identify relevant modes of action that can be used to design targeted testing approaches based on, among others, microphysiological systems and computational models.

The ensuing round table discussion with the speakers included discussion on translating research results to models that companies can use, validating new approach methods based on reliability and relevance instead of by comparing them to animal tests, and the potential role of AOPs in reshaping the validation process and current challenges of organs-on-a-chip technology.

The book "Animal Experimentation: Working Towards a Paradigm Change" was introduced by **Kathrin Herrmann** (CAAT, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA). While animal experiments are hailed to be indispensable to progress in human healthcare, less than 12% of drugs entering human trials after preclinical testing gain market approval. Studies also have reported that 50-90% of preclinical studies are not reproducible. The book, which is aimed at both scientists and laypersons, reviews the current use of animals in science and presents approaches towards achieving change.

After the break-out sessions, **TJ Bozana** (ToxTrack, Inc., Baltimore, MD, USA) explained how cheminformatics technologies are increasing access to big data, i.e., fusing and analyzing large databases. UL Cheminformatics is a suite of predictive models for nine health hazards based on read-across structure activity relationships (RASAR) developed from machine learning approaches. The analysis of large databases on toxicological data showed that six animal toxicity tests have an average sensitivity of 70%, while the RASAR technology reaches an average sensitivity of 89% across the same end-points with a coverage of 100% of all chemicals.

Bianca Marigliani (INMETRO, Federal University of São Paulo, Brazil) called for abstaining from using animal-derived products, such as fetal bovine serum, trypsin, collagen, etc. for nonanimal methods as they cannot be designated as cruelty-free otherwise. Fetal bovine serum (FBS), which is produced from the blood of fetuses upon slaughter of pregnant cows, has an ill-defined composition, may differ from batch to batch, may be contaminated with pathogens, and may influence cellular assays, can be replaced with human serum or with chemically defined medium. Different formulations of chemically defined media should be tried and cells may need to be adapted to a chemically defined medium by a gradual adaptation strategy.

Alison Gray (Afability, UK) stated that millions of animals are still used worldwide to develop antibodies although cruelty-free methods were developed 20 years ago. The phage-display methodology generates an enormous antibody repertoire with a huge molecular diversity at the antibody binding site from natural or synthetic gene fragments. Clonal selection takes place in bacteria. EU-level guidance and recommendations could help to promote the use of animal-free instead of animal-derived antibodies.

Jan van der Valk (3Rs Centre Utrecht, The Netherlands) spoke on fetal bovine serum, which has been a universal, little questioned cell culture supplement for decades. Replacement of the use of FBS is gaining traction as OECD and FDA are now discouraging its use and recommending the use of chemically defined media. The database "FCS-free" informs on suitable chemically defined media for cells and contains relevant confirmatory data.

Carol Treasure (XCellR8, Cheshire, UK) explained that XCellR8 has established animal product-free methods that are accepted by OECD for testing skin and eye irritation and corrosion by replacing all animal-derived products. There are ethical issues around the use of human-derived reagents in some countries and human serum is far more expensive than FBS, however these reagents make up only a small part of the overall testing costs. In her experience, leading with the science and understanding the practical needs of industry is essential to winning companies over to using cruelty-free methods.

Gill Langley (Humane Society International) closed the conference by summing up the key themes as being technology, multidisciplinarity, interfaces, funding, and positive communication and by thanking the speakers and audience.

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Meeting Report

Advanced *In Vitro* Models Analysis

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Microfabrication techniques and tissue engineering have enabled the development of a wide range of 3D cell culture technologies, including multicellular spheroids, organoids, scaffolds, hydrogels, organ-on-a-chip systems, and 3D bioprinting, each with its advantages and disadvantages. 3D models have been penetrating the early drug discovery process, starting from disease modelling to target identification, validation and screening, lead selection, efficacy, and safety assessment.

However, while challenges remain in the standardization of culture and assay protocols in 2D systems, this is even more pronounced in the more complex 3D cell models, which are less accessible to optical imaging. Therefore, improvements in imaging, data acquisition, and analysis tools including chemical sensing are necessary for broad adoption of 3D cell cultures for screening. Furthermore, regulatory authorities have yet to accept data obtained from 3D cell models as a surrogate or at least as a partial substitution for pre-clinical animal testing.

The Competence Centre TEDD (Tissue Engineering for Drug Development and Substance Testing) is a collaborative innovation platform dedicated to 3D cell culture technology and

organ-like tissue models for drug development, substance testing, and personalized and regenerative medicine. TEDD aims to accelerate science application and technology development in industrial R&D, to validate *in vitro* models, and to highlight Switzerland as an international biomedical industry location. One of TEDD's core goals is the implementation of the 3R (Replacement, Reduction, and Refinement) principles through the introduction of validated technologies as an alternative to animal testing. The TEDD community comprises more than 100 Swiss and international members from academia, clinical medicine, and industry.

The TEDD Annual Meeting 2018 was held at the Zurich University of Applied Sciences (ZHAW) in Wädenswil, Switzerland on October 24-25. It brought together experts from diverse fields with a shared interest in advanced 3D models. The idea was to stimulate exchange and collaborations between 3D cell model developers and experts in advanced analysis methods: microscopy, sensors, data modelling, and high-throughput screening. Sixteen industrial exhibitors were present during the extended networking lunch break at the ZHAW Greenhouse.

Preceding the main symposium, TEDD hosted a Think Tank Meeting on "Advanced *in vitro* model validation. Segmentation and harmonization throughout the drug discovery chain" in collaboration with PreComb Therapeutics. Key opinion leaders included Dr Marc Ferrer from the National Institutes of Health/National Center for Advancing Translational Sciences (NIH/NCATS), USA; Prof. Sue Gibbs from Amsterdam University Medical Centre, The Netherlands; Dr Patrick Guye from InSphero, and Dr Kasper Renggli from Department of Biosystems Science and Engineering, ETH, Switzerland. Experts discussed how to accelerate the use of advanced *in vitro* models and their incorporation into drug discovery and development processes. Two critical aspects of the discussion round were segmentation according to 3D models' scalability to define validation groups, and harmonisation for efficacy and safety testing that would enable direct comparison and assess their translational value. The meeting leaders, Prof. Michael Raghunath and Dr Jens Kelm concluded the meeting with a projection of further steps and actions needed to facilitate the validation of cell culture models.

Prof. **Michael Raghunath**, the director of TEDD, opened the main symposium on October 25, 2018. He presented the network's goals, structure, activities, and achievements in 2018 to close to 150 TEDD members and collaborators.

Dr **Marc Ferrer** from NIH/NCATS kicked the meeting off with a keynote talk on integrating 3D cell models into the drug development pipeline. In the last decades, the clinical failure rate of drugs from submission to drug approval has remained close to 90% despite significant scientific and technological advances in the fields of biomedicine and biopharmaceutical R&D. Results of current *in vitro* and *in vivo* toxicity and efficacy assays still appear to translate poorly into clinical outcome in humans. To improve *in vitro* physiology, 3D tissue models are being developed for pre-clinical drug testing but their predictive ability must be validated systematically. Ongoing and future studies with thoroughly validated tissues of higher cellular complexity should generate evidence as to how predictive 3D tissue models are of drug activity in humans, and how much they can help to reduce the failure rate and facilitate decision making in the drug discovery and development pipeline. Dr Ferrer illustrated the use of biofabrication techniques at NCATS, and the development of assay readouts in 3D tissues with the aim of creating a catalogue of normal and disease models for toxicity and drug efficacy screening. He also discussed the importance of harmonizing the validation of these models based on throughput capacity and physiological complexity as a requirement to establish their true translational capacity (Kelm et al., 2018).

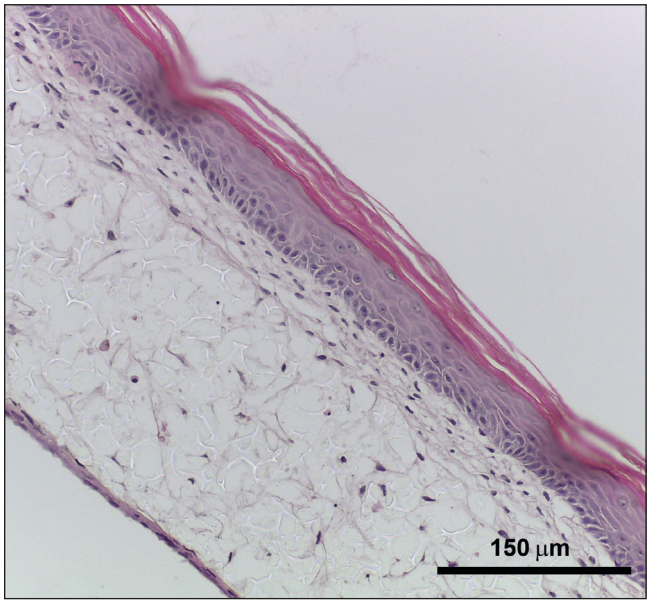

Fig. 1: Skin model developed with the Alvetex platform

Prof. **Stefan Przyborski** from Durham University and CSO of Reprocell Europe Ltd, UK, presented scaffold-based technology for generation of *in vivo*-like tissue models called Alvetex. Cells grown in Alvetex possess a natural tissue-like structure enabling them to function in a more physiologically relevant manner. The scaffold comprises a highly porous polystyrene material, engineered into a membrane in multi-welled plates, and well inserts for use with conventional culture plastic ware and medium perfusion systems (Knight and Przyborski, 2015). He showed examples of application in a neuron-glial co-culture to study neurite outgrowth interacting with astrocytes in the glial scar found in spinal cord injury, and models of a sub-mucosa and skin (Fig. 1). Prof. Przyborski concluded with the statement that these models, which recreate the structure of tissues, promise to become a valuable research tool for use in drug discovery.

Dr **Dieter Ulrich** (CSEM, Landquart, Switzerland) presented an engineering approach for monitoring of cell culture conditions. In the last decades, traditional bioprocesses in large-scale steel bioreactors have shifted to disposable cell culture platforms coupled with individualized cell therapy or drug screening with body-on-a-chip systems. This trend requires innovative standardized analytical solutions to control the bioprocesses, particularly for small-scale bioprocesses. CSEM is working on the development of an at-line cell culture monitoring platform used with, e.g., microbial and mammalian bioprocesses, which uses

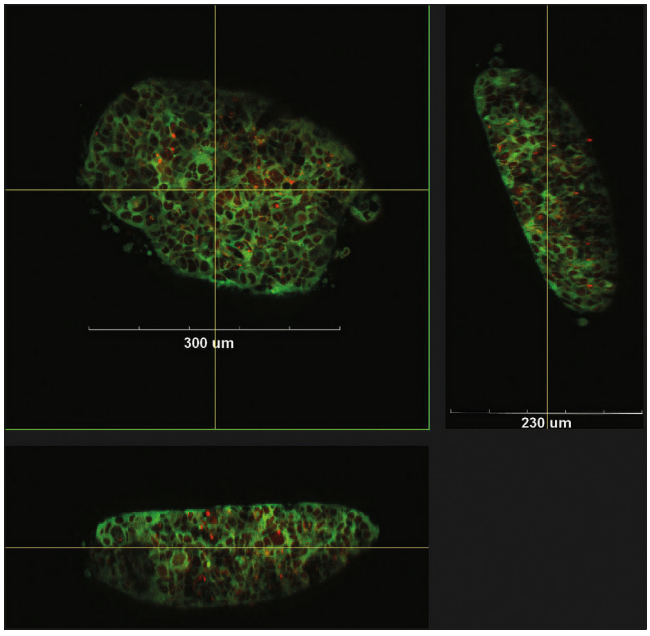

Fig. 2: 3D 2-photon scan of an organoid
The data is part of a multi-day live cell imaging experiment, monitoring the intensity and distribution of a green and red fluorescent protein in the cells.

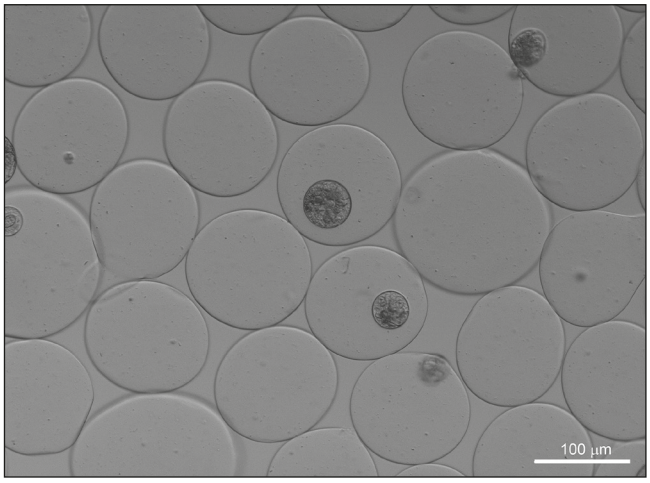

Fig. 3: Microscopic picture of nanoliter-reactors (NLRs) containing clonal spheroids grown from single encapsulated cells of the mesothelioma cell line

low-cost disposable sensors as sensing units. Electrochemical detection of metabolites and ions occurs via an at-line automated sampling platform in quasi-real time. The control of bioprocesses is crucial in order to achieve optimized production, product folding and secretion from fully viable cells, which results in enhanced product quality and higher product titers. Dr Ulrich concluded that CSEM's small-scale analytical platform could be a step towards an inexpensive monitoring technology fulfilling future industrial needs.

The next two talks addressed microscopy techniques. Dr **Martin Rausch** from Novartis presented a 3D cell culture-based compound screening technique based on a 2-photon microscope with fully integrated multi-channel microfluidic perfusion. Until recently, live cell imaging in 3D cell cultures with conventional microscopes or high-content imaging systems was challenging. Limitations include high phototoxicity, insufficient axial resolution, a need for specialized sample handling devices, and lack of tools for precise compound administration. Novartis developed a new imaging setup based on an inverted multi-photon microscope, with a fully water-immersed high-NA (numerical aperture) long working distance lens and compound administration by microfluidic perfusion. It allows generation of high-resolution image stacks from 3D cell cultures for medium-throughput compound screening assays in standard 24-384 well plates over many days (Fig. 2). Novartis successfully applied the system to image tumor and liver spheroids, tissue explants from muscle, skin and gut, as well as different types of co-cultures.

Prof. Dr **Ernst Stelzer** from Goethe University, Frankfurt, Germany introduced light sheet-based fluorescence microscopy (LSFM). Experiments on 3D biological specimens such as cysts, organoids, spheroids, embryonic bodies, tissue sections, and small model organisms require the development of new microscopes and image processing tools/software that are capable of handling millions of large-scale images for various applications. Classical fluorescence microscopy provides high contrast but has several disadvantages such as photobleaching of fluorophores and minimal optical sectioning capability. One of the very few instruments that promises dynamic 3D imaging and addresses these disadvantages of traditional tools, is light sheet-based fluorescence microscopy (LSFM). Particular benefits of LSFM are a good axial resolution, imaging along multiple directions, deeper tissue penetration, high signal-to-noise ratio, unrestricted compatibility with fluorescent dyes and proteins, reduced fluorophore-bleaching and phototoxicity at almost any scale, millions of pixels recorded in parallel, and excellent specimen viability.

The talk of Dr **Andreas Meyer** from FGen, Basel, Switzerland was on a technology platform for high throughput screening of 3D cell cultures using a flow cytometer. The technique addresses the analysis of large cell libraries or complex cell populations, an essential process in pharmaceutical discovery. Nanoliter-reactors (NLRs) encapsulate single cells that proliferate to clonal spheroids or 3D cell clusters (Fig. 3). Flow cytometry is applied to analyze NLRs based on fluorescently-labelled surface markers to select spheroids. The technology platform is intended to be used for the isolation of specific cancer cells from complex cell mixtures such as biopsies for the identification of drug targets by high throughput screening of cell libraries. A mesothelioma model system was presented as proof of concept. Single cells showed high survival and proliferation rates when encapsulated in NLRs. Flow cytometry readily identified and isolated spheroids. Cultivation of the mesothelioma cell line in NLRs with different matrix stiffness showed differential growth behavior, spheroid size, and expression of specific cancer markers.

Dr **Sylke Hoehnel**, CEO of SUN Bioscience, Lausanne, Switzerland took us on a tour of bioscience's path from a working prototype to an industrial-scale product of complex 3D tissue models. Patient-specific, stem cell-derived organoids allow testing for the final efficacy of treatments rather than relying on predictions based on incomplete biomarker sets. However, organoids are still a research tool, and are produced manually, with high variability and high associated costs. SUN Bioscience has developed Gri3D®, a universal organoid culture platform that allows standardization of organoids for their time- and cost-effective use in pharmaceutical screening and clinical diagnostics. A translational pilot study using the platform is currently underway that aims at understanding treatment response variability using intestinal organoids grown from cystic fibrosis patients.

Dr **Carmel B. Nanthakumar**, Fibrosis Disease Biology and Imaging, GlaxoSmithKline, UK, presented *in vitro* platforms for anti-fibrotic drug development. Matrix remodeling is a critical component of tissue fibrosis, and excessive deposition of a collagen-rich extracellular matrix (ECM) leads to loss of tissue compliance and, ultimately, organ failure. At the cellular level, the myofibroblast is central to the pathogenesis of fibrosis irrespective of tissue origin. Upon activation, this cell type drives remodeling by coupling tension and ECM synthesis. Existing models of experimentally induced lung fibrosis currently fail to recapitulate clinical features. Patient tissue- and disease-derived primary cells offer several advantages to pre-clinical fibrosis drug discovery and may be used to progress intervention strategies that block myofibroblast activation and arrest ECM synthesis. A cornerstone of establishing these complex phenotypic and physiologically relevant models was the early adoption of macromolecular crowding, a technology spearheaded by Michael Raghunath to facilitate *in vitro* ECM deposition. The current *in vitro* models at GSK are used to study clinical biomarkers in cell and tissue preparations intending to bridge pre-clinical findings to clinical development in patients.

The last presentation, given by Prof. **Sue Gibbs** from Amsterdam University Medical Centre, highlighted how *in vitro* skin can be used as a screening tool. Understanding the healthy and diseased state of skin is essential in many areas of basic and applied research. The field of skin tissue engineering is advancing by incorporating more cell types into different models. While the simplest skin models consist of only differentiated stratified keratinocyte layers, full thickness models incorporate a dermal compartment with fibroblasts and additional cell types such as immune cells. The major challenge is now to develop optimal biomarker readouts (proteomics, transcriptomics and metabolomics) suitable for the throughput of the different skin models, either extracted from tissue or collected from the culture medium. However, while many technologies are available for small-scale discovery experiments, validated panels of biomarkers will be required for robust and cost-effective screening purposes. Prof. Gibbs pointed out that a compromise is required, which enables full exploitation of the advanced skin models' physiological potential with available screening procedures that comply with future regulatory acceptance.

Prof. Raghunath closed the Annual Meeting by announcing upcoming events and activities of TEDD in 2019 (3D cell culture SMI London, TEDD symposia at TERMIS-EU). The next TEDD Annual Meeting scheduled for October 24, 2019 promises again a vibrant atmosphere, a top-quality scientific program, and a mix of participants from academia, industry and clinics.

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