A multi-organ-chip co-culture of human liver equivalents and neurospheres for long-term substance testing

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Abstract
Current in vitro and animal tests for drug development are failing to emulate the organ complexity of the human body and, therefore, to accurately predict drug toxicity. In this study, we present a smartphone-sized, self-contained multi-organ-chip (MOC) platform capable of co-cultivating up to three organ equivalents inside a combined media circuit. A peristaltic on-chip micro-pump reproducibly operates a PDMS-embedded microcirculation system, emulating the systemic arrangement of organs within the human body. It could be shown, that the multi-organ-chip is capable of supporting long-term co-cultures of human artificial liver microtissues and neurospheres. Cultures were successfully maintained functional over a period of up to 14 days. Liver cell polarity was restored as shown by the expression of specific transporters, tight junctions and the formation of rudimentary canaliculi-like structures. Vitality of the cells was assessed by TUNEL/Ki 67 staining and was markedly increased compared to static controls. Neurospheres derived from the Ntera-2 (NT2) cell line were strongly positive for neuronal markers MAP2 and β-Tubulin III after 14 days of culture in the MOC as assessed by immunohistology and qPCR. Chronic exposure of the cultures to 2,5-hexanedione over 14 days revealed a dose-dependent toxicity on MOC co-cultures.

Experimental Set Up
Generation of tissue equivalents
Liver aggregates containing 4.8 x 10⁴ HepaRG cells and 0.2 x 10⁴ human hepatic stellate cells (HHStSc) were formed in Perflect3D® 384-Well Hanging Drop Plates (3D Biomatrix, USA). After two days of hanging drop culture, 20 aggregates were loaded into a single tissue culture compartment of the micro-bioreactor.

Neurospheres were produced from undifferentiated NT2 cells. Therefore, 7x10⁴ cells/ml were inoculated in a silanized 125 ml spinner vessel equipped with ball impeller. Differentiation was induced by 10 µM retinoic acid for 3 weeks. During the differentiation period, a 50% medium exchange was performed every 2-3 days.

Bioreactor culture
Each circuit of the micro-bioreactor device contained 700 µl medium in total. Daily samples were collected for respective analyses. Experiments were stopped at day 14 and tissues were subjected to immunohistochemical stainings. Experiments were conducted with four replicates.

Tissue exposure to 2,5-hexanedione
Co-cultures were exposed to 0 mM, 16 mM and 32 mM 2,5-hexanedione, starting on day 6 of MOC culture. Substance application was repeated at 24 h intervals simultaneously with the medium change.

Results
Co-cultures of human artificial liver microtissues, consisting of HepaRG cells and HHStSc, and neurospheres have successfully been cultivated over 14 days in the novel microfluidic bioreactor. Glucose consumption and lactate production indicated an aerobic metabolism which reached a steady state after 6 days. Immunohistochemical staining revealed the expression of phase I metabolic enzyme cytochrome P450 3A4 throughout the whole liver equivalent and a network-like arrangement of tight junction protein ZO-1 indicating the polarization of hepatocytes. Neurospheres showed strongly positive for neuronal markers MAP2 and β-Tubulin III, whereas the embryonic stem cell marker TRA-1-60 was negative in all samples. The intensity of staining was comparable to control neurospheres at day zero, which were not cultivated in the MOC. Furthermore, the cultures revealed a dose-dependent response to a 9-day exposure to the toxic substance 2,5-hexanedione as shown by TUNEL / Ki67 staining.

Summary
A promising tool for long term co-culture of human liver equivalents and neurospheres has been developed. The simple MOC design presented, operated cultures at a total on-chip volume of 700 µl medium at recirculation rates of 40 µl/min assisted by an on-chip micropump.

The prediction of toxicity profiles of compounds was demonstrated possible by exposing the cells to different concentrations of 2,5-hexanedione. This platform is designed to generate high-quality in vitro data predictive of substance safety in humans. Tissue cultures can be exposed to pharmaceutical substances at regimens relevant to respective guidelines, currently used for subsystemic substance testing in animals.