APPLICATION NOTE

Drug metabolism in a gut-liver microphysiological system

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Introduction

Studies investigating the efficacy and safety of candidate drugs use a variety of animal models to translate research from the bench to clinical trials and then to the clinic. These models remain an important part of pre-clinical research, but have limitations that include high cost, questions over their ethical use and their weakness as predictors of human biology[1].

Microphysiological systems (MPS), also known as organ-on-chips (OOC), aim to represent the structural and functional characteristics of human tissue. Compared to traditional cell culture on 2D plastic, MPS can utilise multiple cell types of an organ, cultured in 3D scaffolds, under perfusion to mimic blood flow in tissues. They can be used in pre-clinical drug absorption, distribution, metabolism and excretion (ADME) studies, to obtain relevant human data and help inform parameters such as dosage regime and effective drug concentrations.

To-date, these systems have mainly been single-organ tissue models, often built from multiple cell types. To further improve the prediction of *in vivo* pharmacokinetics and pharmacodynamics, more complex MPS models that incorporate multiple tissues related to ADME - including the gut, liver and kidney - are required. Multi-organ MPS offer a unique capability to study organ-organ interaction and crosstalk. For ADME, combining the liver with a gut model, orally administered drugs can be studied in a single system that account for both compound permeability through an intestinal barrier and hepatic metabolism.

Aim

Here, we introduce a multi-organ gut-liver MPS, using the MPS-TL6 consumable plate. This plate is compatible with CN Bio's PhysioMimix® Core System and consists of six wells, each with two compartments, a Transwell® and liver. Liquid flow can be independently controlled in each compartment and in the interconnecting channel from the liver to transwell. The gut barrier is produced using a mix of intestinal epithelial and goblet cell lines cultured on a permeable Transwell® membrane. We aimed to demonstrate the kinetics and metabolism of diclofenac when dosed via the apical side of the gut barrier (to mimic an oral drug dose). Mathematical modelling was used to describe concentration profiles in the MPS-TL6 and allowed for optimisation of drug dosing regimen.

Materials & Methods

Cryopreserved plateable primary human hepatocytes (PHH) were obtained from BioIVT®. 0.6 x 10^6 hepatocytes were seeded into each well on the PhysioMimix MPS-TL6 plate (CN Bio Innovations Ltd) and were cultured in standard hepatocyte media containing 500 nM hydrocortisone (Sigma). At day 4 of PHH culture, media was changed and a gut Transwell® barrier containing a mixture of Caco-2/HT-29 cells (ReadyCell) was added to the MPS-TL6 plate. The MPS-TL6 plate was split into three conditions, gut only (no PHH), liver only (no gut barrier, with drug dosed into a blank Transwell with no cells) and gut-liver co-culture. At day 5, 40 µM diclofenac (Sigma) was dissolved in serum free Caco-2/HT-29 media and dosed by adding this media to the apical side of the Transwell.

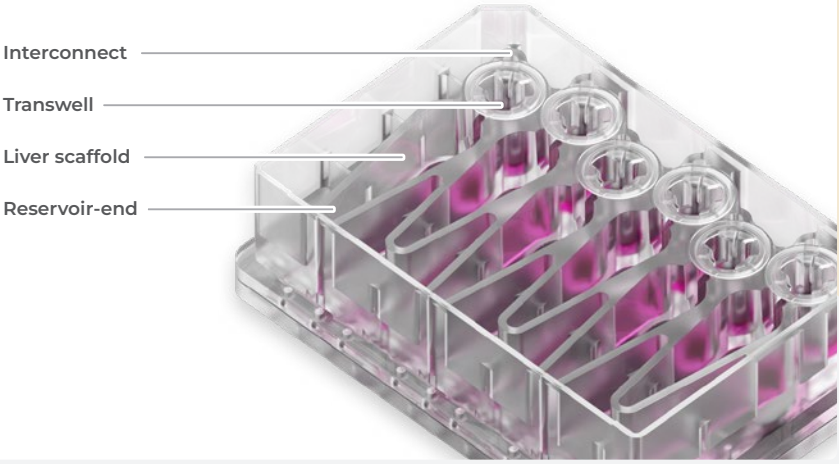
Samples of media were taken at 0, 1, 6, 24, 29 and 48 hours and sent to Sygnature Discovery to perform LC-MS analysis on the concentration of diclofenac and its primary metabolite, 4'-hydroxydiclofenac in the liver compartment.

The metabolic potential of PHH was assessed using a P450-Glo™ CYP3A4 Assay (Promega) at day 4 and 7. Media samples were collected also at day 4 and 7 and a Lactate dehydrogenase (LDH), Urea and Albumin ELISA assays performed. Transepithelial electrical resistance (TEER) was measured to assess barrier stability before and after the drug dosing experiment. TEER was also assessed in control gut Transwells cultured statically in a 24-well plate, in the same apical (caco-2/HT-29 media) and basolateral (liver media) media as the MPS-TL6 experiment.

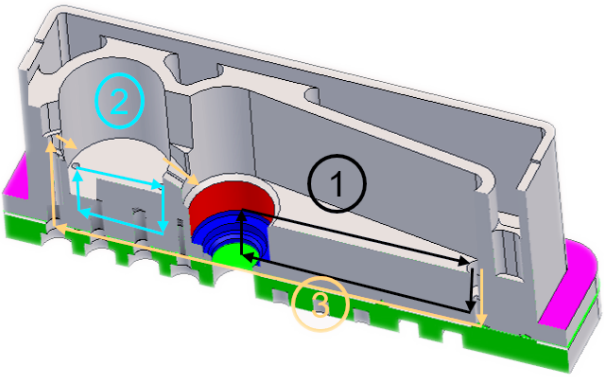
Mathematical modelling was conducted using R programming language with ordinary differential equations (ODE) written to describe the concentration profiles in the MPS-TL6 plate. The package, RxODE was used to simulate the ODEs in R[2]. Diclofenac clearance rates and permeability through a gut Transwell barrier were obtained from literature[3].

Figure 1 – Gut-Liver multi-organ MPS.

A: The *in vitro* MPS model utilises the PhysioMimix Core system. It controls fluid flow independently in the liver MPS and Transwell compartment (gut MPS), and in the interconnecting channel between the two.



B: Image of the MPS-TL6 plate. There are 6 independent culture wells surrounded by reservoir wells that limit liquid loss from evaporation.



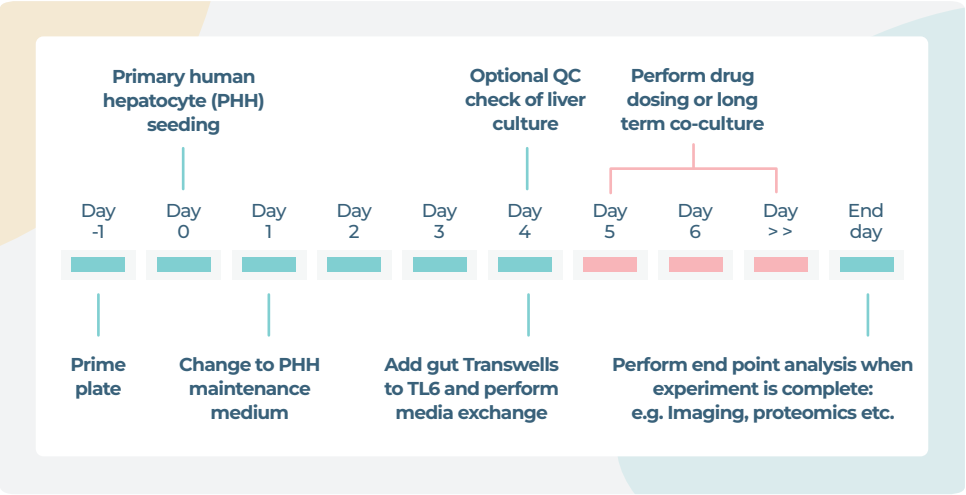
3 active pumps:

1. Recirculating flow through liver scaffold

2. Recirculating flow underside of Transwell®

3. Interconnect flow between liver and Transwell compartments

C: Cross-section of the MPS-TL6 plate showing the fluidic path in each compartment and between compartments.



D: Timeline of a gut-liver MPS-TL6 assay. PHH are seeded into the 3D engineered scaffolds in the liver MPS and medium changed at day 1. At day 4, media samples are taken and QC metric assessed. Gut Transwells are added to the gut MPS and either a drug dosing assay performed, or long-term co-culture undertaken.

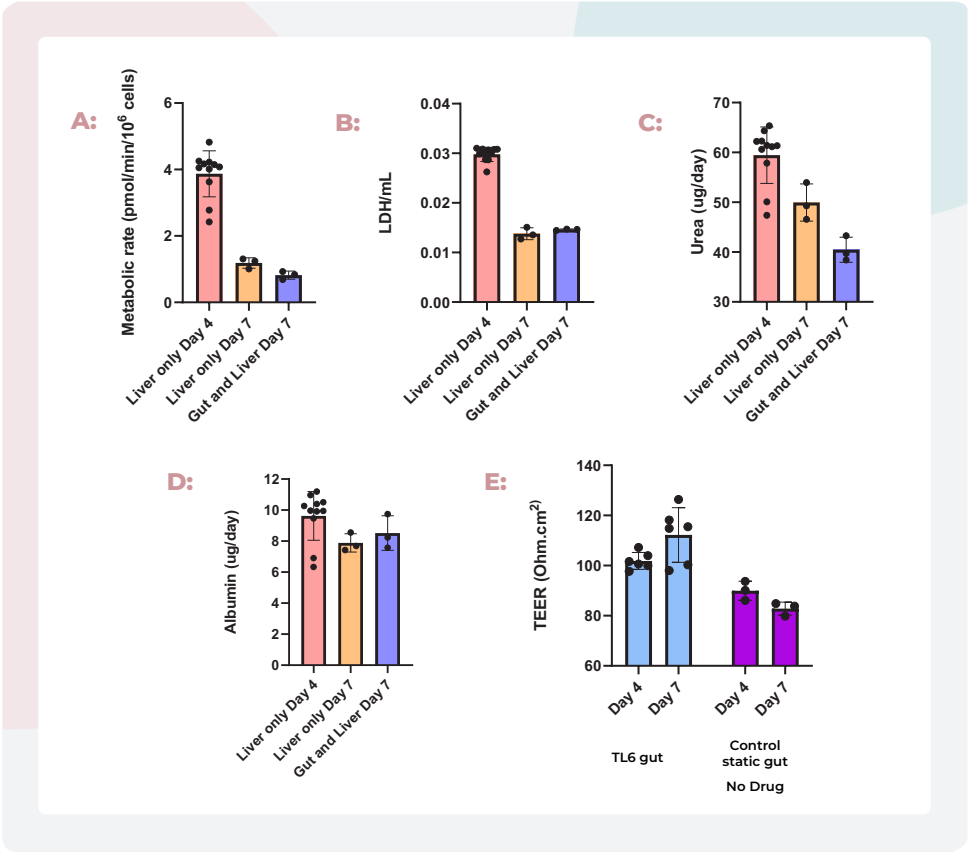


Figure 2 - Liver and gut functionality markers are maintained during culture in MPS-TL6.

Liver functionality markers were assessed at day 4 (liver only) and day 7 (liver MPS only and gut & liver MPS). A) The metabolic capacity was assessed using P450-Glo™ CYP3A4 assay and was maintained in liver MPS throughout. B) Low levels of LDH, a measure of cell toxicity were detected at day 7 in the liver MPS. ELISA assays were conducted to quantify C) Urea and D) Albumin production by PHH and both remained stable throughout the culture. E) The epithelial barrier stability was maintained in gut MPS, assessed by taking TEER measurements before gut Transwells were added to MPS-TL6 (day 4) and at the completion of the study (day 7). Stability was comparable to the control Transwells that were cultured statically, in a 24-well plate with identical media conditions. Data are mean ± SD.

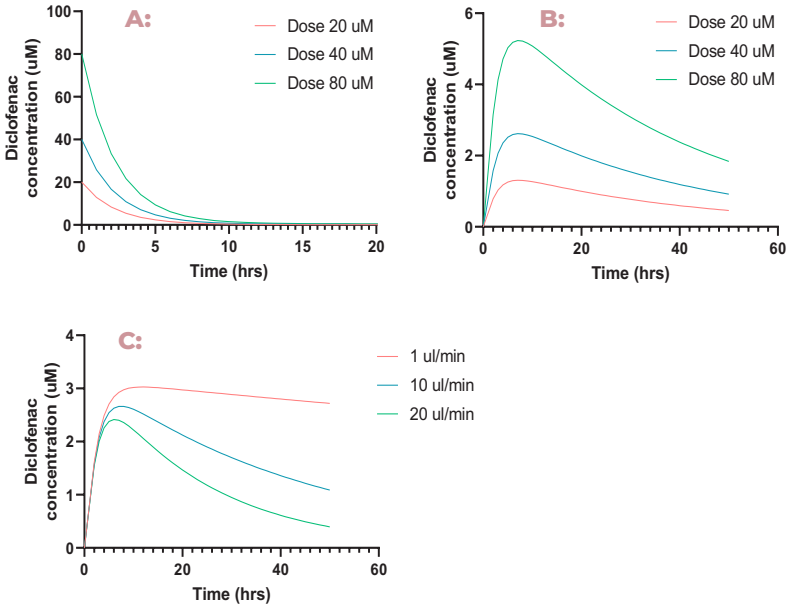


Figure 3 - Mathematical modelling can be used to predict drug clearance in Gut-Liver MPS.

ODEs were written to describe the concentration profiles in the MPS-TL6 plate with gut and liver cultures and simulated using R programming language and the package, RxODE. The concentration of diclofenac in different plate compartments was predicted when dosed with 20, 40, 80 µM in A) the gut Transwell apical compartment and B) the liver compartment. C) The effect of different diclofenac metabolic clearance rates by PHH on the concentration profile in the liver compartment was simulated to validate the model.

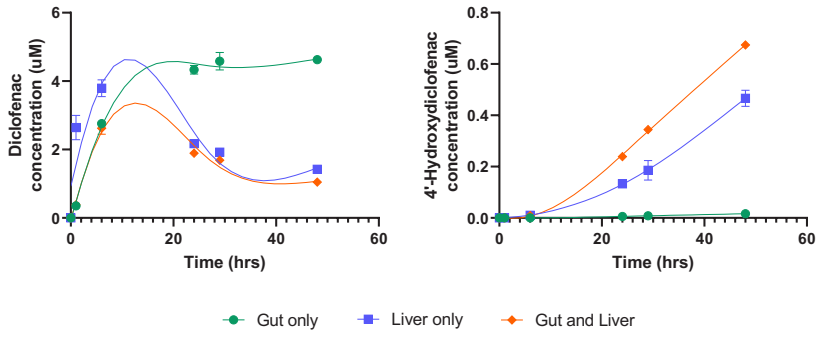


Figure 4 – First-pass metabolism of Diclofenac can be modelled in Gut-Liver MPS

Diclofenac was dosed into the apical compartment of the gut MPS (to mimic oral dosing) in the MPS-TL6 plate and media samples were taken at 0, 1, 6, 24, 29 and 48 hours from the liver MPS and the concentration of A) Diclofenac (parent molecule) and B) its primary metabolite, 4'-hydroxydiclofenac, were quantified by LC-MS. The MPS-TL6 was cultured with the following conditions: gut MPS only (no PHH), liver MPS only (no gut barrier, with drug dosed into a blank Transwell with no cells) and both gut and liver MPS. Time dependent concentrations of both the parent and metabolite were detected in the liver MPS. Data are mean ± SD, from three wells per condition.

Conclusion

We have demonstrated the multi-organ gut-liver MPS-TL6, controlled by the MPS platform PhysioMimix Core System is able to recapitulate the pharmacokinetics of the anti-inflammatory compound diclofenac. PHHs were cultured in 3D engineered scaffolds in the liver MPS before the addition of gut MPS Transwells, which are a mix of intestinal epithelial

and goblet cells that form a barrier. Liver functionality markers CYP3A4, albumin and urea were maintained in the MPS-TL6 during the drug dosing experiment. The integrity of the gut barrier was also maintained evidenced by TEER measurements.

Diclofenac was added to the apical compartment of the gut MPS Transwells where it permeates through the barrier and is primarily metabolized by the liver. We demonstrate the effect of a gut barrier on the bioavailability of diclofenac and subsequent clearance by PHHs. By culturing the tissue models as single and multi-organs in MPS-TL6, we can assess the contribution of metabolite production by both the liver, gut and when combined as a co-culture. Strikingly, the highest levels of metabolite are produced in the co-culture gut-liver MPS which are greater than the sum of the single-organ MPS, suggesting that organ-organ crosstalk promotes tissue functionality.

We have used mathematical modelling to understand the pharmacokinetic behaviour of diclofenac in the MPS-TL6 set up. This was achieved by combining drug specific parameters (e.g., hepatic clearance, gut permeability) with physical characteristics of the plate (e.g., flow rates, compartment volumes). This enables optimisation of experimental parameters such as drug dose and sampling time-point. Mathematical models combined with parameter fitting are essential in the translation of *in vitro* data into relevant ADME parameters that can be used to better predict appropriate drug dosing regimens to be used in human clinical trials.

References

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