Microphysiological systems for studying interactions between the liver, gut and immune system

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INTRODUCTION

Microphysiological systems (MPS), also known as organ-on-chips, are small scale in vitro cell cultures which mimic facets of tissue or organ level function (1). MPS frequently utilise primary human cells, often cultured in 3D, to obtain highly functional, physiologically relevant models. MPS can be utilised alone, but can also be connected through fluidic circuits to create advanced multi-MPS that can model the interactions between organ systems, allowing greater analysis of molecular pathways and disease mechanisms (2).

Several liver MPS systems have been developed that allow the culture of highly metabolically active primary hepatocytes *in vitro* (3, 4). However, most current models are simple mono-cultures of hepatocytes and do not have the complexity to analyse the interactions between different tissue types and determine how these interactions drive specific pathologies. We have developed a novel system for the in vitro culture of hepatocytes in a perfused three-dimensional format, with a separate co-culture allowing interactions to be studied with a second MPS. To demonstrate the utility of this model we have generated two liver multi-MPS models.

AIM

We aimed to establish MPS for the study of liver interactions with other organ cell types. The systems utilise a transwell which was used to culture either mixed immune cell populations or Caco-2 gut epithelia, alongside, cultures of functional primary human hepatocytes.

METHOD

Cryopreserved human hepatocytes (PHH) were obtained from Life Technologies (USA) and cultured in Ad-DMEM medium containing supplements. 2 x 10^5 PHH were seeded onto collagen-coated plastic disks in static conditions, after 24h disks were transferred into T18 microfluidic plates and combined with other cell types after 4-7 days. PBMCs (Stem Cell Technologies) were cultured in transwells (50k cells per well) in Ad-DMEM medium + 100 IU/ml IL-2 and cultured for 4 days. PBMC transwells were combined with PHH in T18 wells in Ad-DMEM medium +/- 100 IU/ml IL-2, 81 nM PMA, 1.3 µM Ionomycin. PHH were infected with adenovirus expression GFP (MOI: 10) (Vectorlabs) 24h before cells were combined with PBMC cultures. Cells were imaged on a Nikon Eclipse Ti–E inverse fluorescent microscope. Caco-2 cells were seeded onto transwells (20k cells per well) and differentiated for 21 days in DMEM, before combining with PHH. Caco-2 transwells were dosed apically with 25 µM Diclofenac and the presence of the compound and its metabolite were detected on the basolateral side (PHH-side) of the transwell by LC-MS/MS.

Total RNA was extracted using Trizol (Ambion) and RNA was converted to cDNA using the high capacity cDNA synthesis kit (Life Technologies). Gene expression profiles were quantified in equivalent cDNA samples using Taqman assays on an ABI QuantStudio 6 real time PCR system. Human albumin and IFN-gamma were measured in cell cultured medium using commercially available ELISA kits (R&D systems). TEER measurements of Caco-2 barrier integrity were made using a EVOM2 epithelial voltohmmeter (World Precision Instruments). LDH release was measured using the Cytox96 assay kit (Promega).

RESULTS

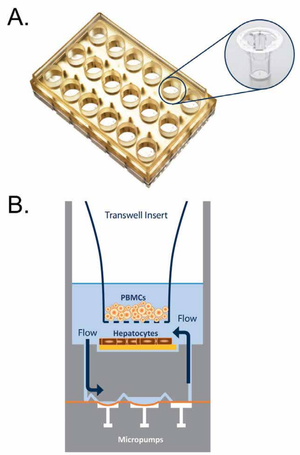

Figure 1 – T18 MPS hardware.
A) The T18 plate is an 18 well perfused cell culture system. B) Media is recirculated in the system by pneumatically operated micropumps. The speed and direction of flow can be accurately controlled using an electronic controller. The system allows for the culture of many cell types on the transwell on 2D base inserts (used here for the culture of PHH).

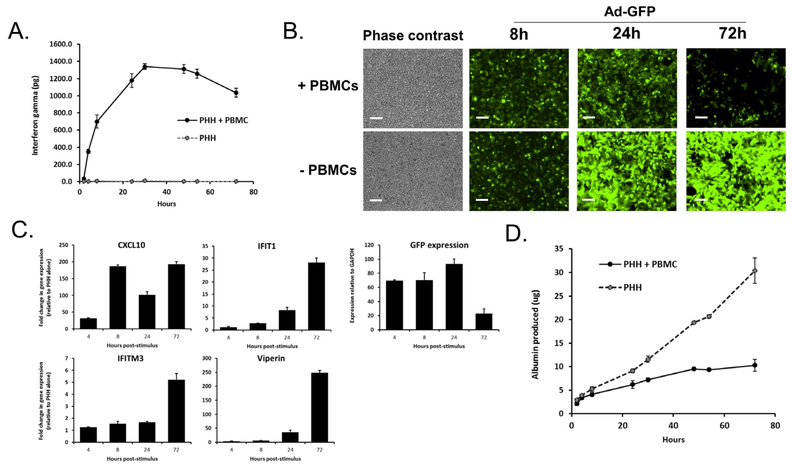

Figure 2 – Liver and peripheral immune system MPS.
PHH and PBMCs were cultured in a T18 platform (PBMCs in a transwell) for 72h and stimulated with IL-2, PMA and Ionomycin. As a control PHH were cultured in the same way with blank transwells. Prior to the co-culture PHH were infected with Ad-GFP for 24h. A) Production of IFN gamma in the basolateral media of the system. B) Fluorescent microscopy images of PHH during the co-culture, scale bar = 100 µm. C) Gene expression of interferon stimulated genes in PHH following culture with PBMCs from total RNA, normalised to GAPDH housekeeping gene and shown relative to PHH alone condition. D) Albumin production in culture media. Data are mean ± SD, n=3.

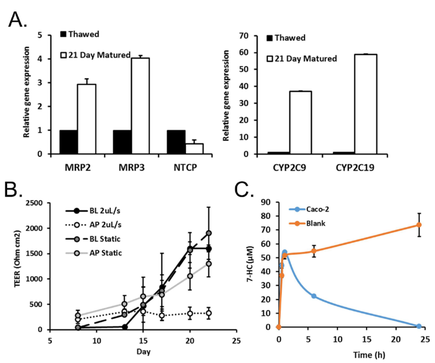

Figure 3 – Establishing a Caco-2 gut model.
Caco-2 cells were differentiated to form epithelial monolayers on transwells in the T18 system. Cells were differentiated on the basolateral or apical side of transwell +/- 2 ul/sec media flow. A) Gene expression analysis of mature cells compared to freshly thawed, analysed from total RNA, normalised to GAPDH and shown relative to the thawed cells. B) TEER measurements of epithelial integrity during differentiation. C) Metabolism of 7-hydroxycoumarin (7-HC) from cell culture medium, after cells were dosed into the apical side of transwells. Concentration of 7-HC was determined by absorbance at 450 nm.

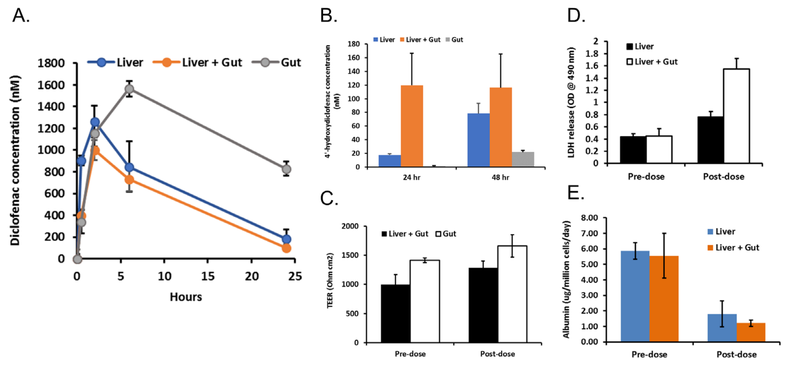

Figure 4 – Liver and gut MPS.
Caco-2 gut transwells (apically seeded) were cultured alongside PHH in the T18 MPS platform for 72h. The apical side of the transwell was dosed with bolus Diclofenac. A) Diclofenac concentration was measured by LC-MS/MS in the basolateral culture medium over 72h (only first 24h shown). B) The presence of the primary metabolite, 4-hydroxydiclofenac was measured in the basolateral medium by LC-MS/MS after 24h and 48h. C) TEER measurements of Caco-2 barrier integrity before and after dosing with Diclofenac. D) LDH release before and after dosing. E) Albumin production from PHH before and after dosing.

RESULTS

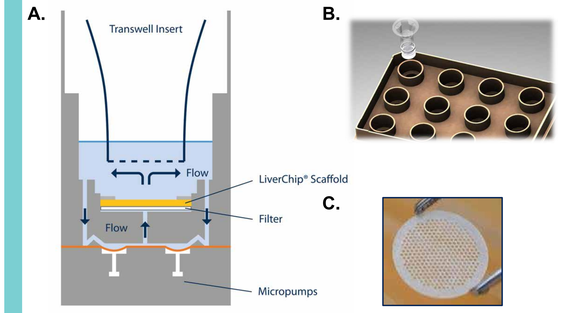

Figure 5 – Next generation T12 MPS hardware.
The T12 plate is a 12 well perfused cell culture system, that allows that culture of three dimensional PHH cultures alongside a second organ. The system has a reduce working volume <400 µl to allow for scaled interactions between organs. A) Media is recirculated in each well by two micropumps which move the media through the PHH containing scaffold. B) Transwells sit in the top of each well and can be used to culture a variety of cell types. C) LiverChip scaffold use to culture 3D PHH in the T12 plate, each scaffold cultures 600k PHH.

CONCLUSIONS

The multi-organ MPS supported the culture of PHH with either a gut or immune models, with both demonstrating interaction between the different organ systems. The PBMCs in the immune model were stimulated to produce IFN gamma, which elicited anti-viral responses in the PHH leading to the clearance of a model adenoviral infection and a reduction in albumin production. The gut model was used to demonstrate absorbance and metabolism of the probe compound Diclofenac. Metabolism was observed in both the liver and gut, as both express CYP2C9 and provide PK profiles like those observed *in vivo*. Dosing of the co-culture did not affect the integrity of Caco-2 epithelia but did reduce function of PHH. The next generation T12 MPS developed will now allow more advanced studies as it supports 3D liver microtissues in a reduced volume.

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