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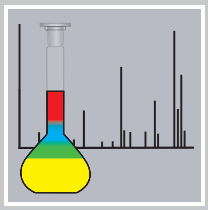

Highlights of Analytical Sciences in Switzerland

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Mass Spectrometric Proteome Analysis of Small Three-Dimensional Microtissues Allows for the Quantitative Description of Toxic Effects of Drugs

Nathalie Selevsek* and Ralph Schlapbach

*Correspondence: Dr. N. Selevsek, Functional Genomics Center Zurich, ETH Zurich and University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Tel: +41 44 635 39 61, E-Mail: selevsek@fgcz.ethz.ch

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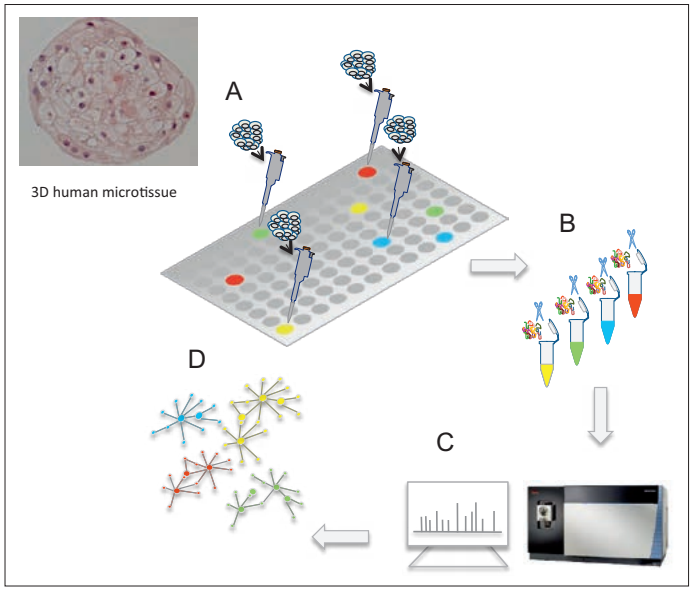
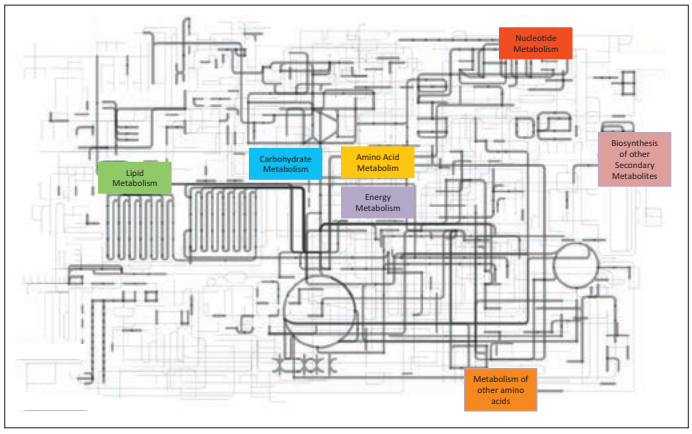
In vitro tissue models are essential for safety testing of novel candidate molecules developed in the pharmaceutical, cosmetic, and chemical industries. Recently, 3D cellular tissue models have been established for improved testing of chronic exposure toxicity, featuring longer lifespans and greater stability compared to 2D monolayer cultures. Besides, their 3D architecture displays more organ-like function than conventional monolayer cell cultures and are often referred to as spheroids, due to their spherical shape.

In the context of a European research project aiming at developing integrative *in silico* tools for predicting human liver and heart toxicity after drug administration, corresponding spheroid tissues are challenged with prototypical hepato- or cardiotoxicants to reveal the biomolecules, such as proteins, involved in the toxic phenotype.



Highly sensitive analytical workflow for the proteomics analysis of drug-treated human spheroids. A: Treatment of liver and cardiac spheroids with prototypical hepato- or cardiotoxicants. B: Protein extraction from drug-treated human spheroids and preparation for proteomics analysis. C: Mass spectrometric analysis of digested proteins for proteome identification and quantitation. D: Protein network analysis.



Metabolic protein pathways identified by MS-based proteomics in liver spheroids (*http://pathways.embl.de/iTuby/*).

Thanks to the rapid development of robust and sensitive mass spectrometers together with computational tools, mass spectrometry-based proteomics has become a powerful analytical approach to study large numbers of proteins up to rather complete proteomes isolated from living cells and organs. Using a latest generation proteomics approach, our analyses were carried out by digesting the protein extracts by trypsin, separation of the resulting peptides by hydrophilic interaction liquid chromatography, and finally mass spectrometric analysis using a Thermo Orbitrap Fusion system. In the mass spectrometer, the peptides are fragmented by collision with a neutral gas and the resulting precursor and fragment-ion spectra are analyzed. Using this method, 3000 proteins can be identified and quantified from as little as the protein equivalent of 1000 cells per measurement. Combining the data from multiple treated spheroids allows to quantitatively map out more than 5000 proteins of the spheroid proteome.

Currently ongoing work focuses on the in-depth analysis of the drug dosage effects on proteomes from drug-exposed liver and cardiac spheroids revealing their drug-specific toxic responses. **The generated data illustrates that with new spheroid fabrication techniques together 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.**

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Reference

Hecatos (Hepatic and Cardiac Toxicity Systems Modeling), Framework Programme (HEALTH-F4-2013-602156).

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Please contact: Dr. Veronika R. Meyer, Unterstrasse 58, CH-9000 St. Gallen
Tel.: +41 71 222 16 81, E-mail: VRMeyer@bluewin.ch