human fibroblasts maintained as spheroids become primed to respond to small molecules. Specifically, human fibroblasts could be reprogramed into stem cells strongly expressing Oct4, Sox3 and Nanog using three small molecules. The same approach was used to rejuvenate senescent human mesenchymal stem cells and fibroblasts. Senescent cells, cultured as spheroids and treated with a single small molecule, began to proliferate and regained their multipotency and differentiation efficiency into bone cells. These observations have important implications for pharmaceutical companies on how they should conduct cell-based screening for small bioactive molecules.

The afternoon session started with the keynote of Prof **Zhongze Gu**, in which he presented the activities of Southeast University (SEU) in China for the TEDD community similarly as the day before at the workshop. Then, Dr **Wing Chang**, director of research and development at STEMCELL Technologies, Cambridge, UK talked about robust and efficient tools for pluripotent stem cell and organoid research.

Dr **Parto Toofan** from REPROCELL Europe Limited, presented the company's efforts to produce GMP-grade iPSCs for drug screening and translational medicine using a safe mRNA strategy. iPSCs produced in GMP-grade for drug screening and translational medicine have the potential to provide patient-specific scalable biologic material of various tissue types, useful for investigating the pathophysiology of rare disorders. iPSCs not only serve as excellent stem cell models, but they also can differentiate into a wide variety of cell types for preclinical studies, providing an unlimited source for the development of healthy or diseased cell models in which to study the effectiveness and toxicity of pharmaceuticals.

After the industry session, the physical Competence Centre TEDD embedded in the Centre for Cell Biology and Tissue Engineering at the ZHAW presented its core competencies, including:
- Cell biology with a focus on stem cell research, cell-based assays, cell differentiation, glycobiology, and cellular engineering
- Applied matrix biology and biophysics (macromolecular crowding, supramolecular aggregates) to develop metabolically active human models such as fatty tissue and skeletal muscle
- Establishment and analysis of tissue equivalents on scaffolds used in the fields of regenerative medicine and substance testing
- 3D cell culture model development that is suitable for the pharma industry and personalized medicine, including technologies such as bioprinting

Dr Markus Rimann concluded meeting with an outlook for the TEDD network. The goal of the network is to grow further and to closely collaborate with other networks and associations to integrate new stakeholders from different industry sectors.

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Meeting Report

Virtual Summer School: Alternative Methods and Models in Science: A Multidisciplinary *In Vitro* Approach

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The international Virtual Summer School: Alternative Methods and Models in Science: A Multidisciplinary *in vitro* Approach[1], targeted at PhD students and young scientists was focused on *in vitro* advanced and innovative methodologies, their application in different disciplines, from toxicology to microbiology, and future perspectives. The two-day event held on June 3-4, 2020 was chaired by Francesca Caloni, Università degli Studi di Milano, Department of Environmental Science and Policy, and was attended by 26 international participants with different scientific backgrounds.

Giulio Casati, director of the Lake Como School of Advanced Studies, opened the event by presenting the activities and the mission of the school.

Francesca Caloni gave an overview on "*Alternative models and* in vitro *strategy*", stressing the importance of a predictive interdisciplinary science for risks related to the environment, hu-

[1] https://amms.lakecomoschool.org/

man and animal health, together with the application of the 3Rs principle. Alternative *in vitro* models, either as stand-alone methods or as parts of an integrated testing strategy, as simple barrier models or more complex 3D cultures, were described in the context of their role in risk assessment evaluation.

Helena Kandárová, CEM & FChFT Bratislava, gave a lecture on "Skin irritation testing in vitro – *3D reconstructed human skin models*". Human skin models (RHSM) and respective *in vitro* protocols have been validated and adopted by regulatory agencies as full or partial replacements of animal experiments, i.e., for skin corrosion and skin irritation as OECD Test Guidelines (TG 431 and 439) and for chemical testing (EU REACH regulation). Recently, the ICH guideline S10 implemented RHSM for phototoxicity assessment of topically applied substances as part of a tiered testing strategy. RHSM are predominantly used for the evaluation of hazard, but they can be applied also in the risk assessment process if appropriate protocols and exposures are used. The cosmetic industry is using RHSM not only to assess the skin tolerance of their products but also to test the efficacy of novel ingredients and formulations. Most recently, RHSM were recognized as useful tools in the assessment of human skin irritation effects of medical devices (De Jong et al., 2018; Kandarova et al., 2018). Wound healing and efficacy studies conducted with RHSM were also discussed.

Marisa Meloni, CEO VitroScreen, Milan, Italy, presented a talk entitled: "*Eye irritation: an endless challenge for* in vitro *science*". The process leading to replacement of the Draize rabbit test with alternative non-animal approaches, such as the Integrated Approach on Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation (OECD, 2017), has taken almost 20 years. The industrial need to evaluate the tolerance of ophthalmic formulations in a more relevant, sensitive and reproducible way compared with the *in vivo* Draize rabbit test has driven the development of alternative approaches that better assess these products under realistic exposure conditions. A multiparametric approach (MEA) with defined acute and repeated protocols on reconstructed human corneal epithelium (HCE) has been used to assess delayed cytotoxicity effects of eye drops and exclude any potential chronic damage to the ocular surface structure. Information on the mechanism of action and reversibility of the damage can be obtained by investigating the expression level of occludin, a functional protein of the tight junctions (Meloni et al., 2010). The results confirmed the reliability, sensitivity and predictivity of the MEA approach on HCE in detecting subclinical signs of cellular toxicity also for so-called "soft" preservatives, suggesting the need to include delayed toxicity evaluation in the biocompatibility and risk assessment of ophthalmological formulations intended for long-term use (Meloni et al., 2019).

Arno C. Gutleb, Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology, gave a lecture entitled: "*Current status of* in vitro *models to evaluate pulmonary toxicity*". Inhalation of chemicals can have a wide range of effects from irritation in the airways, development of respiratory sensitization, to systemic effects. *In vitro* air-liquid interface (ALI) cell culture models have been developed to assess inhalation toxicology relevant endpoints. Such models range in complexity from single cell lines to complex 3D models consisting of several cell types. Adequate exposure techniques are essential when it comes to exposing cells grown at the ALI, and an overview of available exposure devices was presented. ALI cultures can serve as an excellent test case to show the impact of replacing animal-derived products, such as foetal calf serum, which are known to be a source of high experimental variability. The status of development, validation, and needs for further improvements of *in vitro* models and their potential to replace animal experiments in a regulatory context was also discussed.

Hassan Rashidi, NIHR Great Ormond Street Hospital Biomedical Research Centre, UCL Great Ormond Street Institute of Child Health, University College London, provided a short history of 2D and 3D cell culture techniques and described the development of a 3D platform based on hepatocytes generated from human pluripotent stem cells (hPSCs) as an *in vitro* tool to evaluate liver toxicity of new lead compounds. 2D-derived HLCs exhibit foetal features and transient phenotype *in vitro*, limiting their clinical application (Cameron et al., 2015). However, novel 3D liver organoids are not suitable for clinical application due to their reliance on animal-derived and undefined biological components. A novel platform was developed to generate hPSC-derived 3D hepatospheres (3D Heps) under xeno-free and GMP-ready conditions (Rashidi et al., 2018). The 3D Heps downregulated the expression of alpha-fetoprotein, a foetal marker, and remained metabolically active and drug-inducible for over a year in culture, providing an *in vitro* platform to evaluate long-term hepatotoxicity. Notably, generated 3D tissues provided critical liver support in tyrosinemia type-I animal models, indicating that these tissues may be able to treat certain liver diseases in the future.

Yula Sambuy, CREA-Research Centre for Food & Nutrition in Rome, Italy, introduced the most common *in vitro* intestinal barrier models and some promising advancements in this field. The human intestinal Caco-2 cell line has been used for thirty years to study intestinal toxicology and physiology. Derived from a colon adenocarcinoma, it can be made to differentiate on permeable substrates into a monolayer of polarized cells, coupled by functional tight and adherence junctions and expressing several transport and metabolic features of the absorptive enterocytes of the small intestine. However, cell- and culture-dependent factors strongly affect the expression of fully differentiated features (Sambuy et al., 2005). Newer approaches involve co-culture models of Caco-2 with other intestinal cell types, and 3D-intestinal preparations from normal human tissue have become available. Human induced pluripotent stem cells and organoid technologies (Nakamura and Sato, 2018) promise to be the next step towards obtaining human tissue-relevant models of the intestinal mucosa.

Teresa Coccini, Laboratory of Clinical and Experimental Toxicology, ICS Maugeri, IRCCS Pavia, Italy, presented a talk entitled "*Human cell-based models for neurotoxicity*". *In vitro* cell-based models for neurotoxicology research are moving on from simple *in vitro* systems with single cell types to more advanced models including 3D models (spheroids), cell co-cultures and primary human cells. Human umbilical cord lining (CL) can be used to isolate pluripotent mesenchymal stem cells (hCL-MSCs), which have strong, long-term proliferative ability (self-renewal) and show stable amplification *in vitro*, are widely available without ethical restrictions, and can be easily differentiated into specific cells. In view of the limited access to human tissue, stem cells are likely to gain further importance as an alternative to primary human cells.

Doris Wilflingseder, Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, gave a talk on "*Studying infectious diseases in animal free human 3D models*". 3D animal-free barrier/immune cell systems were developed to study interactions of respiratory barriers with pathogenic fungi, SARS-CoV-2 or influenza or to test novel approaches against HIV-1 infection of a mucosal model. Confocal microscopy and high content screening of 3D respiratory/mucosal samples provide high-resolution pictures and enable quantitative analyses of a high number of cells (Zaderer et al., 2019). Culture under perfused conditions results in accelerated differentiation of barrier models (Chandorkar et al., 2019). Primary respiratory cells of the bronchial and small airway epithelial tract can be cultured over a period of more than two years without losing epithelial integrity, impacting mitochondrial fitness or affecting mucus production by goblet cells. Interactions with fluorescent beads, fungi and viruses can be studied in such cultures.

An interactive debate with the participants on the perspectives and future role of alternative *in vitro* models on replacement concluded the event.

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References

Cameron, K., Tan, R., Schmidt-Heck, W. et al. (2015). Recombinant laminins drive the differentiation and self-organization of hESC-derived hepatocytes. *Stem Cell Reports 5*, 1250-1262. doi:10.1016/j.stemcr.2015.10.016

Chandorkar, P., Posch, W., Zaderer, V. et al. (2017). Fast-track development of an in vitro 3D lung/immune cell model to study Aspergillus infections. *Sci Rep 7*, 11644. doi:10.1038/s41598-017-11271-4

De Jong, W. H., Hoffmann, S., Lee, M. et al. (2018). Round Robin study to evaluate the reconstructed human epidermis. (RhE) model as an in vitro skin irritation test for detection of irritant activity in medical device extracts. *Toxicol In Vitro 50*, 439-449. doi:10.1016/j.tiv.2018.01.001

Kandarova, H., Willoughby, J. A., De Jong, W. H. et al. (2018). Pre-validation of an in vitro skin irritation test for medical devices using the reconstructed human tissue model EpiDerm™. *Toxicol In Vitro 50*, 407-414. doi:10.1016/j.tiv.2018.02.007

Meloni, M., Pauly, A., De Servi, B. et al. (2010). Occludin gene expression as an early in vitro sign for mild eye irritation assessment. *Toxicol In Vitro 24*, 276-285. doi:10.1016/j.tiv.2009.08.016

Meloni, M., Balzaretti, S. and Ceriotti, L. (2019). Medical devices biocompatibility assessment on HCE: Evidences of delayed cytotoxicity of preserved compared to preservative free eye drops. *Regul Toxicol Pharmacol 106*, 81-89. doi:10.1016/j.yrtph.2019.04.022

Nakamura, T. and Sato, T. (2018). Advancing intestinal organoid technology toward regenerative medicine. *Cell Mol Gastroenerol Hepatol 5*, 51-60. doi:10.1016/j.jcmgh.2017.10.006

OECD (2017). Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation. *Series on Testing and Assessment, No. 263*. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2017)15&doclanguage=en

Rashidi, H., Luu, N. T., Alwahsh, S. et al. (2018). 3D human liver tissue from pluripotent stem cells displays stable phenotype in vitro and supports compromised liver function in vivo. *Arch Toxicol 92*, 3117-3129. doi:10.1007/s00204-018-2280-2

Sambuy, Y., De Angelis, I., Ranaldi, G. et al. (2005). The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol Toxicol 21*, 1-26. doi:10.1007/s10565-005-0085-6

Zaderer, V., Hermann, M., Lass-Flörl, C. et al. (2019). Turning the world upside-down in cellulose for improved culturing and imaging of respiratory challenges within a human 3D model. *Cells 8*, 1292. doi:10.3390/cells8101292

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