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Molecular and *in vitro* Toxicology at the FHNW

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Abstract: In the recently established Molecular Toxicology laboratory at the Institute for Chemistry and Bioanalytics of the School of Life Sciences in the FHNW, we aim to develop and apply *in vitro* models for the investigation of toxic effects, with a primary focus on liver and kidney. In collaboration with other institutions, we are developing multicellular type 2D and 3D cell culture assays to be able to closely mimic relevant *in vivo* situations. In parallel we are broadening the choice of available endpoint analyses.

Keywords: Molecular *in vitro* toxicology · Organotypical 3D-cultures

In the past years, there has been a continuous interest in the use of *in vitro* methods that could serve as alternatives to animal experimentation for drug discovery research in general and toxicity testing in particular. Several industrial sectors, including cosmetic, chemical, pharmaceutical and consumer care products are currently striving towards *in vitro* toxicology assays to perform risk assessment. The new European regulations under REACH (Registration Evaluation Authorisation Chemicals), as well as the ban of animal testing for cosmetic ingredients, and the late failures (Phase 2 and 3) of pharmaceutical drugs due to safety issues have fuelled the interest in animal-free, patient-relevant toxicity assessment methods.

A major challenge in *in vitro* toxicology remains the need for suitable *in vitro* systems with adequate cells and physiologically relevant culture conditions. Also, specific qualitative and quantitative endpoints are needed to enable safety scientists to go beyond hazard identification and move towards the field of relevant risk assessment by improving our understanding of mechanisms of toxicity.

Commonly used *in vitro* systems include cell lines and primary cell cultures that often fail to reproduce the *in vivo* situation. Cell lines are often genotypically and phenotypically different from their precursors in a healthy (or diseased) individual. On the other hand, primary cells tend to be short-lived in culture, cannot always be easily obtained and show large donor-to-donor variability. The fast advances in the field of stem cell research and the availability of reprogramming and differentiation protocols have opened new opportunities to generate *in vitro* systems that are suitable for toxicity testing.

In addition to the use of more physiologically relevant and human-derived *in vitro* systems, major efforts are currently focused on the establishment of organotypical, multicellular co-culture models that can partly mimic the tissue architecture and cell–cell interactions. These characteristics have a great impact on disease development and response to pharmacological or toxicological stimuli.

Specific parameters for a variety of endpoints such as cellular status, oxidative stress, cellular respiration need to be included in the evaluation, as simple cytotoxicity assays need to be complemented by more functionally relevant cellular endpoints that can better reflect the organ physiology.

In the recently established Molecular Toxicology laboratory at the Institute for Chemistry and Bioanalytics of the School of Life Sciences in the FHNW, we aim to develop and apply *in vitro* models for the investigation of toxic effects, with a primary focus on liver and kidney. To this end, we apply state of the art cell culture systems involving primary cells, commercially available cells and engineered cell lines. It is foreseen that we will also pursue the use of stem cell derived (SCD) systems in the future. Currently, we focus in two major ways of improving the relevance of the cell cultures for toxicity assessment: suitable cell culture systems and appropriate endpoints.

We are working on the generation of organotypical, 3-dimensional (3D)-cultures that contain several cell types that should better mimic the physiology of the organ, including aspects such as cell–cell interactions. With these systems, we aim to study mainly liver toxicity (hepatotoxicity) as well as clinically relevant liver diseases such as fibrosis and cirrhosis. Also, organotypical cultures are being implemented to mimic the function of kidney tubular cells, where metabolic and transport activities must be maintained.

To evaluate the toxicity potential of a substance, we assess the effects of xenobiotics on cellular systems by a variety of relevant parameters, such as cell death, secretion of specific biomarkers, cell proliferation and cellular respiration parameters. As an example, we have compared the responses of several human cell lines to compounds with known toxicities by monitoring parameters over several days, including impedance measurements, oxygen consumption and extracellular acidification rate. The different parameters, together with cytotoxicity assays provided insights into the toxicity mechanisms of the tested compounds. The use of different cell types representing several tissues provided useful information in terms of tissue-specificity of the effects.

We work together with other institutions that are proficient in *in vitro* toxicology systems in Switzerland and abroad. In this context, it is worthwhile to mention our alliance with

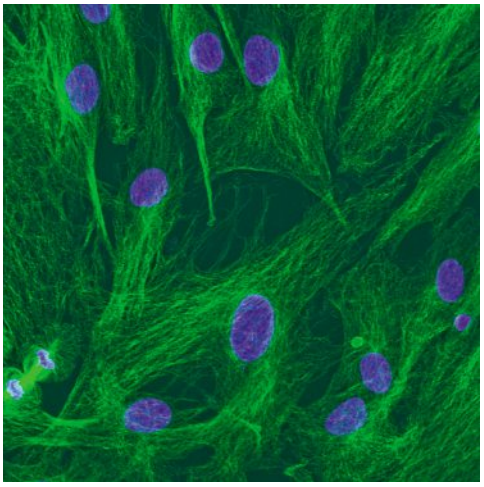

Fig. 1. Immortalized human hepatic stellate cells in culture. Blue: nuclear stain with DAPI, Green: α-Tubulin immunostain (cellular cytoskeleton).

TEDD (Tissue Engineering for Drug Development, part of the Biotechnet Switzerland and the NTN Swiss Biotechnet), for the establishment of 3D-liver culture systems. Also, we work in close collaboration with institutions promoting alternative methods to animal experimentations, such as NC3Rs (National Centre for the Replacement, Refinement and Reduction of animals in research, UK), EPAA (European Partnership or Alternatives to Animal testing), and SCAHT (Swiss Centre for Applied Human Toxicology). In an ongoing collaboration with NCL New Concept Lab GmbH in a SATW supported joint project, we are working on a new high-throughput ready membrane-based device which allows the study cell migration and invasion as well as metastasis. Each of these processes can be followed using multiple parameters. The experimental results of the proof-of-concept have successfully demonstrated the feasibility for cell migration and invasion assays and will be used for further development of the device and expansion of its uses. We are also seeking additional collaborations to establish and validate methods relevant to *in vitro* toxicology.

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