Defining validation criteria for a primary jejunum and primary hepatocyte dual-organ MPS: a promising tool for more predictive studies of human drug ADME and oral bioavailability

Poster 249

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1. Abstract

Efforts to improve the *in vitro* to *in vivo* translation of drug efficacy and safety data has led to the emergence of more physiologically-relevant microphysiological systems (MPS) that consist of multiple fluidically linked organs[1]. Here, we combine two established and well-characterized human MPSs, the RepliGut® Jejunum and PhysioMimix® Liver MPS, in an interconnected dual-organ MPS, to create a Gut/Liver system capable of profiling oral bioavailability.

Defining a cell validation criteria is important to ensure donor lots of liver and intestinal cells are functional in co-culture and meet the threshold for regulatory requirements and market adoption[2].

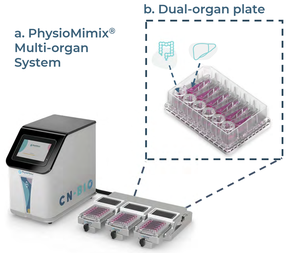
Here, we define cell validation criteria whereby liver and intestinal cells are first validated separately, then as a functional, fluidically coupled co-culture system. We use a test compound to ensure the cells in co-culture are metabolically suitable for ADME studies. Finally, through real-world drug examples we demonstrate how the Gut/Liver MPS can be used to provide a mechanistic understanding of a drug's oral bioavailability *in vitro*.

2. Methods

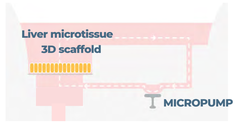
Fig 1. Establishment of the Gut/Liver MPS

a. PhysioMimix® Multi-organ System

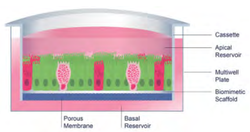
b. Dual-organ plate



c. Liver tissue formed by seeding primary human hepatocytes (PHH)

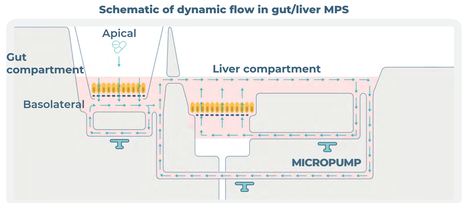


d. RepliGut® Planar-Jejunum: jejunum crypt epithelial stem cells were expanded and differentiated on a biomimetic scaffold



e. The Gut/Liver MPS is established: 4 days post PHH seeding, RepliGut® is added into the gut compartments of Dual-organ plates and fluidically connected to its respective liver compartment

Schematic of dynamic flow in gut/liver MPS

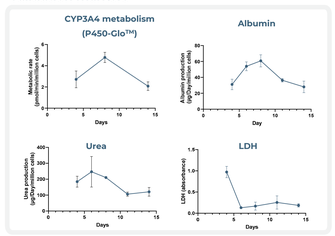


3. Results

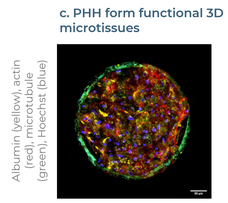
Fig 2. Validation of PHH donor first in a Liver-on-a-chip to confirm metabolically functional tissue.

a. Donor lot validation in a Liver-on-a-chip

b. Liver function and cell health validation markers



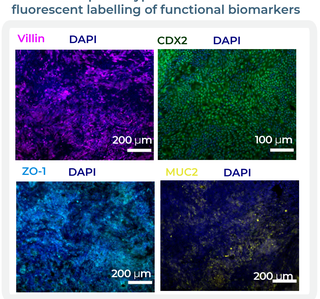
c. PHH form functional 3D microtissues



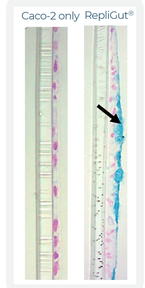
- PHH donors are pre-selected for a Gut/Liver MPS by first validating in a Liver-on-a-chip over a 14-day experiment.
- Functionality is assessed by CYP3A4 activity, albumin and urea production. Lactate dehydrogenase (LDH), a cell health marker, peaks at day 4 after the formation of microtissues, then remains low over the course of the experiment.

Fig3. Validation of RepliGut® donor to confirm tissue functionality and reproducibility.

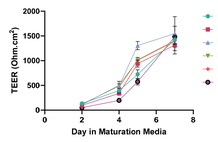
a. Intestinal phenotype confirmed with fluorescent labelling of functional biomarkers



b. Continuous mucus layer (alcian blue stain) with RepliGut®



c. Reproduceable TEER profiles from a single donor and cell lot in static



d. Two cell lots from the same donor show comparable TEER profiles in static

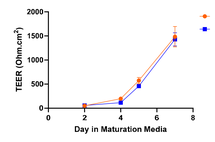


Fig4. PHH functionality is maintained for at least 48 h in co-culture with RepliGut®

a. Liver CYP3A4 metabolism (P450-Glo™)

b. Liver albumin production

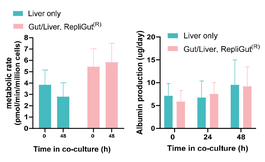


Fig5. Intestinal barrier is maintained in co-culture with liver microtissues

a. Gut barrier Integrity (TEER)

b. Gut permeability to Lucifer Yellow

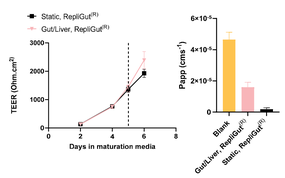
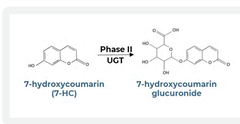


Fig6. Validation with ADME test compound, 7-hydroxycoumarin (7-HC) to confirm intestinal absorption and hepatic metabolism in gut only, liver only and Gut/Liver MPS models.

a. Pathway of 7-HC metabolism



b. 7-HC profiles comparing two lots of the same RepliGut® donor

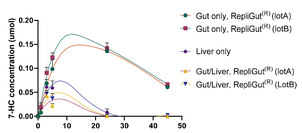


Fig7. Case studies: profiling bioavailability on carboxylesterase (CES) mediated compounds.

a. CES isoenzyme expression is incorrectly expressed by Caco-2 cells[3]

	Human Liver	Human Small intestine	Caco-2
CES1			
CES2			

b. Pattern of CES gene expression in the Jejunum model over time in expansion media (EM) and differentiation media (DM); data courtesy of Scott Magness, UNC Chapel Hill

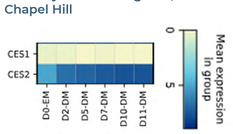


Fig 8. Case study 1, Enalapril: greater resistance to intestinal clearance observed in primary cell Gut/Liver MPS, correlates with isoenzyme expression in the human intestine

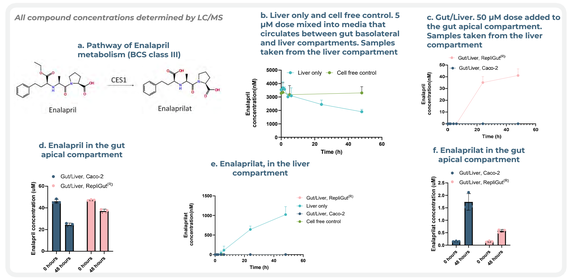
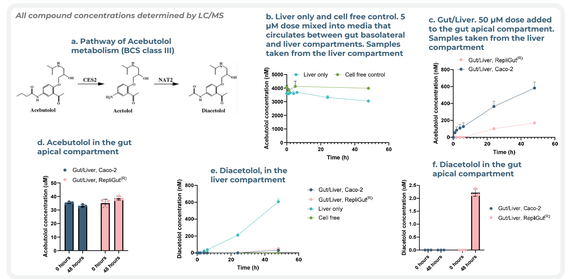


Fig 9. Case study 2, Acebutolol: the combination of intestinal metabolism and low permeability contribute to its low oral bioavailability. This is observed in the primary Gut/Liver MPS and correlates with acebutolol's bioavailability in humans



4. Conclusion

- The Gut/Liver MPS is a pre-clinical assay designed to profile human oral bioavailability *in vitro*.
- We provide validation criteria to ensure donor lots of liver and intestinal cells are functional in co-culture and are metabolically suitable for ADME studies.
- Through real-world drug examples, we demonstrate the assays utility to provide drug developers with a mechanistic understanding of bioavailability *in vitro*, allowing the progression of the most promising drug candidates.

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References

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2. T.K. Baker et al., Drug Metab. Dispos 52, 198-209 (2024).
3. T. Imai, et al, Drug Metab. Dispos. 33, 1185–1190 (2005).

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