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Substance exposure to organotypic human liver and small intestine co-cultures in a Multi-Organ-Chip

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Abstract

Systemic absorption and metabolism of drugs in the small intestine, as well as metabolism by the liver, are key determinates of efficacy and safety for therapeutic candidates. However, these systemic responses of applied substances are ignored in most in vitro tests. In this study, a co-culture of 3D human liver organoids and differentiated primary human small intestine epithelial tissues were combined in a dynamically-perfused Multi-Organ-Chip (MOC) system. An on-chip micro-pump enabled metabolite transport between the 2 organotypic cultures and added physiological shear stress. The liver and primary intestine equivalents were kept viable in a co-culture for 14 days. The small intestine model showed columnar epithelial cell morphology similar to in vivo human small intestine, and showed expression of tight junctions and specific drug transporters. Test chemical troglitazone was applied to the co-culture system daily for 11 days. The effects of apical application onto the small intestine were compared to the effects of direct substance application to the medium to analyse the influence of the penetration and metabolism of the small intestine. An outlook on the 4-Organ-Chip culture for 28 days is given (Figure 5).

Experimental Set Up

Tissue Reconstruction:

EpiIntestinal Tissues: Small intestinal epithelial cells were harvested using enzymatic tissue dissociation. The cells were expanded in culture and seeded directly onto 96-well cell culture inserts, raised to the air-liquid interface, and cultured using a specially formulated culture medium (MatTek Corporation) designed to induce differentiation over a period of 14 days.

Liver spheroids: HepaRG and human hepatic stellate cells were produced in hanging drop plates for 48 h and were then moved to the MOC.

Intestine and liver tissue loading

Liver microtissues (aggregates of HepaRG+human hepatic stellate cells) and MatTek small intestinal models were loaded onto the Multi-Organ-Chip, submerged in media with a total volume of 500 µl (Figure 1). The cultures were allowed 3 days of adaption to shear stress and the shared medium, before substances were added daily for another 11 days.

Multi-tissue exposure to substances

Troglitazone (10 µM) was dissolved in DMSO and was applied daily to the co-culture apically of the intestinal tissue for 11 days (Figure 2).

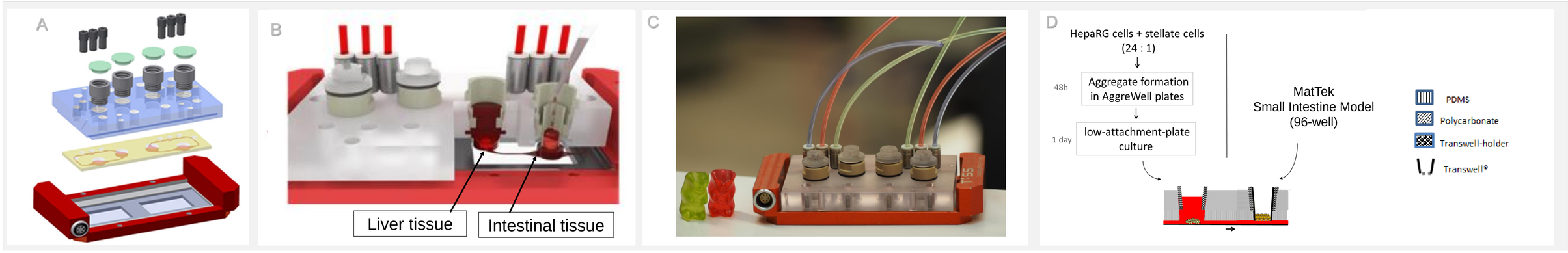

Fig. 1. The microfluidic MOC device at a glance. (A) Exploded view of the device comprising a polycarbonate cover-plate (blue), the PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height: 3 mm) and a heatable MOC-holder (red). **(B)** 3D drawing of substance exposure, either systemic or apical. **(C)** Photo of the 2-Organ-Chip. **(D)** Schematic sections through the tissue culture compartments supporting small intestine cultures in Transwells® and submersed cultures in the fluid flow.

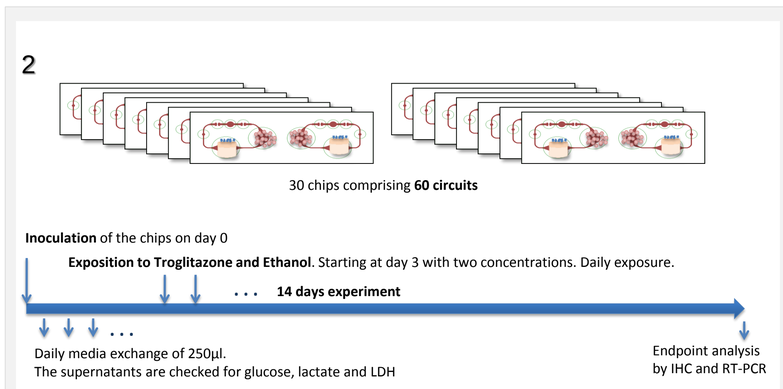

Fig. 2) Set up of culture and troglitazone exposure.

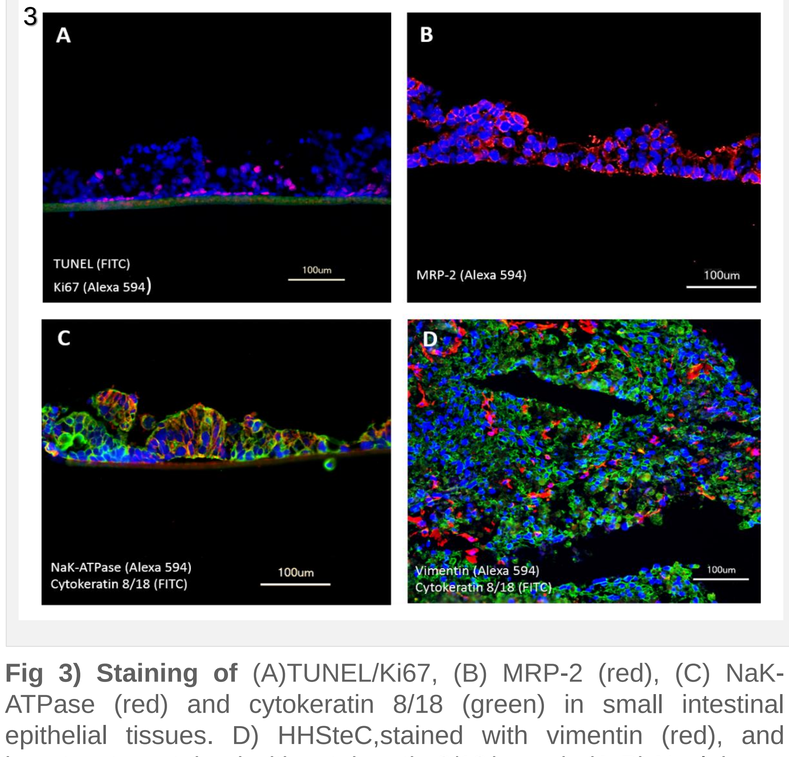

Fig 3) Staining of (A)TUNEL/Ki67, (B) MRP-2 (red), (C) NaK-ATPase (red) and cytokeratin 8/18 (green) in small intestinal epithelial tissues. D) HHSteC,stained with vimentin (red), and hepatocytes, stained with cytokeratin 8/18(green). (A-D) Nuclei were stained with DAPI (blue).

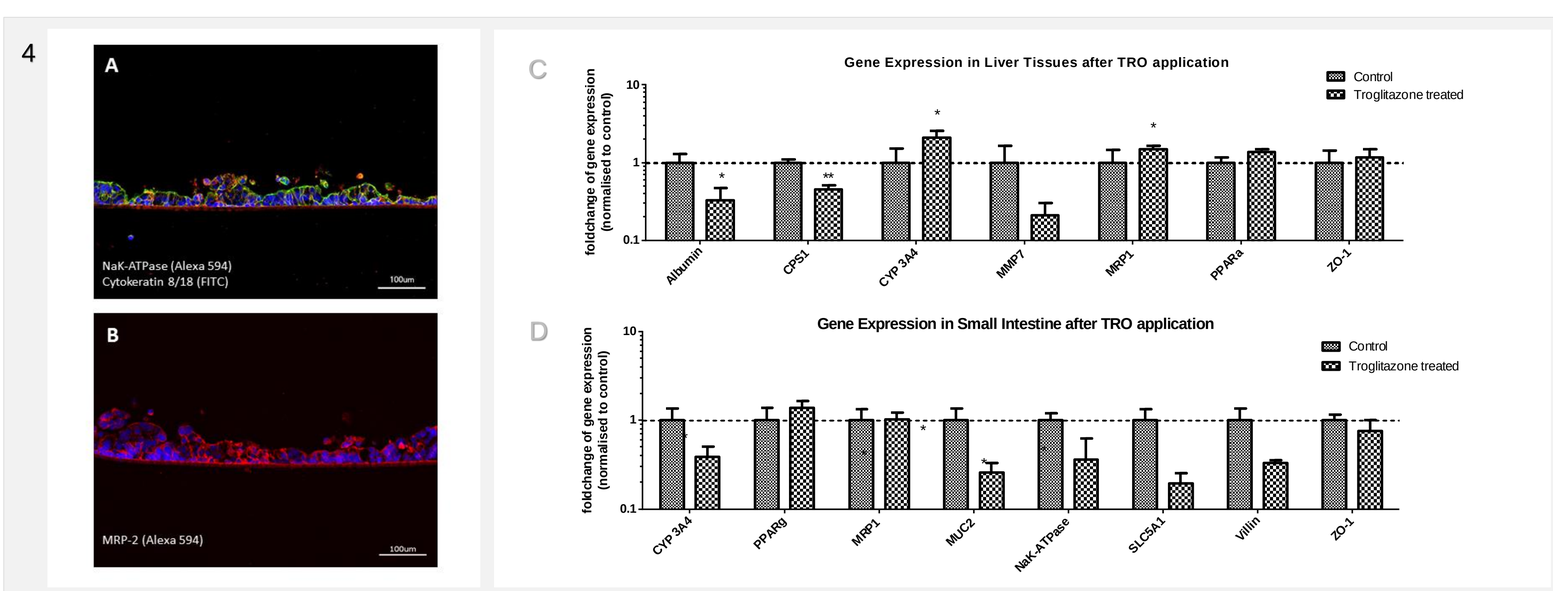

Fig. 4) Stainings and mRNA expression profiles of multi-tissue cultures after chronic substance exposure (A) staining of NaK-ATPase (red) and Ctk 8/18 (green) and (B) of MRP-2 of intestinal tissues. (C) mRNA expression in liver tissues after troglitazone application and (D) in small intestine. (A and B) Nuclei were stained with DAPI (blue). Scale bars 100 µm. (C and D)
Data are mean ± SD of four independent co-cultures. * indicates $p \leq 0.05$

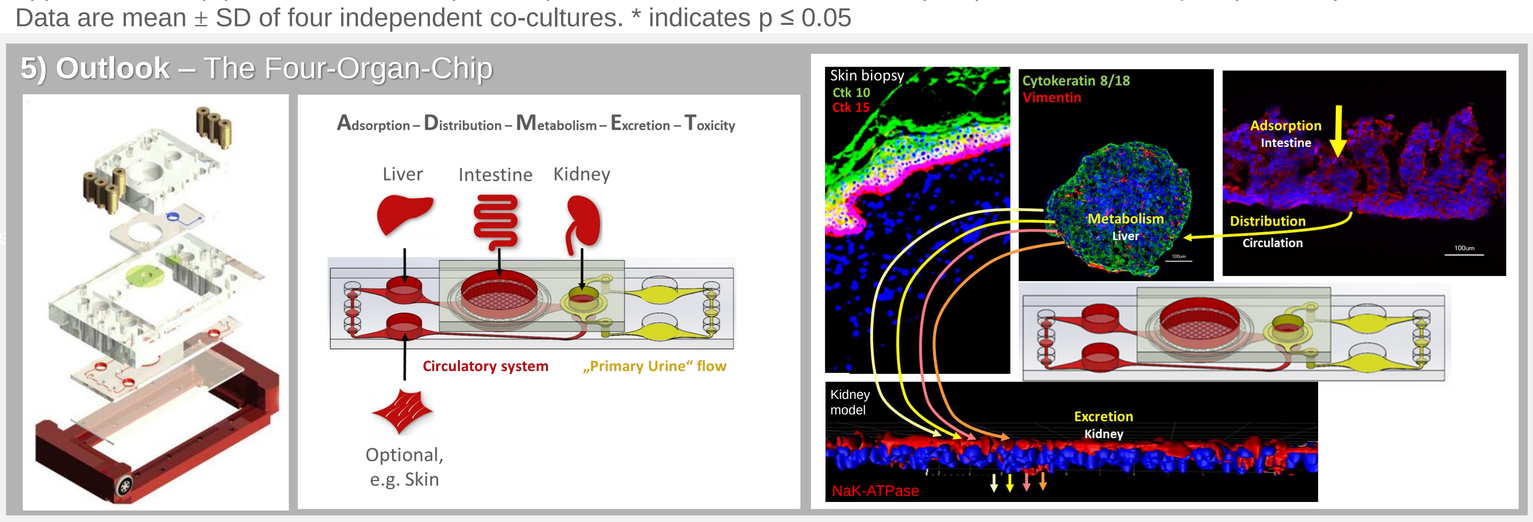

Fig 5) Four organ co-culture on a Multi-Organ-Chip – a 28 day Proof of Concept including cultures of intestinal tissue (MatTek), liver aggregates, skin biopsies and kidney models.

Results

The small intestine model showed columnar epithelial cell morphology similar to in vivo human small intestine and had a height of up to 140 µm. Our primary intestinal epithelial tissues could keep a constant close to physiologic TEER values during the 14-day culture in the MOC (around 50-180 $\Omega*cm^2$). A reproducible and easy to perform oral substance administration protocol was performed over a stretch of 11 days, resulting in a continuous two-week MOC performance at repeated dose testing conditions.

Summary

Fourteen-day co-cultures of human 3D liver equivalents with human intestinal barriers have successfully proven the capability of the microphysiological MOC system to reliably and reproducibly interconnect a parenchymal organ equivalent with a human barrier organ model. The liver equivalents responded properly to the apical applied troglitazone challenge, as demonstrated at the mRNA level. To summarize, our findings suggest that the MOC platform is capable of comprehensively maintaining various miniaturized human organ equivalents over long culture periods

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