Modelling the intestine-liver axis and the role of FXR agonists in a multi-organ microphysiological system model of non-alcoholic fatty liver disease

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INTRODUCTION

The human liver and intestine are uniquely linked, and this "gut-liver axis" plays a crucial role in controlling the pathology of liver disease. If homeostasis is disturbed, dietary factors and microbiota can alter intestinal barrier function, leading to the release of microbial metabolites, and components, which translocate to the liver contributing to inflammation, fibrosis and cancer (1). It is proposed that the progression of non-alcoholic fatty liver disease (NAFLD) is tightly linked to changes to the gut-liver axis (2). There are currently no FDA approved drugs for the treatment of NAFLD and its more severe form non-alcoholic steatohepatitis(NASH) and there is a requirement to better understand the mechanisms that drive disease progression to better inform therapeutic R&D programs.

Currently available preclinical models of NAFLD and NASH, be it *in vivo* or *in vitro*, have a range of limitations and do not fully represent the key aspects of the human disease state (3). Very few accurately capture the human gut-liver axis and enable studies into the molecular mechanisms that drive NAFLD progression.

We have developed fully human microphysiological system (MPS) models for liver, intestine and a multi-organ MPS platform that connects the intestine and liver. Together these advanced *in vitro* models allow the investigation of NAFLD/NASH and the gut-liver axis.

AIMS

1. Demonstrate that a liver MPS can be induced to recapitulate key aspects of a NAFLD/NASH phenotype. Additionally demonstrate the model is responsive to the FXR agonist - Obeticholic acid (OCA), a semi-synthetic bile acid analogue .

2. Demonstrate that the intestine-liver MPS can crosstalk and mimic the oral absorption of therapeutic compounds.

3. Demonstrate OCA treatment of the intestine-liver MPS induces physiological responses in both tissues

MATERIALS and METHODS

Cryopreserved primary human hepatocytes (PHH), human Kupffer cells (HK) and human hepatic stellate cells (HSC) were obtained from Life Technologies (USA) and cultured in HEP-Lean or HEP-Fat medium. Cells were co-cultured at physiologically relevant ratios in the PhysioMimix™ Multi-organ System for up to 15 days. Fat accumulation was measured by Oil Red O staining of fixed microtissues and normalised to total protein content. Production of IL-6, TNFα, and albumin were all measured by ELISA (R&D systems). Cytokine profiles analysed in cell culture samples by Luminex analysis using Milliplex map Human cytokine/chemokine magnetic bead panel – premixed 38 plex (Merck Millipore).

Fibrosis in 3D microtissues were stained and imaged using a Yokogawa CV7000 high content imaging system. Data generated was quantified using automated MatLab scripts. For transcriptomic analysis RNA-seq or QPCR was used to analyse cDNA using an Illumina NextSeq500 benchtop sequencer or QuantStudio 6 analyser.

Intestine MPS microtissues were generated using CacoGoblet (ReadyCell) Transwells and cultured with primary hepatocytes in PhysioMimix™ Dual-organ (MPS-TL6) plates in optimIzed MPS media. CYP activity and drug metabolism data were all generated using quantitative LC-MS analysis. TEER values determined using a EVOM2 probe (World Precision Instruments).

RESULTS

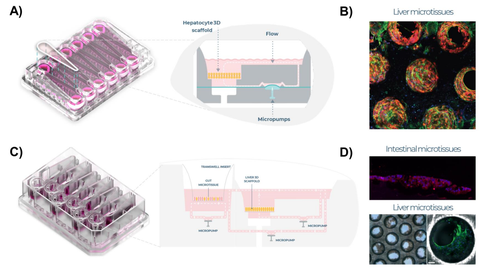

Figure 1 – Liver MPS and intestine-liver MPS set up

A) Liver MPS designed for PhysioMimix™ OOC system, uses open well plates (PhysioMimix™ MPS-LCT2 plate) for the culture of primary liver cells in a 3D engineered scaffold, with 12 replicates per plate. Schematic representation of each replicate shown. B) Highly functional 3D liver microtissue formed over time within embedded scaffolds (IF staining; red – albumin, green – cytoskeleton). C) Intestine-liver MPS (Dual-organ MPS-TL6 PhysioMimix™ plate) with six replicates per plate and fluidic link between the liver and intestine MPS, schematic representation shown of replicate. D) Intestinal microtissues cultured on Transwell® inserts (IF staining: red – ZO-1) and liver microtissues (equivalent to those cultured in the liver MPS) were cultured in the Dual-organ MPS.

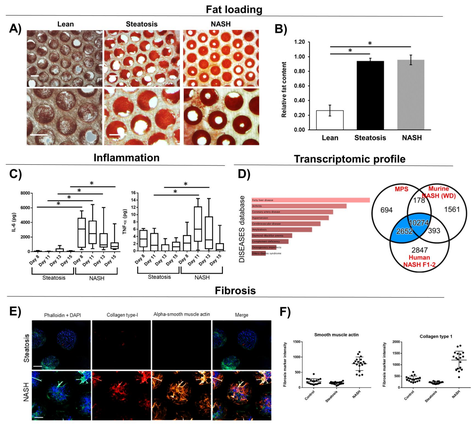

Figure 2 – Liver MPS microtissues loaded with fat display NASH phenotype

PHH, HK and HSC were culture in the liver MPS in either lean or fat conditions. Steatosis controls contained 10-fold less HK and HSC than NASH co-cultures. Microtissues analysed for A + B) Fat loading via quantified Oil Red O staining. C) Inflammatory biomarkers were measured in cell culture medium; D) Transcriptomic profile of NASH microtissues was analysed via DISEASEs database and compared to profile of human NASH biopsy samples and western diet (WD) NASH mice – numbers highlight number of overlapping DEGs. E) Fibrosis was measured and quantified using high content confocal microscopy. Microtissues were imaged (representative images shown) and F) images quantified. All scale bars 300 µm. Data are mean ± SD, n = minimum 6. * = P < 0.05.

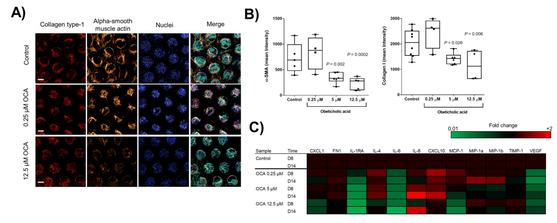

Figure 3 – OCA treatment of liver MPS microtissues reduces NASH phenotype

Liver microtissues were cultured in the Liver MPS for 15 days and after 4 days cultures were dosed daily with the FXR agonist OCA or vehicle (0.1% DMSO). A) Confocal microscopy images of microtissues from each condition at the end of study (representative images shown). For each scaffold 8 regions of interest acquired and each biological condition has N=4. B) Quantification of staining for collagen type 1 and alpha-SMA in microtissues. Data are mean ± SD, N = 4, * = P < 0.05. C) Key cytokines expressed in different conditions at two timepoints during experiment, shown as Log2 fold change compared to vehicle control samples.

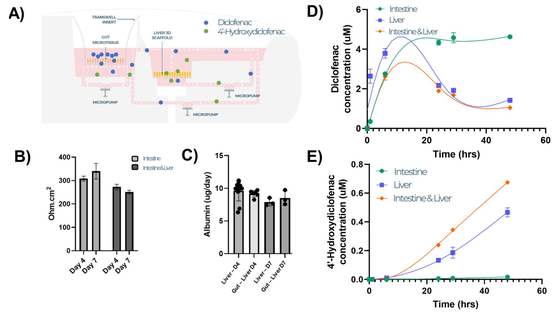

Figure 4 – Intestine-liver multi-organ MPS mimics the human gut-liver axis and enables inter-organ crosstalk and first-pass metabolism studies

Intestinal and liver microtissues were co-cultured in the Dual-Organ plate for seven days and dosed with Diclofenac (10 µM) in the apical compartment of the intestine MPS (to mimic an oral dose) and cultured for 48 hours. A) Schematic representation of transfer of diclofenac and its primary metabolite between the two tissue models. B) TEER measurements of intestinal culture, C) albumin production by liver culture. Concentration of diclofenac (D) and its primary metabolite 4-hydroxydiclofenac (E) were measured by LC-MS in the circulating medium across time, demonstrating drug absorption and metabolism in the same linked MPS platform. The MPS was run with single-organ and multi-organ set-ups for comparison. All data shown is a minimum of three biological replicates and shown as mean ± SD.

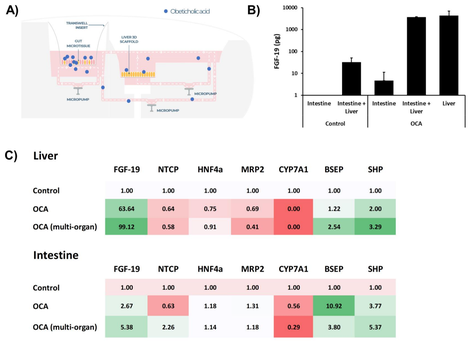

Figure 5 – Treatment of intestine-liver MPS with FXR agonist induces synergistic responses from both organ models

Liver MPS and intestine-liver MPS were cultured for seven days, in either the PhysioMimix Liver or Dual-organ plates, and dosed with FXR agonist OCA - 20 µM in the apical compartment of the intestine MPS (to mimic an oral dose) and cultured for 48 hours. A) Schematic representation of the transfer of OCA between the two tissue models. B) Fibroblast growth factor (FGF-19) production measured via ELISA from single or multi-organ cultures with and without treatment. C) Transcriptomic changes to key FGF-19 target genes in liver and intestinal tissue following OCA exposure. Data shown as fold change compared to control/untreated tissue. All data a minimum of three biological replicates and shown as mean ± SD.

CONCLUSIONS

Utilising the PhysioMimix™ liver MPS we have generated a fully human *in vitro* model of NASH. Co-cultures of PHH, HK and HSC were cultured in fat containing medium which induced key features of clinical disease including, fat loading, inflammation and fibrosis. The transcriptional profile of the model more closely matched that of human F1-2 NASH patients than the profile of western diet mice. When treating the NASH liver MPS with OCA we were able to demonstrate clear dose responsive effects on both inflammatory and fibrotic endpoints.

We also demonstrated the use of the dual organ intestine-liver MPS was able to maintain the linked tissue models for more than one week of culture and is able to recapitulate first pass metabolism following oral drug delivery. The dosing of the multi-organ MPS with OCA induced synergistic responses with stronger responses observed in intestinal and liver tissues when co-cultured together rather than in isolation. OCA dosing on the intestinal cultures induced significant FGF-19 production and a significant change to gene expression particularly in the liver microtissues with loss of CYP7A1 expression and upregulation of BSEP and SHP, mimicking *in vivo* observations.

This work demonstrates that these MPS model(s) are well suited to exploring the molecular mechanisms that underlie the development of NASH and can be useful tools for analysing the efficacy of novel therapeutic strategies.

References:

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