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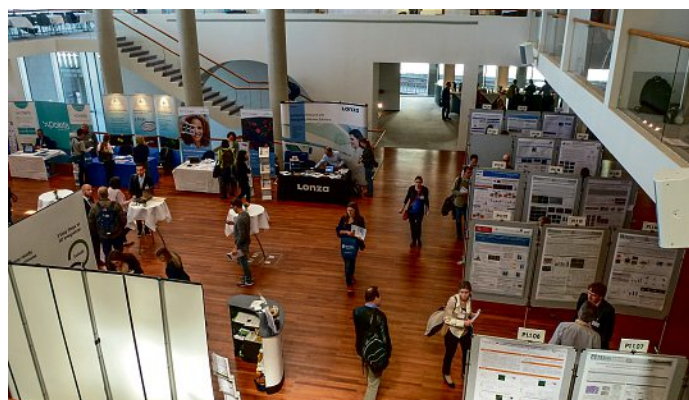
The State of the Art of 3D Cell Cultures

DECHEMA Conference in Freiburg
on April 19–21, 2016

Elsbeth Heinzlmann, journalist science + technology

Abstract: Where are we today with 3D cell cultures as predictive, physiologically relevant model systems? Are there any successful applications, innovative developments or yawning gaps? The DECHEMA April conference assembled international experts to take a look behind the scenes and reveal relevant disease models, applications of 3D models in clinical practice and industry, predictive cell models for compound characterization and enabling technologies.

Keywords: DECHEMA conference 2016 · Swiss Biotechnet



Scenes of the DECHEMA 3D Cell Culture 2016 event in Freiburg. The conference highlighted successful applications and newest developments in the key areas of 3D cell culture (Copyright X. Wu, DECHEMA e.V.).

The DECHEMA conference in Freiburg, held from 19–21 April 2016, was jointly organized by:

- VBU Association of German Biotechnology companies, <http://v-b-u.org/en/>
- TEDD Tissue Engineering for Drug Development and Substance Testing, www.zhaw.ch
- National Thematic Network Swiss Biotech, www.swissbiotech.org/www.biotechnet.ch

3D cell culture has been used for decades and currently plays a key role in cancer and stem cell research. The rapid development of the biopharmaceuticals sector has delivered a real leap in innovation and accelerated the demand for cell culture products for drug discovery and safety testing. But where do 3D cell cultures add most value today, and what challenges do we have to face? The expert speakers at the DECHEMA conference in Freiburg gave a deeper insight into the state of the art.

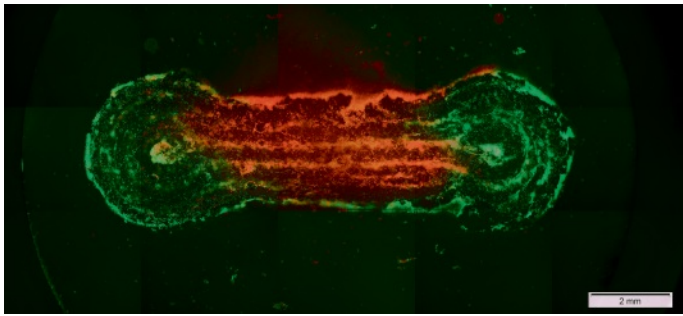
Pioneer in Laser-Assisted Bioprinting

It's fine to have creative ideas, but it's much better to put them into practice, as epitomized by Dr. **Fabien Guillemot**, CEO and founder of Poietis, the first Laser-Assisted Bioprinting company in the world and a leader in Regenerative Medicine. We could define bioprinting as the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 3D organization to produce bio-engineered structures for regenerative medicine, pharmacokinetic and basic cell biology studies. "From a technological point of view, the Laser-Assisted Bioprinting (LAB) technology is an alternative to inkjet and (micro)extrusion methods and overcomes some of their limitations in patterning living cells and biomaterials with micron-scale resolution and high cell viability", explains Fabien Guillemot. "By harnessing this high cell printing resolution, we observe that tissue self-organization depends on the cell patterns initially printed by LAB, as well as cell types." Drawing on his experience acquired from *in vitro* and *in vivo* experiments he has introduced the 4D bioprinting paradigm. "The 4th dimension relies on programming self-organization, *i.e.* determining appropriate 3D micro patterns of tissue components so that a specific tissue function emerges (matures) with time through interactions between components – internal – and interactions with the host – external."

www.poietis.com

A Clever Way to Bioprint Tissues for Substance Testing

At Zurich University of Applied Sciences (ZHAW), Dr. **Markus Rimann**, Head of 3D Tissue and Biofabrication, has been working with the Novartis Institutes for BioMedical Research, Weidmann Medical Technology and regenHU on the development of a novel bioprinting solution for producing tissues



Co-printing of two cell types in the same construct. Primary tenocytes and myoblasts were fluorescently pre-stained prior to printing (tenocytes: green; myoblasts: red) and then printed in the same tissue model. The picture shows cell distribution directly after the printing process (Source Markus Rimann ZHAW).

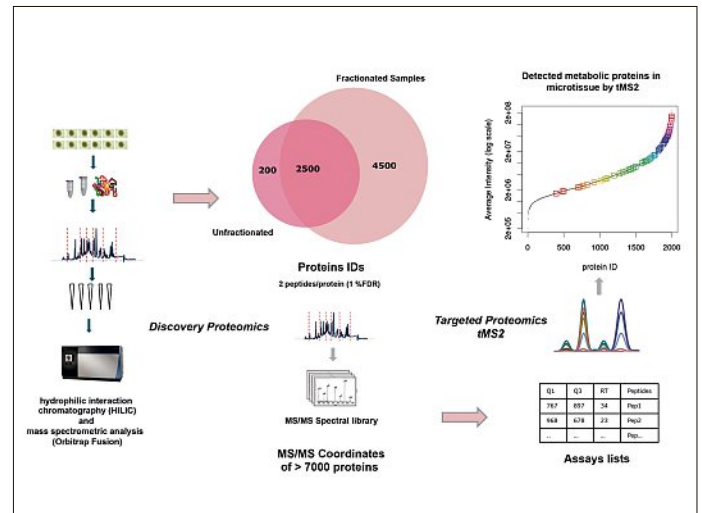
for substance testing. To this end, he generates tissues by printing one layer of bioink that is subsequently polymerized to provide a stable support for the subsequent printed cell layer. “This process is alternated to produce a multi-layered 3D tissue construct”, the biologist explains. “In a proof-of-concept-study we established robust protocols for printing full thickness skin equivalents for future use in the cosmetic industry.” In the current industry-driven research project the research partners are developing an *in vitro* tool for drug assessment to treat muscle/tendon-related diseases. “The idea is to provide an all-in-one solution for producing and analysing printed *in vitro* muscle/tendon tissues in a well plate”, according to the scientist. “The specialized 24-well plate harbours two posts in each of the wells. The ultimate objective is to print muscle/tendon precursor cells around, and in between, the posts to induce tissue formation with tendon around the posts and muscle fibres between the posts.” First, monocultures of primary human myoblasts and primary rat tenocytes were printed separately in a dumbbell shape around the posts. After cell differentiation, the myoblasts were stained positive for myosin heavy chain (MHC), and myotubes were developed. For tendon tissue, the characteristic collagen I distribution around the cell nuclei was detected. “The printed muscle tissue contracts on electrical stimulation, demonstrating physiological functionality.” Next, the co-cultures of tenocytes and myoblasts were printed around the posts and in between the posts, respectively, to generate differentiated tendon and muscle tissues. The optimized culture conditions allowed both cell types to differentiate into the respective tissues as verified by histological analysis. The bioprinting technology shows a huge potential to produce organotypic tissue models that mimic the native tissue to a high degree.

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Scientific Progress in Toxicogenomics

At ETH Zurich, Prof. **Ralph Schlapbach** is Managing Director of the Functional Genomics Center and actively involved in the European HeCaToS project (Hepatic and Cardiac Toxicity Systems modelling), in which researchers are developing integrated *in silico* tools for predicting human liver and heart toxicity. As drug-induced hepatotoxicity and cardiotoxicity are currently responsible for the market withdrawal of drugs and the exclusion of drugs in clinical phases, researchers are developing an integrated modeling framework, by combining advances in computational chemistry and systems toxicology, for modelling toxic perturbations in liver and heart across multiple scales. “We populate models with data generated using advanced Omics



Workflow of proteome analysis (Source Ralph Schlapbach ETHZ).

technologies applied to *in vitro* 3D liver and heart spheroids that are challenged with prototypical hepato- or cardiotoxicants”, explains Ralph Schlapbach who has experience as a Technology Group Leader at the Max Planck Institute for Infection Biology in Berlin. To identify and monitor the molecular pathways and toxicological endpoints, his team developed a highly sensitive and robust analytical workflow for the proteomic analysis of drug-treated human 3D spheroids. “Using state-of-the-art mass spectrometric analysis, 3000 proteins can be identified and quantified from as little as the protein equivalent of 1000 cells per measurement”, the scientist recalls. The data from multiple treated spheroids can be combined to quantitatively map out more than 5000 proteins of a 3D microtissue proteome covering the relevant molecular protein pathways.

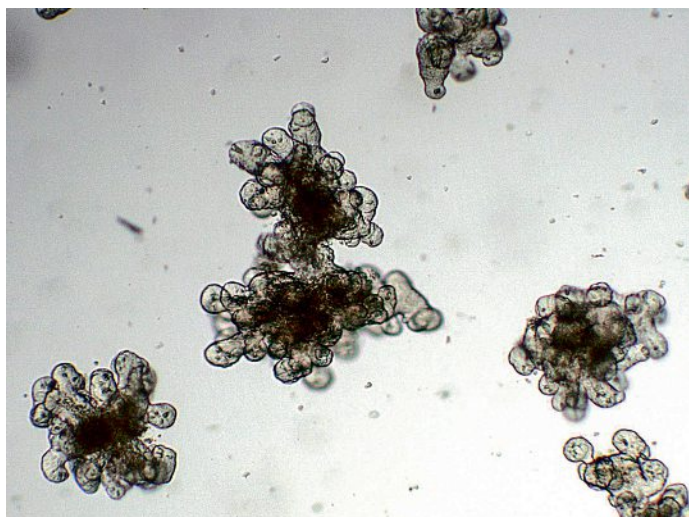
The biochemist concludes: “Using novel 3D cell culture systems in combination with sensitive and accurate MS-based technologies and sophisticated bioinformatics methods, the comprehensive analysis of drug mechanisms and toxicity is feasible at the proteome scale and can be applied to various types of 3D tissue model systems in the future.” Together with data generated at the epigenome, transcriptome and metabolome levels, the resulting proteomics data enables comprehensive mapping of molecular regulation and the precise description of cellular and tissue behaviour.

<http://www.fgcz.ch>

<http://www.hecatos.eu/>

The Organoid – A Unique Platform for Drug Development

The Hubrecht Organoid Technology (HUB) is a not-for-profit organization founded on the pioneering work of Prof. Hans Clevers: he discovered how to grow stem cell-derived human epithelial ‘mini-organs’ – organoids – from tissues of patients with various diseases, including cancer and cystic fibrosis. “Healthy or diseased tissue from patients, such as cancerous tissue, can be efficiently grown in the lab for use in developing effective personalized treatments, or as a model system for drug development”, explains Dr. **Robert G. J. Vries**, Managing Director of HUB, who studied neural stem cells at Stanford University (USA). His statement is based on the previously discovered identity of adult stem cells in many human tissues, such as liver, pancreas, breast and lung at HUB. “With



The organoid is a unique new platform for drug development, for precision medication for patients in the clinic, and a possible new source for cell therapy (Source HUB Robert Vries).

the identification of these stem cells we were able to develop a culture system that enabled the virtually unlimited, genetically and phenotypically stable expansion of the cells from several animal models, including humans.” The HUB researchers found that the intestinal organoids are a very powerful tool for studying cancer, fibrosis and inflammatory bowel disease. The models represent previously unavailable *in vitro* models and patient-specific samples for drug development, patient stratification and diagnostics. “This makes the organoid a unique new platform for drug development, for precision medication for patients in the clinic, and a possible new source for cell therapy”, concluded Robert Vries.

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Pioneers in Engineered Human Myocardium (EHM)

At the Institute of Pharmacology and Toxicology of the University Medical Center Göttingen, researchers are aiming to obtain a better understanding of complex cellular processes underlying heart failure development in order to define novel therapeutic strategies. As their recent research has shown, human myocardium can be engineered under defined conditions for drug screening applications. Key components of engineered human myocardium (EHM) include cardiomyocytes from pluripotent stem cells, a defined pool of stroma cells and collagen type I. “Automation of EHM production, culture and analysis is feasible and allows for phenotypic screens with enhanced throughput and high reproducibility”, says Prof. **Wolfram-Hubertus Zimmermann**, the director of the institute. He is one of the pioneers in the development of manufacturing techniques for creating artificial heart tissue. “Advanced maturation of EHM with physiological properties similar to those observed in adult human non-failing myocardium is an important prerequisite for applications in predictive drug screens. Applications of pluripotent stem cell-derived cardiomyocytes and/or stroma cells from patients with cardiomyopathies, as well as simulated neurohumoral overstimulation, enable us to tissue engineer heart failure models.”

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MPS-based Co-cultures Forge New Paths to Toxicity Testing

Dr. **Jochen Kuehnl**, Head of Laboratory Experimental Toxicology at Beiersdorf AG Hamburg, attracted a lot of attention in the media when he and his co-workers stimulated skin to produce its own protective free radical scavengers with a cosmetic ingredient and with licochalcone A, a type of natural flavonoid. As he states, skin serves as a key role model in terms of exposure to cosmetic ingredients and everyday items such as household products, perfumes, volatile organic compounds and fertilizers. Today, *in vitro* methods are accepted for studying toxic effects such as skin irritation, but risk assessments for more complex aspects such as skin sensitization and systemic toxicity still represent a challenge. That’s why Jochen Kuehnl points out the characteristics of skin models and focuses on the evaluation of new approaches for systemic toxicity assessment based on co-cultures of skin models and liver organoids in dynamic micro physiological systems (MPS).



Dr. Jochen Kuehnl with his collaborators Silke Gerlach and Thamée Rings (in the middle), holding the developed chips (Source: Beiersdorf AG).

“At Beiersdorf, we performed case studies for skin and liver-relevant compounds with the aim of comparatively analysing the outcome and the *in vivo* relevance of simple and complex *in vitro* systems. The results indicate differences between systemic vs. topical substance application regarding epidermal and hepatic cell activation and metabolite formation.” Extensive *in vitro* time-course studies, carried out in cooperation with the biotech start-up TissUse, displayed the influence of skin models on the bioavailability of compounds and link this outcome to differences in the expression of detoxification-associated genes and the formation of respective metabolites. “MPS-based co-culture systems have displayed long-term *in vitro* stability of skin models, as well as hepatic organoid functionality, and allowed for repeated substance application”, explains Jochen Kuehnl. “Although MPS-based assays will require thorough evaluation and extensive formal validation efforts prior to full industrial adoption, MPS-based co-cultures provide new opportunities for long-term systemic toxicity testing of substances due to biologically relevant function and interactions or organoids.”

www.beiersdorf.de

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