Characterization of rainbow trout hepatic 3D spheroids for next generation ecotoxicity testing

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There is an increasing demand for toxicity testing of chemicals with animal alternative using new approach methods (NAMs). At present, there are many established *in vitro* tests using both primary and continuous cell cultures. However, few are suitable for evaluating chemical long-term toxicity. A recent advancement using a more complex three-dimensional (3D) spheroid model, derived from rainbow trout primary hepatocytes (RT-HEP), has shown potential as it retains physiological properties over an extended period of time.

To determine its suitability within toxicity testing, an extensive characterization of the RT-3D hepatic cell culture system is required. For this, high-resolution multiphoton fluorescence microscopy (MFM) was used to examine various parameters like morphology, DNA integrity, and inner-core hypoxia. Additionally, comprehensive characterization utilizing OMICS techniques and various bioassays was conducted to assess RT hepatic 3D spheroids response to toxins.

Assessment involved exposure to model chemicals representing different modes of toxicity (MoT), such as copper, 17 β-ethinyl estradiol (EE2), pyrene, 3,4-dichloroaniline (DCA), and carbonyl cyanide m-chlorophenyl hydrazone (CCCP). One of the aims of this study was to assess the RT-3D model's sensitivity, responsiveness and reproducibility to above mentioned toxins by measuring endpoints such as viability, metabolic activity, ATP synthesis, vitellogenin (Vg) induction, and oxidative stress and comparing it towards *in vitro* literature. Furthermore, transcriptomics (RNA-seq) and untargeted metabolomics (mass spectrometry-based) approaches were employed to unravel the molecular landscape of the 3D spheroids when exposed to chemicals with diverse MoT.

Preliminary results indicate the reliability of the RT hepatic 3D spheroids in evaluating cytotoxicity, cell membrane integrity, and oxidative stress. Additionally, confirmation of the absence of hypoxia at the spheroid core was confirmed. These ongoing characterizations display the potential use of the RT hepatic 3D spheroids as an ecotoxicity screening tool for screening a variety of chemical with different toxic MoT.