkof[2], Armand Mensen[1]

[1] Swiss 3RCC, Bern, Switzerland
[2] Paulin Jirkof, University of Zurich, Switzerland

Tunnel handling emerges as a new non-aversive handling method that could replace traditional tail-handling in animal facilities due to its benefits to animal welfare and research outcomes. In a recent survey from the 3RCC assessing 231 respondents (animal caretakers, AWO, technicians, veterinarians, scientists) across various Swiss animal facilities, near 58% of these had already used tunnel handling. However, while many have experienced it, tunnel handling still has not replaced tail handling in animal facilities due to a number of misconceptions. Here, we gather the knowledge from four Swiss Institutions that piloted tunnel handling in order to provide recommendations for a facilitated implementation. We also present the

results from survey performed on 20 animal care-takers that experienced tunnel handling and rated their satisfaction over traditional tail-handling. Overall, we report that animal care takers prefer transparent tunnels over red ones because transparency facilitates health checks. One pilot observation suggests that tunnels clipped on grid are preferable for habituation and hygiene. When routinely implementing tunnel handling, trained staff self-assess time spent on cage changes to be equal between tunnel and standard tail-handling (hands or forceps) routines. Observations point to advantages in progressive implementation of the use of tunnels (e.g. first on few cages, then on a rack, then on an entire room, and then on several rooms). Recommendations will be available on our website.

Poster 11

3D-printed Mouse Tail Models to promote the 3Rs in i.v. injection training

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Intravenous (i.v.) injections are a very common experimental procedure in mice to deliver substances. However, mice have a very limited number of easily accessible veins. Additionally, the success is highly dependent on the operator's skill. For reliable results, it is necessary to undergo extensive training on multiple mice. Personnel lacking any experience tend to require even higher numbers of mice to train on to get used to tail- and syringe handling before being able to fully concentrate on the injections themselves. Training these first steps on artificial mouse tail models could not only prepare trainees better for injections on live mice in terms of handling and speed but ultimately also bear the potential of reducing the number of animals required for training overall. Currently available animal training models are in use nowadays, but these models have proven to be unsatisfactory in regards of anatomical accuracy and feel. Hence, a collaboration between animal research scientists and mechanical/electrical engineers proficient in various 3D-printing technologies spawned a newly designed mouse tail that combines anatomical accuracy with more realistic tactile feedback. The next goals are therefore to collect long term user experiences and setting up a reliable and market-ready product for further distribution.

Poster 12

Same-sex housing affects the estrous cyclicity and hippocampal structural plasticity of female mice

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The underrepresentation of female mice has resulted in new policies that require equal representation of both sexes in preclinical research. This opened questions about the implementation of this initiative in animal facilities. Should males and females be housed together or in separate rooms? This information is rarely reported, although this important aspect of the animals' environment may strongly influence physiology and behavior, and hence experimental outcomes. Here we studied how same-sex and mixed-sex housing affects female reproductive cycle, hippocampal structural plasticity, and behavior. To do so, we housed female mice of two inbred strains (C57BL/6J and Balb/c) either with (mixed-sex) or without males (same-sex) in the same room, from weaning until adulthood. We show that female estrous cyclicity was affected by housing condition, whereby females housed under same-sex conditions exhibited suppressed or prolonged estrous cycles. We also found strain-specific alterations in ventral-hippocampus volume that likely contributed to observed differences in exploratory behavior in the open field test. Our findings suggest a complex interaction between housing conditions and mouse genetic background in determination of reproductive and neurobehavioral outcomes, with implications for the validity of animal models and the design of animal experiments.

EXHIBITORS

VIGILITECH
ADVANCED ANIMAL MONITORING

NAGI
BIOSCIENCE

ECTICA
TECHNOLOGIES

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3 R
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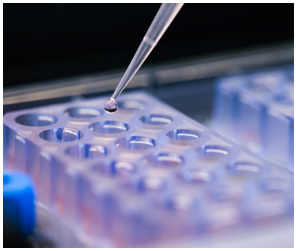
Swiss 3R Competence Centre

DRIVING 3Rs ADVANCEMENT FOR BETTER ANIMAL WELFARE AND SCIENCE



We fund research,
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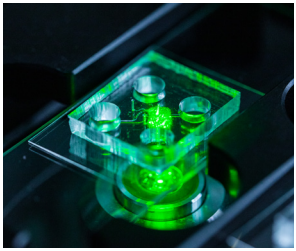
we educate,
we disseminate,
we award progress



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