Connecting the human intestine and liver: a primary jejunum and primary hepatocyte multi-organ MPS for more predictive studies of human drug ADME and oral bioavailability

CN-BIO

ALTIS BIOSYSTEMS

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Abstract

Traditional immortalized intestinal cell lines and suspension hepatocytes have absent or low levels of metabolic enzyme expression, and thus fail to predict first pass human metabolism and oral bioavailability.

Efforts to improve the *in vitro* to *in vivo* translation of drug efficacy and safety data has led to the emergence of more human relevant microphysiological systems (MPS) that consist of multiple, fluidically linked organs[1]. Here we describe a new MPS that links the jejunum (RepliGut® planar - jejunum) and liver using the PhysioMimix® Multi-organ System, with both cell types being of primary human origin.

To demonstrate improved predictive capacity, we investigated two drugs where current models fail to adequately predict human ADME behaviour. Temocapril, which is a prodrug and is designed to be resistant to intestinal hydrolysis[2] and midazolam, which is known to undergo intestinal clearance[3].
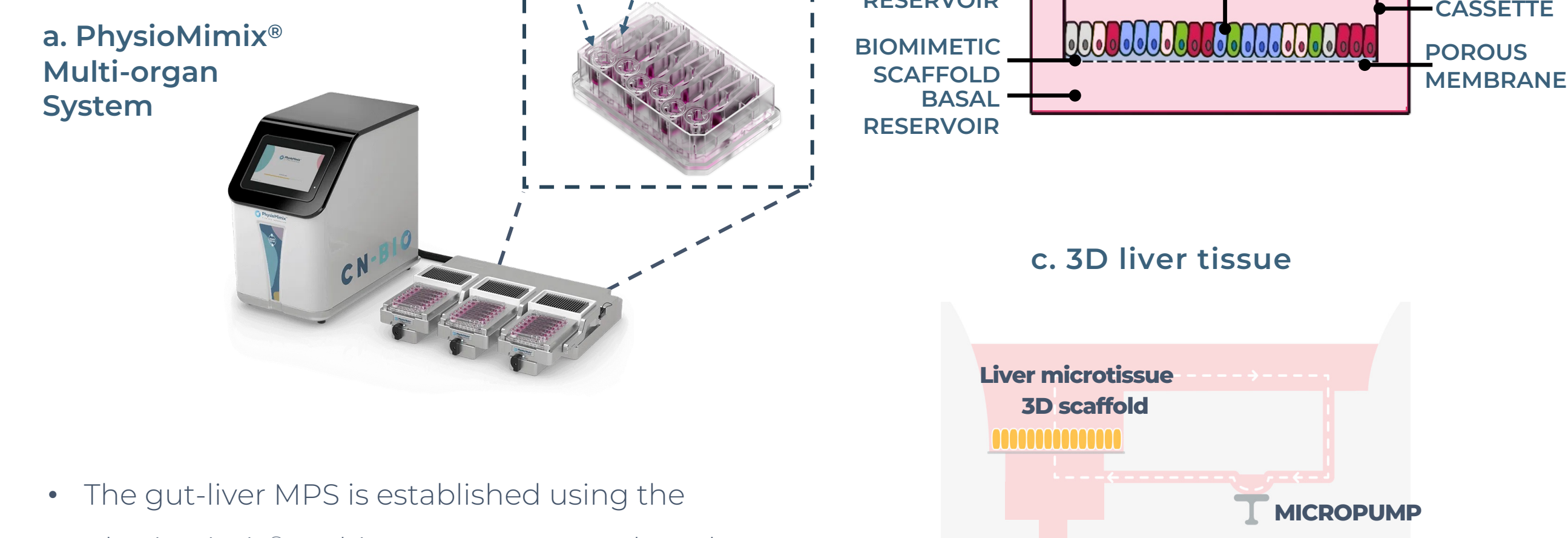
Objectives

The objectives of this study are:

- Develop a gut-liver MPS made up of entirely primary human cells
- Demonstrate the functionality of liver and intestinal tissues
- Deliver a protocol to conduct drug ADME and bioavailability studies
- Demonstrate improved predictive capacity with two drugs where current models fail to predict human ADME behaviour

Methods

Fig 1. Establishment of the gut-liver MPS

a. PhysioMimix® Multi-organ System

b. RepliGut®

EPITHELIAL MONOLAYER
LUMINAL RESERVOIR
BIOMIMETIC SCAFFOLD
BASAL RESERVOIR
CASSETTE
POROUS MEMBRANE

c. 3D liver tissue

Liver microtissue 3D scaffold
MICROPUMP

- The gut-liver MPS is established using the PhysioMimix® Multi-organ System and Dual-organ plate (Figure 1 a).
- Liver microtissues are formed by seeding primary human hepatocytes (PHH) on a 3D collagen coated scaffold (Figure 1 c).
- For the intestinal barrier, jejunum crypt epithelial stem cells were expanded and differentiated on a biomimetic scaffold, in static conditions (RepliGut®, Figure 1 b).
- 4 days post PHH seeding, RepliGut® is added into the gut compartments of Dual-organ plates and fluidically connected to its respective liver compartment. (Figure 2). ADME studies begin.

Fig 2. ADME Study

Drug dosed (temocapril= 100 μM, midazolam= 50 μM) in the gut apical compartment. After transport through the barrier, the drug is mixed into the gut basolateral and liver compartments

Gut compartment
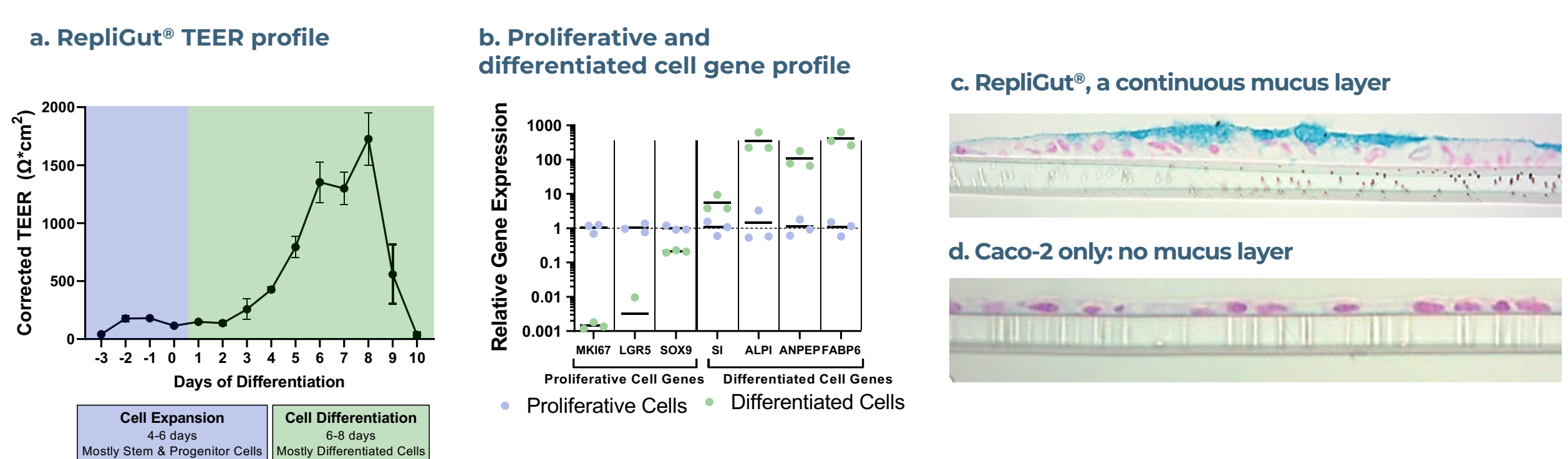Liver compartment
MICROPUMP
MICROPUMP
MICROPUMP
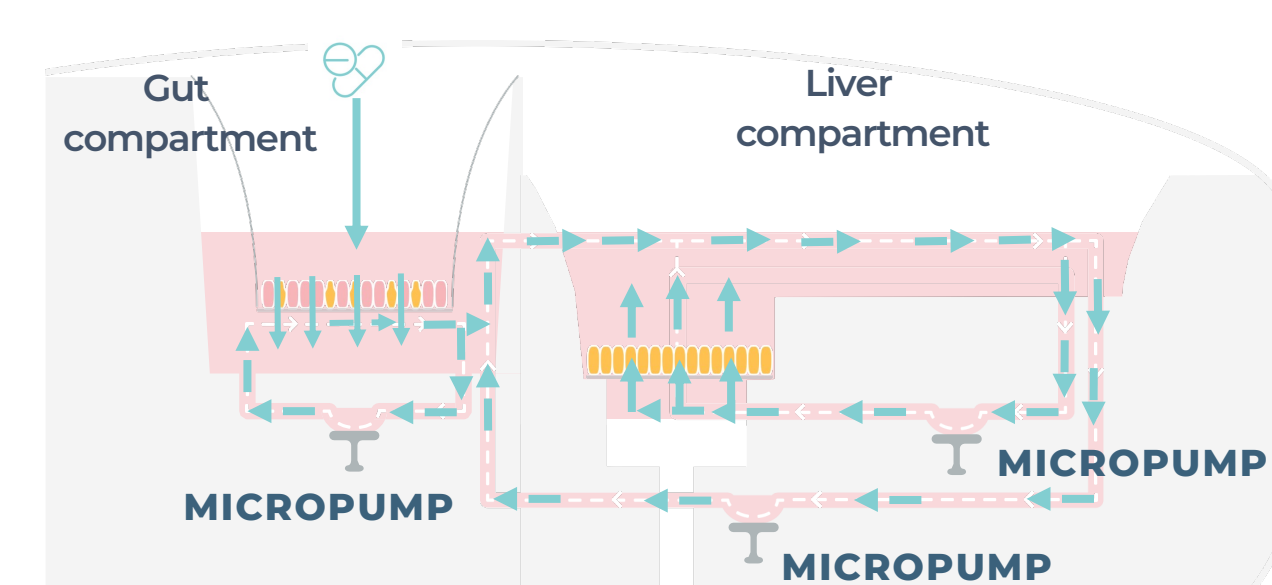
Conclusion

The gut-liver MPS recapitulates the physiological conditions of oral drug dosing in the human (Figure 3-5). Made up entirely of primary human cells, this model enables for more predictive ADME and bioavailability studies compared to standard *in vitro* models (Figure 7).

This human relevant gut-liver model offers a vast improvement in the methods used to study the pharmacokinetics of prodrugs that undergo CES metabolism, such as temocapril (Figure 6).
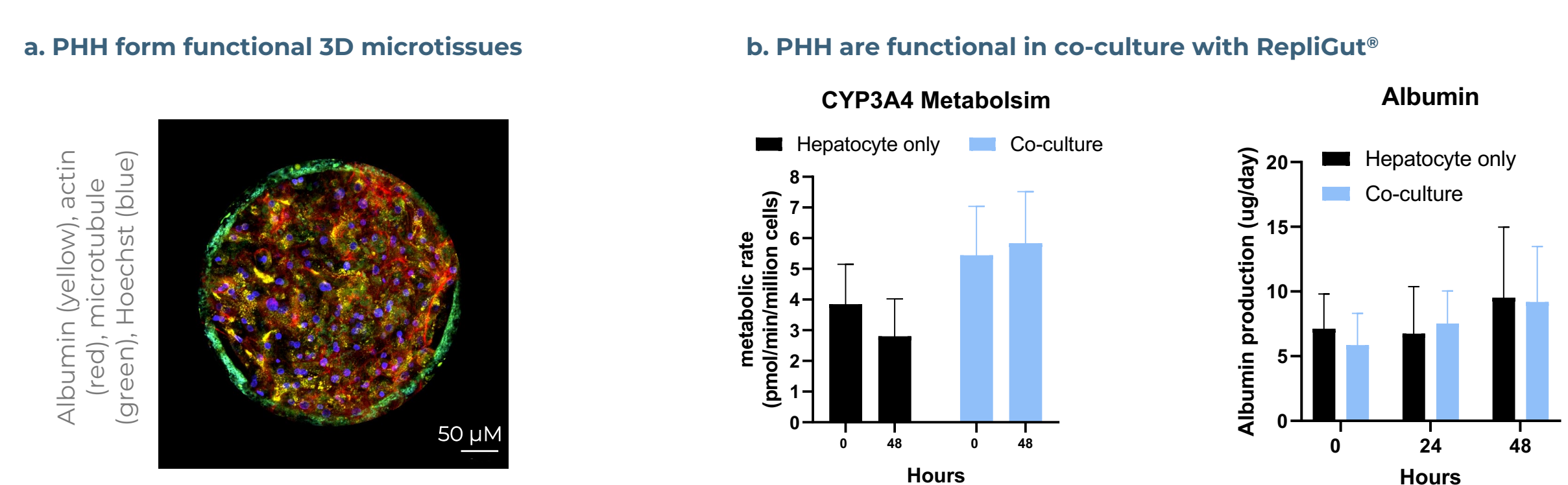
Results

Figure 3. RepliGut® exhibits distinct proliferative and differentiated cell populations and a continuous layer of mucus on its apical surface
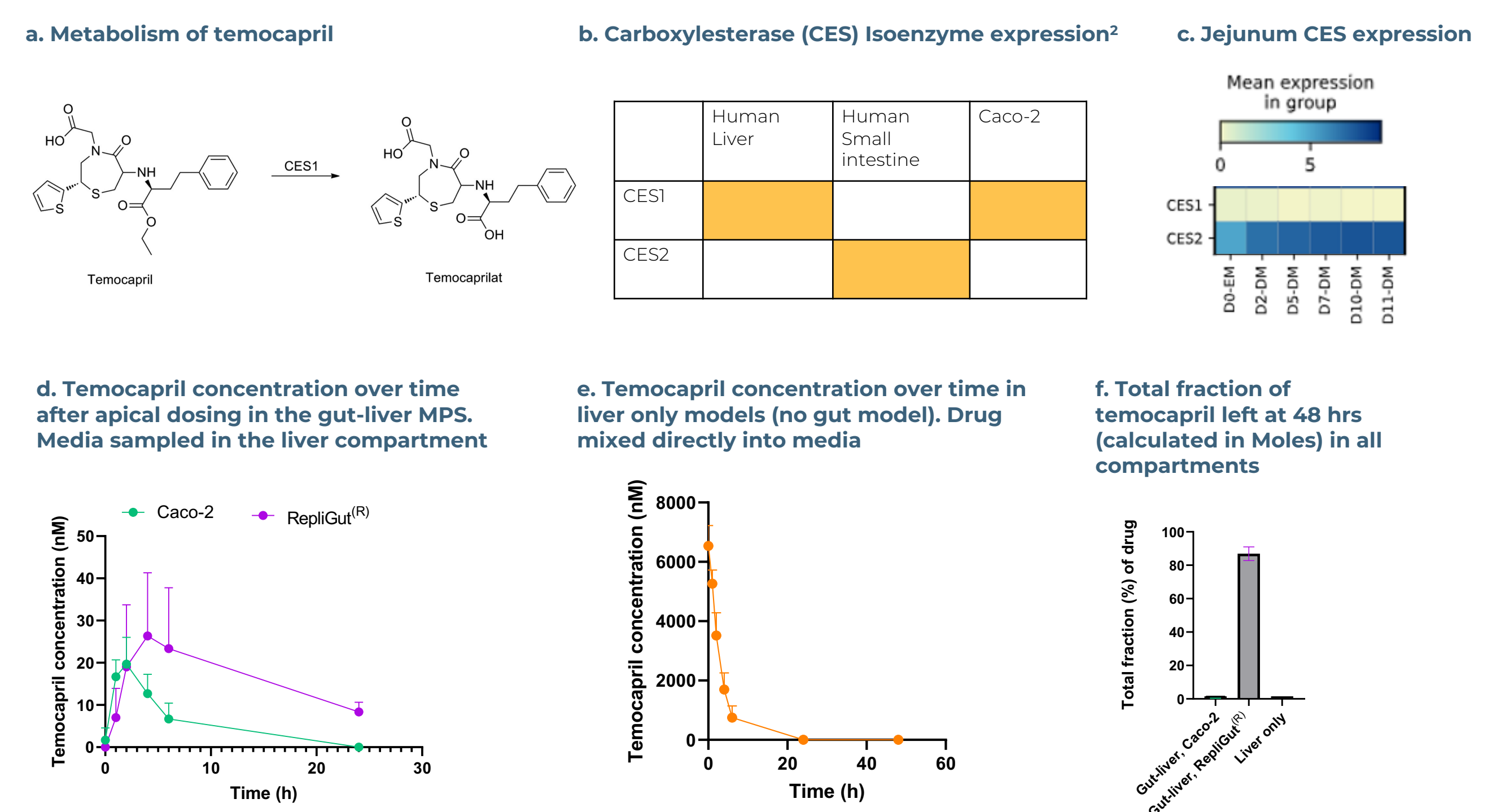

a. RepliGut® TEER profile
b. Proliferative and differentiated cell gene profile
c. RepliGut®, a continuous mucus layer
d. Caco-2 only: no mucus layer

Figure 4. Improved metabolic and transporter gene expression with primary gut cells vs caco-2 cell line

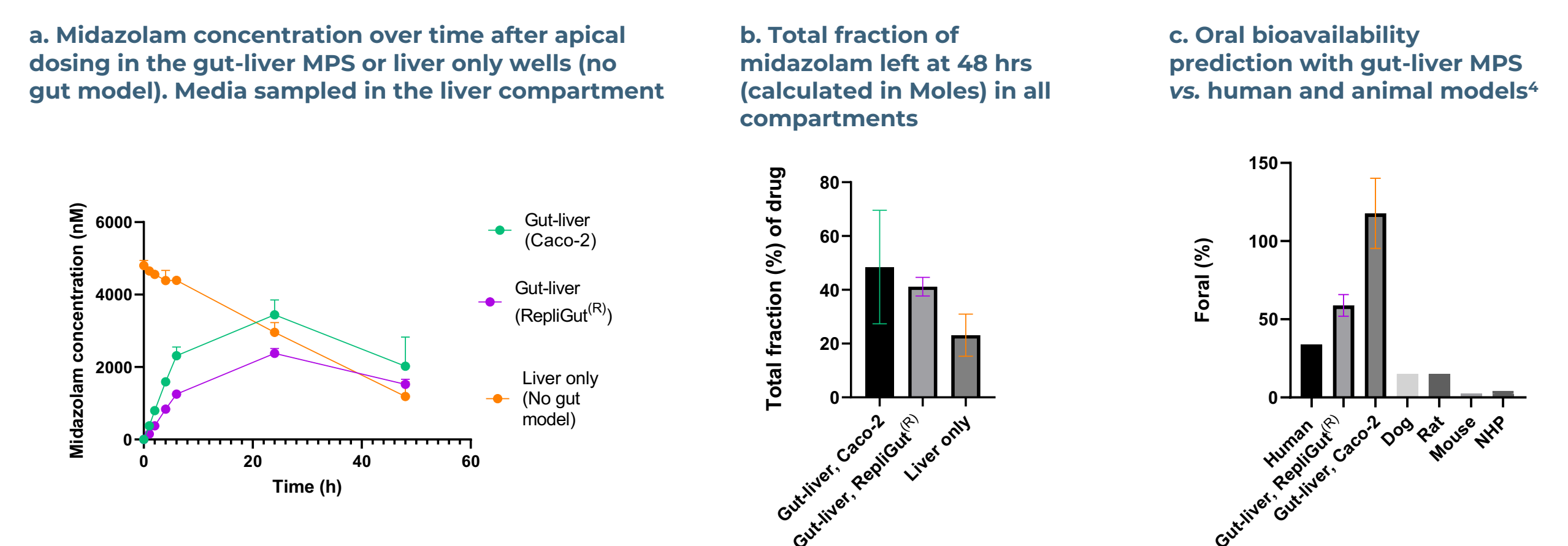CYP3A4 | SLC15A (PEPT1) | CYP2C19 | ABCB1 (PG-P) | ABCG2 (BCRP) | UGT1A1-1A10
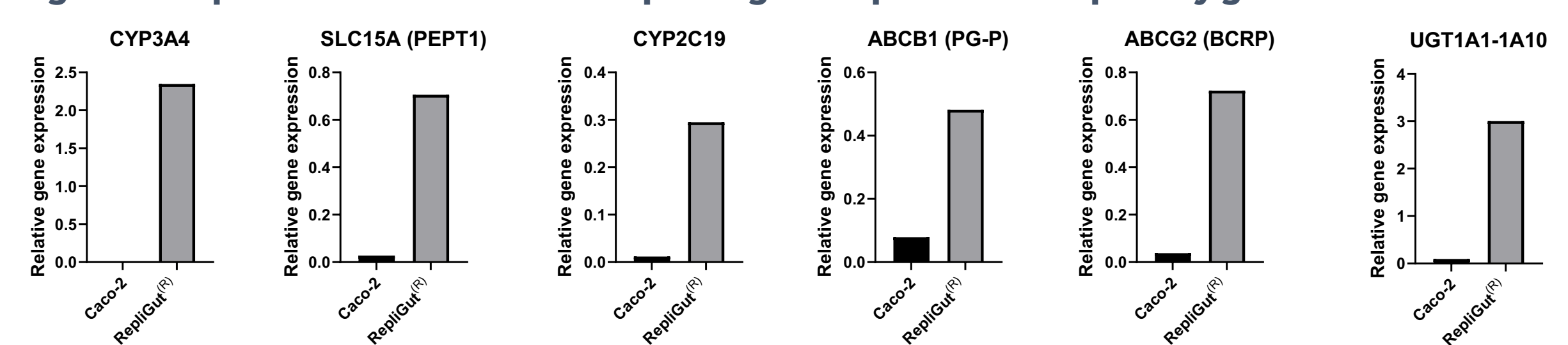
Figure 5. 3D liver tissues are functional and metabolically active in both liver only and gut-liver co-culture.

a. PHH form functional 3D microtissues

Albumin (yellow), actin (red), microtubule (green), Hoechst (blue)
50 μM

b. PHH are functional in co-culture with RepliGut®

CYP3A4 Metabolism
Albumin

Figure 6. Clearance of temocapril correlates with human isoenzyme expression in the gut-liver MPS with primary cells only

a. Metabolism of temocapril

b. Carboxylesterase (CES) Isoenzyme expression[2]

	Human Liver	Human Small Intestine	Caco-2
CES1			
CES2			

c. Jejunum CES expression

Mean expression in group

d. Temocapril concentration over time after apical dosing in the gut-liver MPS. Media sampled in the liver compartment

e. Temocapril concentration over time in liver only models (no gut model). Drug mixed directly into media

f. Total fraction of temocapril left at 48 hrs (calculated in Moles) in all compartments

Figure 7. Improved bioavailability prediction of midazolam in the gut-liver MPS with primary cells

a. Midazolam concentration over time after apical dosing in the gut-liver MPS or liver only wells (no gut model). Media sampled in the liver compartment

b. Total fraction of midazolam left at 48 hrs (calculated in Moles) in all compartments

c. Oral bioavailability prediction with gut-liver MPS vs. human and animal models[4]

References

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