Multi-organ *in vitro* Organ-On-Chip models for intestinal drug absorption and metabolism

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INTRODUCTION

The drug development process is known to be inefficient and time consuming, with only 1:10 drug candidates progressing through clinical trials due to toxicity or lack of efficacy. This inefficiency is partly caused by a lack of physiological relevant pre-clinical models able to accurately predict human responses. Understanding gut barrier permeability of new compounds and their subsequent metabolism by the liver is an important part of the drug development process.

The Caco-2 (Caucasian colon adenocarcinoma) cell line is a widely accepted model for intestinal drug absorption, which whilst offering advantages such as availability and ease of culture, there is debate as to the effectiveness of the model to accurately mimic the intestinal drug response of humans. A lack of mucosal cells and decreased paracellular transport (due to very tight epithelia barrier) is understood to limit the efficacy of the simple Caco-2 gut model.

Development of a more translational relevant intestinal *in vitro* model for accurate prediction of drug absorption could improve the time, cost and overall efficiency within the drug development process. This study explores the use of Caco-2 and HT29-MTX co-cultures in a perfused organ-on-chip/ microphysiological system (MPS) to study paracellular transport and more closely mimicking human intestinal absorption. The model was also extended to include a liver module of primary human hepatocytes to create a multi-organ liver-intestinal model to study drug absorption and metabolism in a single system.

AIM

Utilise a microfluidic organ-on-chip platform to develop a human intestinal model containing Caco2/HT29-MTX cells for prediction of drug absorption. Further develop platform to include a liver model to allow linked drug absorption and metabolism studies.

MATERIALS & METHODS

A perfused co-culture intestinal model was developed encompassing Caco-2 epithelial cells and goblet like HT29-MTX cells. These were seeded onto 0.4 µm pore PET transwell inserts (Corning). Intestinal cultures were maintained either in standard 24-well plates (static) or within the perfused MPS system (PhysioMimix™ - CN Bio Innovations) (Figure 1) to form a functional barrier.

A multi-organ model was developed, containing Primary human hepatocytes (ThermoFisher) which were cultured on removable collagen-coated inserts on the base of wells in the perfused MPS system (Figure 1). For compound dosing studies the intestinal model within the transwell inserts was suspended above the liver inserts, with recirculating flow to connect the two organ systems.

Trans-epithelial electrical resistance (TEER) readings were all performed using WPI EVOM2 Epithelial Volt/Ohm Meter. Gene expression was performed using TaqMan assays on a Applied Biosystems QuantStudio 6 Flex Real-Time PCR System. Dextran permeability was performed using dextran fluorescein, 10,000MW (Fisher scientific). Aminopeptidase-N assay was performed using a 1.5 mM solution of L-Alanine-Nitroanilide hydrochloride (A4N) (Sigma #A9325) incubated for 2 hours and then absorbance read at 405 nm. Fluroscently imaging was performed using Nikon Eclipse Ti, Digital sight – DS-Qi1MC. All histological sectioning and staining was performed by Aquilla biomedical (Edinburgh, UK) and LC-MS quantification was performed by Xenogesis (Nottingham, UK). Albumin assays were performed using Assay Pro Human Albumin AssayMax ELISA Kit. LDH release was measured using CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, UK).

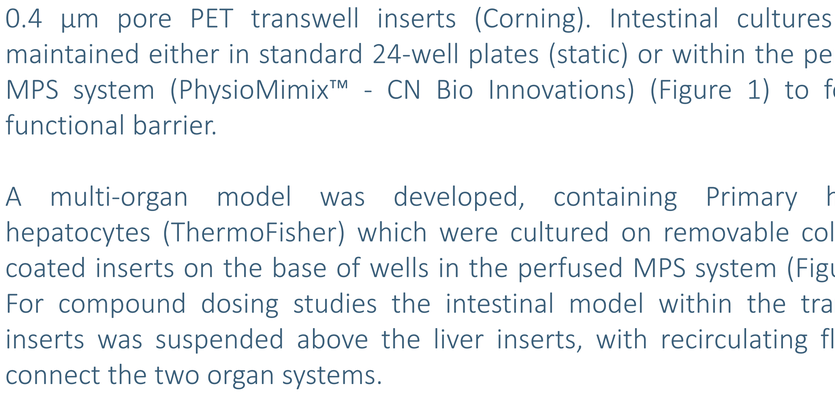

Figure 1 – Organ-on-chip/MPS platform used for perfused intestinal model

A) PhysioMimix™ MPS platform is a benchtop device that uses consumables plates for the culture of different tissue-mimic cultures. The T12 consumable plate was used for all studies. The PhysioMimix™ hardware contains a controller unit that sits outside a standard incubator and MPS-drivers that sit inside the incubator. B) Schematic representation of a single well on a T12 plate used to perform intestinal only cultures showing a cross sectional view of the transwell insert and direction of fluid flow. C) Schematic representation of the T12 gut-liver chip with a cross sectional view of the transwell insert and the PHH containing scaffold, showing the 'gut' cultured on the transwell inserts suspended above the hepatocytes, arrows display the direction of fluidic flow as modulated by the micropumps.

RESULTS

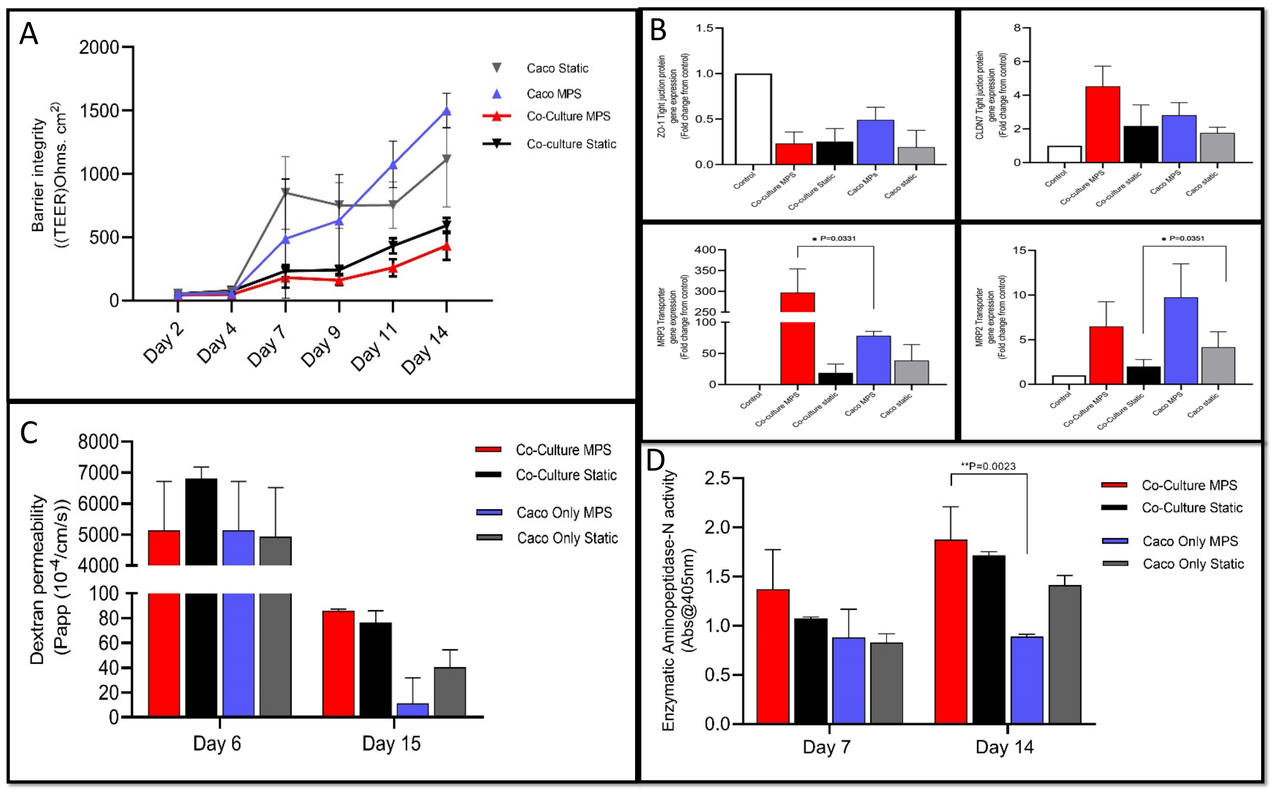

Figure 2 – Perfused intestinal co-culture model shows increased permeability and increased differentiation compared to standard static mono-culture model

Evaluation of Caco-2 mono-culture and Caco-2/HT29-MTX co-cultures in either a static or perfused (MPS) conditions. A) Tight junction integrity quantified by measuring TEER. B) Expression profiling of key intestinal functional genes, as determined by QPCR, normalised to GAPDH. C) Paracellular permeability (Papp) measured by quantifying Dextran transport through intestinal cultures at day 6 and day 15. D) Measurement of cell differentiation by quantification of brush border aminopeptidase-N activity in cultures after 7 or 14 days respectively. All data shown is a mean ± SD, minimum of N=3 (TEER data N=6), P-value determined by two-way ANOVA.

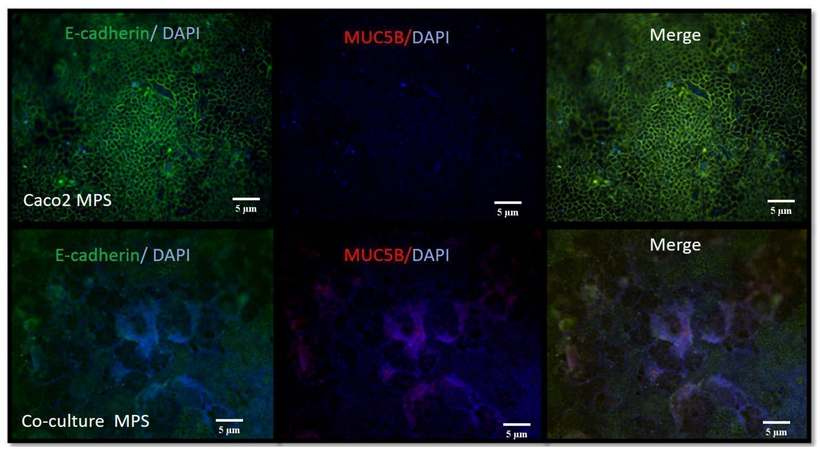

Figure 3 – Perfused intestinal co-culture model expresses mucus and has an altered tissue morphology. Fluorescent microscopy images showing immunostaining of E-Cadherin, DAPI and MUC5B in Caco-2 mono-cultures or Caco-2/HT29-MTX co-cultures generated in perfused MPS platform for 18 days. Representative images shown, scale bar = 5 µm.

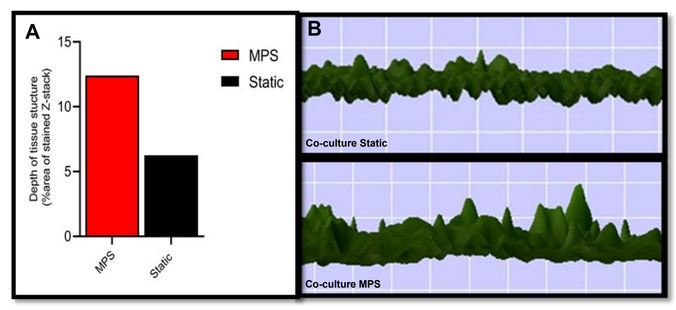

Figure 4 – Intestinal co-culture model under perfused conditions has increased depth of tissue structure.

Caco-2/HT29-MTX co-cultures were cultured in perfused MPS platform or under static conditions for 18 days. Cultures were imaged by fluorescent microscopy for the presence of E-cadherin and Z-stack acquisition used to determine fluorescent staining throughout tissue structure. A) Percentage of tissue depth stained with fluorescent E-cadherin marker. B) Representative Z-stack acquisition and 3D render showing depth of tissue staining.

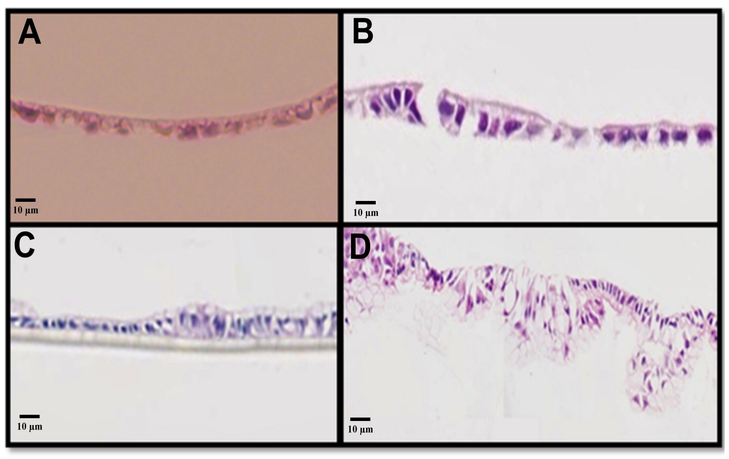

Figure 5 – Intestinal co-culture model under perfused conditions has increased complexity and depth of tissue structure.

Histological staining of Caco-2 or Caco-2/HT29-MTX cells after culture for 20 days. A) Caco-2 mono-culture in a static condition. B) Caco2 mono-culture perfused MPS condition. C) Caco-2/HT29-MTX co-culture in a static condition or D) Caco-2/HT29-MTX co-culture perfused MPS system. All sections were stained using Haematoxylin and eosin (H+E). Blue represents haematoxylin nuclear staining and the pink (Eosin) represents cytoplasm and extracellular matrix. Representative images shown, scale bar = 10 µm.

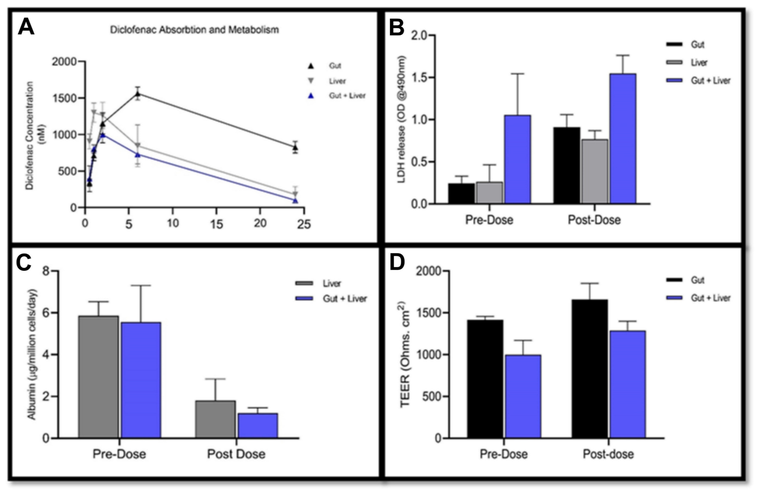

Figure 6 – Intestinal and liver multi-organ model can be used to model drug absorption and metabolism

Intestinal (Caco-2 monoculture) and liver (hepatocyte) cultures were either cultured individually or together in the perfused MPS platform. Cultures were initially pre-prepared and then bought together for 48 hours of compound dosing. A) The apical side of the transwell/intestinal model was dosed with Diclofenac (DCF) (to mimic oral dosing) and concentrations were measured by LC/MS in the basolateral medium (liver compartment). B) LDH release from Caco-2 monoculture, PHH mono-culture and mutli-organ culture medium pre- and post-dosing with DCF. C) Albumin production from PHH before and after dosing with DCF. D) TEER measurements of Caco-2 barrier integrity before and after dosing with DCF. Data shown as mean ± SD, n=3.

CONCLUSIONS

This study explored an organ-on-chip/MPS approach to developing a more translational relevant intestinal model for drug absorption applications. The importance of perfused co-cultures was demonstrated to decrease epithelia barrier integrity, promote mucus production and increase functional endpoints for intestinal cultures. Perfusion of intestinal cultures altered cellular morphology, creating more complex 3D structures. The same MPS platform was used to create a intestinal-liver co-culture which was able to support Diclofenac absorption and metabolism (by the liver) in a simple multi-organ model. Further work will demonstrate the wider utility of the intestinal model for drug absorption, disease modelling and organ-cross talk studies.