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Hot from the press!

Advanced Cell Culture Systems: Exactly what Academia and Industry Need!

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After many years in pharmaceutical research and five years at the FHNW, Professor ***Laura Suter-Dick*** combines crucial skills in both basic and applied research. Her recent appointment as President of biotechnet Switzerland places her in an excellent position to strengthen research interactions between the private sector and academia in Switzerland. Let's take a peek behind the scenes of her work at the School of Life Sciences.

Laura Dick-Suter is Professor for Molecular Toxicology in the School of Life Sciences at the University of Applied Sciences Northwestern Switzerland and, since January 2017, President of biotechnet Switzerland. Photo Roche.

Advanced cell cultures for the future: mimicking tissue architecture

Most cell culture systems are based on two-dimensional (2D) monolayers on plastic dishes. These systems can be useful but differ substantially from the reality of a tissue or organ in a patient. The Molecular Toxicology Group at the School of Life Sciences (FHNW) focuses primarily on advanced *in vitro* systems that could provide alternatives to animal experimentation. Prof. Laura Suter-Dick, a European Registered Toxicologist (ERT), is an active and convinced promoter of the 3Rs (Replace, Reduce and Refine animal experimentation) in drug discovery research. "Complex, fit-for-purpose cell culture systems are definitely the biological system of the future, but these systems can also already be applied here and now", she says.

Entering the third dimension in oncology research

Conventional methods to test cytostatic agents use cultured adherent 2D cell monolayers. However, tumours naturally grow in 3D, and the spatial distribution of cells affects their mutual interactions. In the context of a student exchange programme with the Universitã degli Studi di Palermo, the group headed by Laura Suter-Dick made use of alginate to generate 3D, spherical cell cultures containing cancer cells. "As alginate is well suited for cell encapsulation, we developed 3D microparticles for the culture of MCF-7, a breast adenocarcinoma cancer cell line", explains Laura Suter-Dick. "Our goal was to implement 3D cell culture systems to evaluate cell proliferation, viability and response to treatment." The first step was the production and characterisation of cell aggregates generated in high-throughput. They showed homogeneous size and distribution of cells, as well as cell proliferation within the aggregates. The scientists subsequently observed that Epithelial to Mesenchymal Transition (EMT), a mechanism whereby cells lose their epithelial characteristics and acquire migratory properties, was influenced by the cell culture architecture: MCF-7 cells in monolayers display an epithelial phenotype, while 3D cultures promote a mesenchymal phenotype. The differences in cellular phenotype also resulted in differences in the responses to cytostatic agents, a key finding in terms of using the system for assessing potential new anticancer drugs. "As our data show, the proliferation rate of MCF-7 cells in 3D cultures was lower than in monolayers", says a visibly happy Laura Suter-Dick. "This was probably due to the differentiation process occurring in 3D cell culture. Immunofluorescence and western blot analysis revealed that E-cadherin, a protein that plays a key role in cellular adhesion, was expressed to a lesser extent in 3D cultures than in monolayers. Loss of this function has been associated with a greater potential for tumour metastasis. In addition, analysis of the cell defence factor NRF2 shows that MCF-7 grown in 3D cultures display a higher chemo-resistance capability compared to monolayer MCF-7 cultures". In fact, the data show that the response

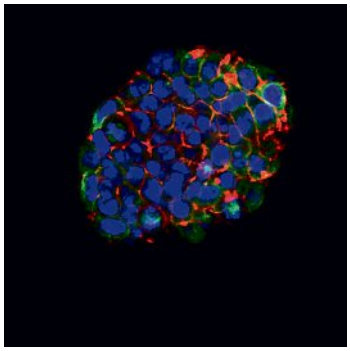


Photo-micrograph of MCF7-alginate aggregates after immunofluorescent staining. The cells build a compact 'mini-tumour'. The cell nuclei are stained blue (DAPI), green and red are the immune-stains of the cytoskeletal proteins tubulin and actin, respectively. Photo FHNW.

of MCF-7 to the anticancer drug doxorubicin is stronger in 2D than in the more realistic 3D alginate aggregate. "This finding is key, as it indicates that the less physiological 2D systems might lead to an overestimation of the anticancer efficacy of drugs", Suter-Dick concludes.[1]

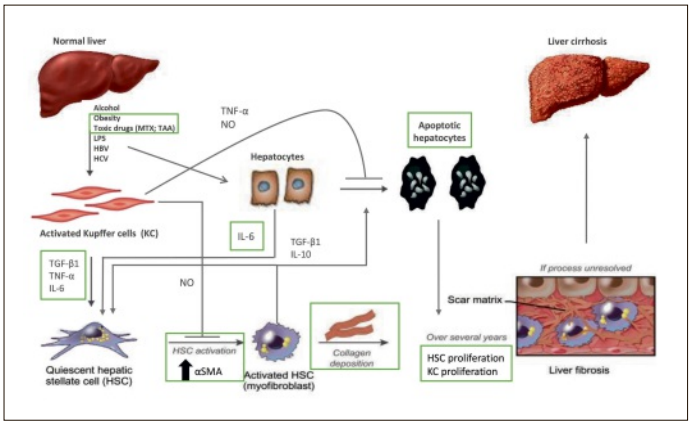
The human kidney on a chip: combining 3D and fluidics

Our kidneys play an important role in the elimination of drugs and their metabolites. Consequently, they are exposed to high concentrations of xenobiotics and are therefore a common target

of drug toxicity. No wonder that 25% of all cases of acute renal failure are caused by drug-induced kidney injury (DIKI). Together with colleagues from the Netherlands, Laura Suter-Dick and her team are working on improved model systems to predict DIKI. To this end, they are applying the organ-on-a-chip technology from Mimetas (*https://mimetas.com*), an *in vitro* system reproducing the 3D micro-environment to culture cells under physiological flow conditions. The consortium (FHNW, Radboud University and Mimetas) funded by NC3Rs, UK (*www.nc3rs.org.uk*) aims to bridge the gap between traditional 2D well plate assays and the *in vivo* situation, with the ultimate goal of replacing or reducing animal experimentation for toxicological drug screening. "In this project we implement microfluidic cell and tissue cultures amenable to widespread use in biological applications", elucidates Laura Suter-Dick. Physiologically adequate 3D kidney models amenable to screening can advance pharmaceutical research by enhancing predictivity, accelerating research, reducing animal experimentation and limiting the costs of development of new and safer drugs. A major gap in the application of such complex systems is the lack of suitable biomarkers to assess the effect of drugs on the cells. The group at the School of Life Sciences (FHNW) is therefore focusing all their efforts on implementing new sensitive biomarkers. Preliminary results look promising as several miRNAs (small non-coding micro RNAs), as well as transcripts and proteins, are showing robust responses to compounds known to cause renal injury. Laura Suter-Dick looks ahead with optimism: "Research should focus on finding the optimal balance between a physiologically relevant design, sensitive and robust biomarkers, and high-throughput technologies. Such a validated kidney-on-a-chip will ultimately bring us safer drugs".[2]

How to roll up sleeves for breakthrough results in liver fibrosis

It is known that liver fibrosis is a scarring process that results in growth of connective tissue, inflammation and liver cell death. Progression of liver fibrosis to cirrhosis often leads to liver failure and the need for a liver transplant. Since no currently available *in vitro* system is capable of recapitulating the cellular events leading to liver fibrosis, most research in this domain involves animal studies. In a collaborative project with InSphero AG (*https://insphero.com/*), and supported by the CTI (Commission for Technology and innovation), Laura Suter-Dick's group generated a system containing the three key cellular players of liver fibrosis: hepatocytes, Kupffer cells and stellate cells. These human cell lines are co-cultured using the InSphero hanging drop technology to generate scaffold-free 3D micro-tissues. Exposure of the micro-tissues to pro-fibrotic compounds such as TGF-β1 (Transforming growth factor beta 1), MTX (methotrexate), and TAA (thioacetamide) for up to 14 days elicited a fibrotic phenotype characterised by the secretion of cytokines, the increased deposition of extracellular matrix proteins and the induction of gene expression of fibrosis markers. The scientists were excited to observe that multicellular 3D micro-tissue cultures can be kept in a non-activated status before being exposed to pro-fibrotic stimuli. Laura Suter-Dick is thrilled with these results: "This is very different than what happens in 2D cultures, where the cells are activated by the contact with the rigid cell culture dish. It demonstrates once again the superiority of cell cultures in more physiological 3D formats". This system is so powerful for the study of fibrosis *in vitro* that it has become the cornerstone of a collaboration between the School of Life Sciences (FHNW) and the Swiss Center of Applied Human Toxicology (SCAHT: *http://www.scaht.org/*). In this exciting project, Vincenzo Prestigiacomo, a talented PhD student, is studying the fibrosis Adverse Outcome Pathway (AOP) and the involvement of the antioxidant response in the activation of the stellate cells. Laura Suter-Dick concludes: "The application of such a system would be a great contribution for the further understanding of the mechanisms of liver fibrosis and for the study of potential anti-fibrotic treatments".[3]



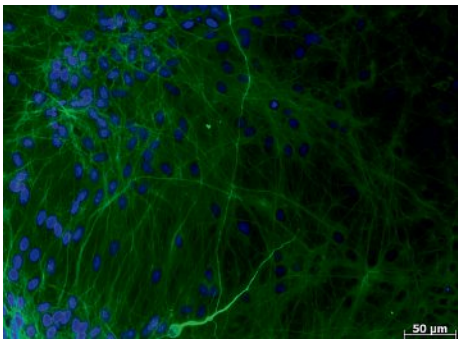
The scheme depicts the complex interactions between matrix-producing hepatic stellate cells and liver-resident macrophages (Kupffer cells) and hepatocytes after liver injury leading to heptic fibrosis. Green boxes highlight processes that can be reproduced in the *in vitro* MT-system. Photo FHNW.

Mimicking Alzheimer's disease in a dish

Around the globe, about 44 million people suffer from Alzheimer's disease (AD) or a related dementia. According to the Alzheimer's Association, the global cost is estimated to be $236 billion in 2016. Therefore, Laura Suter-Dick is also investigating the mechanisms leading to neuronal death in AD. "We started this project in collaboration with F. Hoffmann-La Roche. Within my group, Dr Carine Gaiser applied a human 3D *in vitro* system based on neural precursor cells engineered to carry mutations that are common in familial AD", says Laura Suter-Dick. The cells carry mutations on APP (amyloid precursor protein) and PSEN (presenilin) and can be differentiated into neurons and astrocytes. In patients, these mutations affect the deposition of Abeta peptides, the main component of the amyloid plaques found in the brains of AD patients. They also lead to hyper-phosphorylation of the cytoskeletal protein tau and the formation of intracellular tangles, hallmarks of the disease.[4]

Long-term maintenance of these engineered cells in 3D culture systems leads to the development of AD pathology *in vitro*. The researchers were able to detect increased Abeta secretion, Abeta deposition and tau hyper-phosphorylation. Suter-Dick's group is currently pioneering research on additional factors that modulate AD. For example, the addition of macrophages to mimic neuro-inflammation represents a novel approach that takes this *in vitro* system to an additional level of complexity by including immune cells. THP-1 cells, a human monocytic cell line, are co-cultured with the differentiated neurons and astrocytes by means of bio-printing technologies. Over several days these immune cells proliferate and release immune-inflammatory signals – cytokines – that affect the neuronal cells. This is a step in the right direction for AD research, as several aspects of the disease can be reproduced *in vitro*, including the phenotype of mutations of familial AD and the influence of neuro-inflammation. Although there is still a long way to go, this opens up an unparalleled opportunity to study the mechanisms underlying Alzheimer's disease and the effects of pharmacological interven-

tions. It needs to be kept in mind that, as of today, there are no successful treatments for AD, and that its incidence is increasing due to the rising life expectancy of the population. In addition, animal models are not suitable for addressing certain major, clinically relevant, aspects. So Laura Suter-Dick is delighted to have a tool at hand that can help test potential cures for such a devastating disease, and is open to explore new horizons. "Based on our current knowledge, we are looking for industrial partners to pursue this research and help them generate and assess new pharmacological interventions in AD, therefore directly benefiting the patients".[5]



Fluorescence micro-graph of 15 day differentiated, bio-printed neural progenitor ReN cells. Bio-printing was performed using a printer from regenHU SA. The cell nuclei are stained blue (DAPI), green indicates positive immunostaining for the neuronal differentiation marker Tuj1. Photo FHNW.

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