Meeting Report

Cross-Industrial Applications of Organotypic Models

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Recent advances in microphysiological systems (MPS) promise a global paradigm shift in drug development, diagnostics, disease prevention, and therapy. The expectation is that these systems will model healthy tissue, various diseased stages, and disease progression to predict toxicity, immunogenicity, ADME profiles, and treatment efficacies. MPS will provide *in vitro* models with unprecedented human-like physiological properties, enabling their routine application in the pharma industry and thus reducing drug development costs by lowering the attrition rate of drug candidates.

We showcased MPS application diversity across different industries during the *TEDD Annual Meeting* on October 14, 2021, in Wädenswil, Switzerland. The goal was to promote cross-sectoral collaboration of academia and industry to further pave the way to developing next-generation MPS based on 3D cell culture, organoid, and organ-on-chip technology, and their widespread exploitation. To enable visionary projects and radical innovations, we covered multidisciplinary fields and connected different industry sectors, like pharma, medtech, biotech, cosmetics, diagnostics, fragrances, and food, with each other.

Prof. **Christian Hinderling**, head of the Institute of Chemistry and Biotechnology at ZHAW, who opened the meeting, highlighted that in-person gatherings, now more than ever, support and facilitate collaboration in the current global situation. Dr **Markus Rimann**, head of the TEDD Competence Centre (Tissue Engineering for Drug Development and Substance Testing), pointed out that the 3Rs initiative is at the core of the network, and the network aims for future-oriented systems such as MPS to replace animal testing, an essential goal in the socio-political context. Dr **Armand Mensen** from the Swiss 3R Competence Centre further highlighted the 3Rs and presented the status and future of funding schemes in this area relevant to TEDD Partners.

The scientific part of the meeting started with a keynote by Dr **Olivier Frey**, Vice President and Head of Technology & Platforms at InSphero. He answered the question: What does it take to engineer scalable multi-tissue disease models? Multi-tissue MPS interconnect several organ models using microfluidic technology and enable investigating the effects of compounds in a more systemic, *in vivo*-like fashion. To date, such insights can only be obtained with animal experiments at the preclinical drug development stage. Over the past years, InSphero has developed different technical and biological components, ultimately merging them into a scalable microfluidic multi-organ platform that can be used for a broad spectrum of preclinical studies. An example they are currently advancing is a microphysiological 3D human liver-islet microtissue platform, which models liver-pancreas crosstalk and can provide a better understanding of metabolic diseases (Fig. 1). The liver model consists of a primary hepatocyte-Kupffer cell-stellate cell triple-culture, which retains metabolic and inflammatory function for at least four weeks. The islet model comprises all endocrine cells at a physiological ratio and remains glucose-responsive over the same duration. Moreover, the liver-islet crosstalk improves viability and functional responsiveness of the microtissues in the insulin-free medium. Rapid insulin internalization and degradation by the liver leads to reduced insulin accumulation in the co-culture, closely reflecting the *in vivo* situation. The models are combined in a microfluidic culturing device that enables interconnection of up to ten 3D microtissues. The microfluidic platform is produced by an injection-molding mass fabrication process and uses a gravity-driven tubeless flow concept, enabling parallelization. In addition, a fast and reliable method for loading quality-controlled spheroids using a fully automated robotic pick-and-place transfer enables large-scale multi-tissue experiments. Dr Frey concluded that with the capability of engineering diseased states, including non-alcoholic steatohepatitis and type 2 diabetes, these models can address rising health challenges.

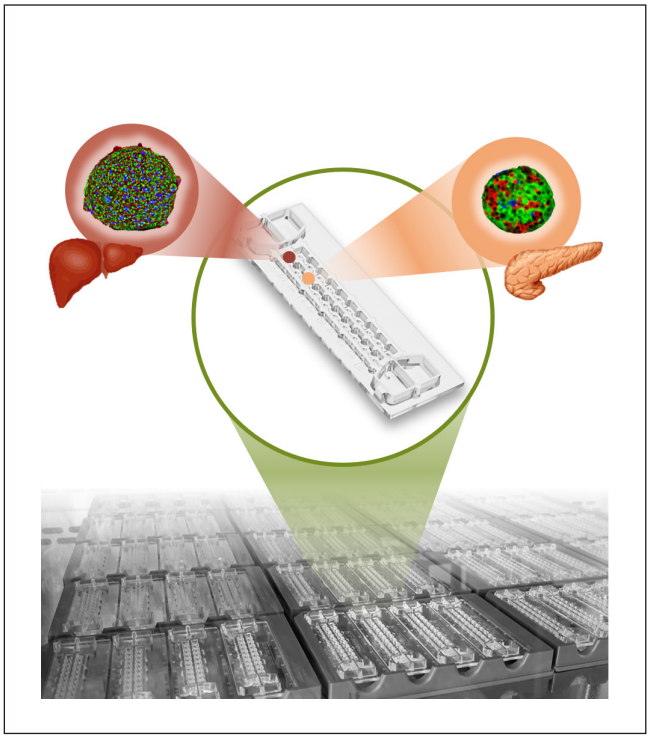

Fig. 1: Modular microfluidic system developed by InSphero with connected liver and islet microtissues

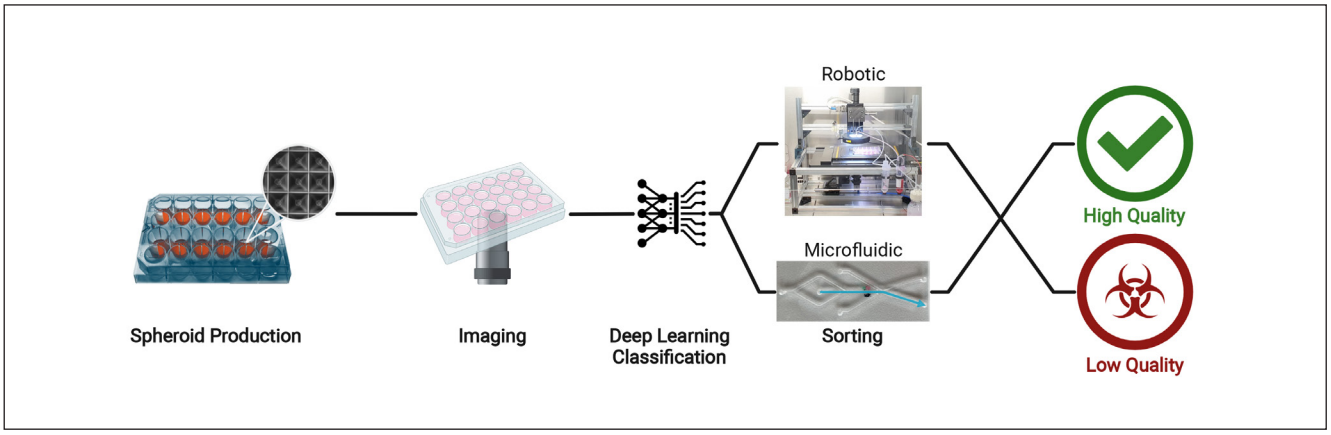

Fig. 2: CSEM high-throughput automated processing for organoid sorting (Created with Biorenders.com)

Prof. **Laura Suter-Dick**, who leads the Cell Biology and Molecular Toxicology Group at the University of Applied Sciences Northwestern Switzerland (FHNW), discussed the study of liver disease using cell line-based spheroid models. There are few therapeutic options for prevalent liver diseases such as nonalcoholic fatty liver disease/non-alcoholic steatohepatitis, fibrosis, and cirrhosis, as their underlying molecular mechanisms are not yet fully understood. Moreover, diagnostic tools for early detection of liver disease are lacking. She described a 3D, multicellular *in vitro* system composed of HepaRG cells, THP-1, and hTERT-HSC that can mimic the main features of hepatic fibrosis as described in the liver fibrosis adverse outcome pathway (AOP:38). Several biomarkers were implemented in her group to assess the physiological characteristics of the liver microtissues in healthy and diseased states and to investigate specific molecular mechanisms. The human cell line-based spheroids display liver-like characteristics such as bile canaliculi formation, albumin secretion, and metabolic capacity (CYP3A4 expression and activity). Upon challenge with profibrotic compounds such as methotrexate, the liver spheroids displayed a fibrotic phenotype characterized by stellate cell activation, a hallmark of liver fibrosis. This activation was not observed in stellate cell monocultures or after treating liver spheroids with acetaminophen, known to induce acute liver injury without fibrosis in humans. The results support the suitability of the model and underline the need for a multicellular system to mimic the expected clinical outcome. In the search for a potential marker of fibrosis, Prof. Suter-Dick's group investigated extracellular miRNAs released in response to methotrexate in comparison to acetaminophen treatment. Functionally, the identified miRNAs induced by methotrexate were able to activate stellate cells upon transfection and to downregulate specific target proteins. Thus, Prof. Suter-Dick concluded that these multicellular spheroids can be employed to investigate liver fibrosis *in vitro* and can help to elucidate its etiology, assess potential therapeutic interventions, and identify novel biomarkers.

We then welcomed Dr **Vincent Revol**, Head of Research and Business Development, Life Science Technologies for CSEM SA Regional Centers. He talked about automation in physiological microenvironments as a critical enabler for industrial and clinical applications. Organoid technology has emerged to play a central role in disease modelling and drug testing, but the transition from the lab to the clinic requires both standardized protocols and automated closed systems. In this context, CSEM is developing tools to overcome current technological limitations in organoid technology by using precision manufacturing and digitalization. They rely on standardized tools to ensure reproducible and homogeneous production of organoids, picking and sorting using multisensor data and deep learning, assembly to combine biomaterials, 3D positioning of the organoids to mimic *in vivo* tissue architecture, and maturation to recreate physiological microenvironments with biomonitoring to control organoid growth. High-throughput organoid sorting based on deep learning algorithms comprises a handling module to harvest and sort organoids (Fig. 2). The imaging module acquires high-resolution video sequences for deep learning classification. Tissue maturation in a physiological microenvironment extends over a few days to months. Standardized perfusion of the living tissues is based on a smart lid approach, which is compatible with most base plates,

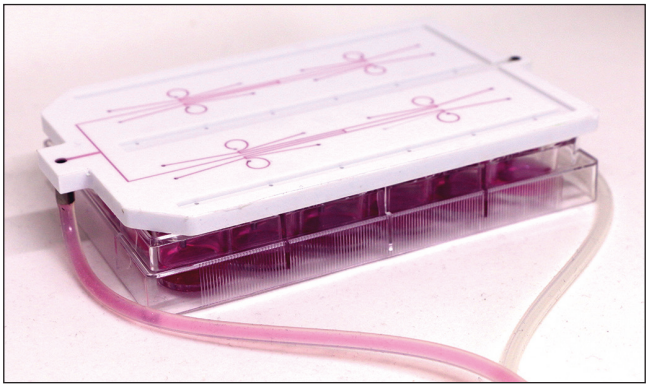

Fig. 3: CSEM perfusion lid of the YOU-ON-CHIP™ platform for the automated maintenance of *in vitro* tissue maturation in multi-well plates

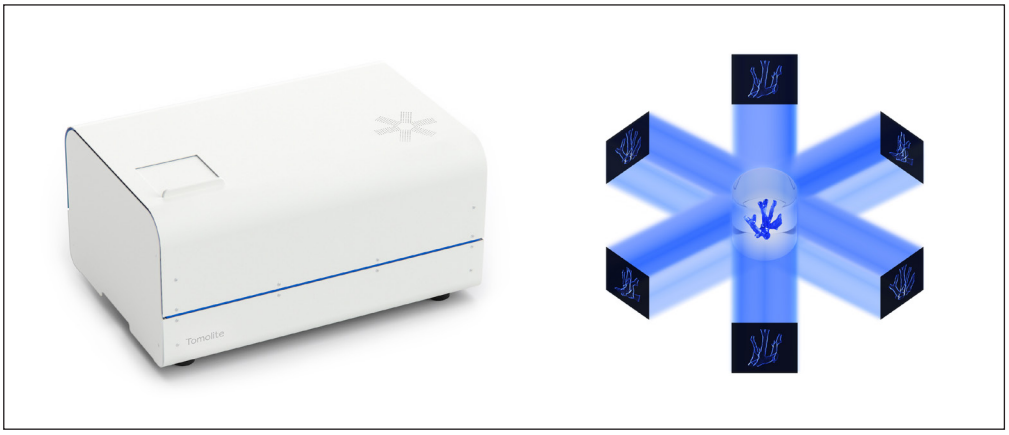

Fig. 4: Readily3D's Tomolite bioprinter with tomographic printing illustration

enables automated medium circulation, and may comprise sensing features. The technology platform YOU-ON-CHIP™ aims at automating tissue maintenance, differentiation, sampling, and monitoring (Fig. 3).

Dr **Damien Loterie**, co-founder and CEO of the spin-off Readily3D, talked about advances in volumetric 3D printing. Today, most 3D printers work in a layer-by-layer fashion, which is slow and limits them to specific materials and geometries. In contrast, Readily3D's tomographic printers create the entire object volume. This results in ultra-rapid build times (30 s) and the ability to print a new range of materials and geometries. Tomographic printing addresses several significant constraints of classical extruders: the process is scalable since it can produce and reproduce centimeter-sized prints in a few tens of seconds, it is entirely contactless, prints are done through sealed, sterile glass containers, protecting bioresins from contamination, and the process is gentle, as it uses very little light during a short time. Hence, the use of more fragile cells that are not compatible with extrusion processes due to shear stress is possible. In a series of recent publications, researchers have demonstrated how to print biostructures using a variety of hydrogels such as GelMA, GelNB, HAMA, and PEG4SH in combination with a range of cell types, including mesenchymal stromal cells, articular chondroprogenitor cells, mouse myoblasts, and human dermal fibroblasts. The printed constructs show up to 95% viability. Readily3D's Tomolite printer with a self-contained volumetric 3D printing system, accessories allowing multiple print diameters, and a straightforward user interface has been commercially available since April 2021 (Fig. 4).

The following two talks highlighted advances in the new field of cellular agriculture. Keynote Prof. **Ori Bar-Nur**, assistant professor in the Department of Health Sciences and Technology at the Swiss Federal Institute of Technology (ETH) Zurich, talked about how researchers harness muscle stem cells for regenerative medicine and cellular agriculture. Current approaches to produce muscle fibers primarily rely on the differentiation of myogenic progenitors termed myoblasts, which exhibit limited proliferation and differentiation capacities. Recent work reported that transient overexpression of the transcription factor MyoD in concert with a small molecule treatment directly converts mouse fibroblasts into induced myogenic progenitor cells (iMPCs). These myogenic cultures consist of a heterogeneous population of muscle fibers and myogenic progenitors that can expand long-term *in vitro* without losing self-renewal and differentiation capacities. In the first part of his talk, Prof. Bar-Nur discussed his comparison of primary myoblasts with iMPCs via multi-omics tools from which he concluded that iMPCs represent an augmented capture of a myogenic stem cell state. iMPCs produced from two Duchenne muscular dystrophy (DMD) mouse models proliferate extensively, express myogenic stem cell markers, and form dystrophin-negative myofibers. Correction of the dystrophin mutation utilizing CRISPR/Cas9 led to *in vitro* protein re-expression, and engraftment of corrected DMD iMPCs into dystrophic limb muscles restored dystrophin expression *in vivo*. Finally, Prof. Bar-Nur presented his team's current efforts to translate the understanding of the mouse system to devise a similar strategy to enhance cow myoblast differentiation and proliferation capacities for cultivated meat production. He concluded that this novel approach bears potential implications for basic research, translational applications, and cellular agriculture.

Next, Dr **Suman Das**, co-founder and Chief Scientific Officer of Mirai Foods, presented the company's advanced tissue engineering concepts for cultivated meat. The company's vision is to become a household brand for cultivated meat, accelerating the transition to an environmentally, ethically, and economically sustainable global food system. Its mission is to provide high-quality cultivated meat at a fair price. The cultivated meat in the form of minced beef can be produced from a small sample of muscle tissue from a cow biopsy or a fresh piece of meat by cultivation of the cells. During the process, the nutritional value of cultivated meat is monitored and controlled, including fat and protein content as well as micronutrients like vitamins and minerals.

Dr **Marko Loparic** is the Chief Medical Officer of ARTIDIS, a company focused on developing a medical device for fast and early cancer (breast, lung and pancreatic) diagnosis based on a unique nanomechanical biomarker. The system consists of the ARTIDIS device for nanomechanical measurements and the digital data platform ARTIDISNET. It enables precise cell and tis-

sue stiffness measurements down to the molecular level, which can be applied to any material or living tissue, providing a broad range of R&D and clinical applications. ARTIDIS aims to leverage the platform to accelerate drug development and deliver personalized patient treatment strategies. ARTIDIS can identify cancerous tissue materials based on their cellular stiffness. Tumor cells are softer than healthy tissue, allowing them to navigate through the basement membrane and fibrillar matrix. Carcinoma-associated fibroblasts remodel the basement membrane and collagen I matrix to promote invasion of the softer tumor cells. The measurement provides a nanomechanical score based on more than 5 million nano-palpations per tissue type, 10,000 measurements per patient's specimen. The system can increase the overall number of patients eligible for targeted treatments, identify the non-responders to become responders, and measure which patients will respond to immuno-, targeted, and cell therapy after radiation and chemotherapy.

The scientific part was closed by **Michela Di Filippo**, a PhD student from University Hospital Zurich, Department of Dermatology, who spoke about the potential of MPS using CRISPR-Cas9-modified cells in 3D skin models. 3D human skin equivalents mimic the sophisticated structure of the human skin, allowing the investigation of complex tissue-specific effects in homeostasis or disease. A scaffold-free 3D model was shown to be comparable to human skin regarding morphology and gene expression of keratinocytes and fibroblasts. The system is stable for months *in vitro*, allowing the study of chronic treatments (for instance drug testing), and possesses high self-regenerative and plastic capacity. Gene-modulated skin equivalents can be built by knocking out or overexpressing specific genes in either primary keratinocytes or primary fibroblasts using the CRISPR-Cas9 technique. For example, the model was used to investigate the role of the NLRP1 inflammasome, a component of the innate immunity expressed by human keratinocytes. To this end, skin equivalents with control keratinocytes or keratinocytes whose NLRP1 inflammasome components had been knocked out using the CRISPR-Cas9 technique were built, and the crosstalk between epidermis and dermis upon pharmacological activation of the NLRP1 inflammasome was investigated via mRNA sequencing to learn how inflammation is induced in human skin and obtain insights into possible therapeutic options. Using a similar approach, scientists can build models of psoriasis, atopic dermatitis, epidermolysis bullosa and other inflammatory syndromes for research use. In addition, the model allows the investigation of the molecular mechanisms underlying skin cancer formation and development using cells derived from healthy skin and skin cancer biopsies of the same patient, thereby eliminating genetic variability between different donors.

In addition to the scientific symposium, sixteen TEDD Partner companies and academic institutions presented their science, services, and products during the extended lunch break sessions. Finally, Dr Markus Rimann closed the meeting announcing the upcoming in-person university and company visits offered by TEDD in 2022.

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