NEXT LEVEL DRUG RESEARCH IN AN EX VIVO TISSUE GUT-ON-A-CHIP MODEL: ADVANCED APPLICATIONS OF THE INTESTINAL EXPLANT BARRIER CHIP

#2P-245

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INTRODUCTION

- The Intestinal Explant Barrier Chip (IEBC) is a gut-on-a-chip model with the unique property of integrating small *ex vivo* intestinal tissue explants between two microchannels.
- Intestinal permeability of small molecule drugs can be studied in the IEBC for 24 hours. Showing good rank order relationship with the *in vivo* fraction absorbed [1].

***Figure** 1. Intestinal Explant Barrier Chip (IEBC) and laboratory set-up for multiple IEBCs.*

AIM

- To study advanced research questions in the field of:
  1. Drug absorption & metabolism.
  2. Host-microbe (immune) interaction

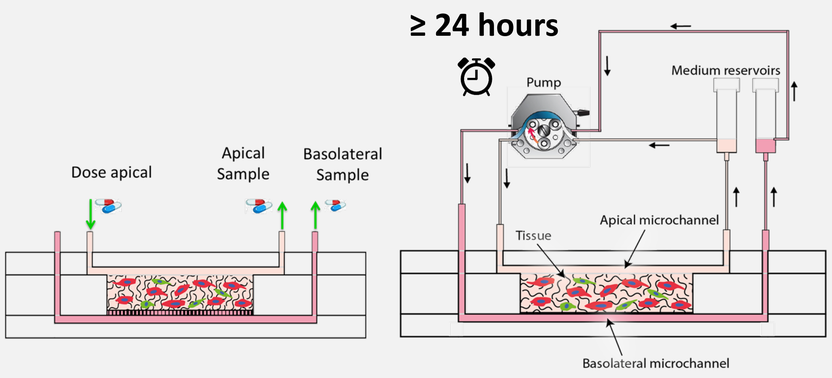

***Figure 2**. Schematic representation of the microfluidic setup and sampling possibilities in the IEBC. Recirculating flow rate: 2 mL/hr. Apical administration of drugs or fecal water containing microbial excretion products [2].*

METHODS

- Human jejunum, ileum or colon tissue explants were fixed in the IEBC and apically exposed to drugs dissolved in standard cell culture medium or in fecal water containing microbial excretion products (iscreen technology [2]) for 24 hours.
  - Phase I and phase II metabolism using coumarin and 7-OH coumarin
  - SCFA enriched iscreen spent medium
- Addition of aerobic-anaerobic interface to study host-microbe interactions in a more physiological environment.

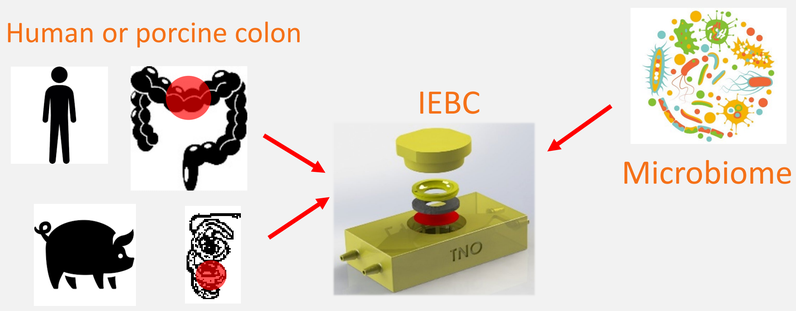

***Figure 3**. Colon tissue of human or porcine origin is added in the IEBC. In host-microbe interaction studies microbiome is added at the apical side of the tissue.*

REFERENCES

[1] *Eslami Amirabadi H. & Donkers J.M. et al., Lab Chip (2022) 22: 326-342*
[2] *Fehlbaum et al., Int. J. Mol. Sci. (2018) 19(10): 3097*

RESULTS

Phase I and Phase II metabolism

- Coumarin was predominantly hydroxylated to 7-OH coumarin (via CYP2A6) at the apical side of the intestinal tissue.
- 7-OH coumarin was metabolized further into 7-OH coumarin glucuronide and 7-OH coumarin sulfate via phase II conjugating enzymes and appeared basolateral at high rates.
- Effective and preserved phase 1 and 2 metabolism was also shown for midazolam, diclofenac, testosterone, and irinotecan.

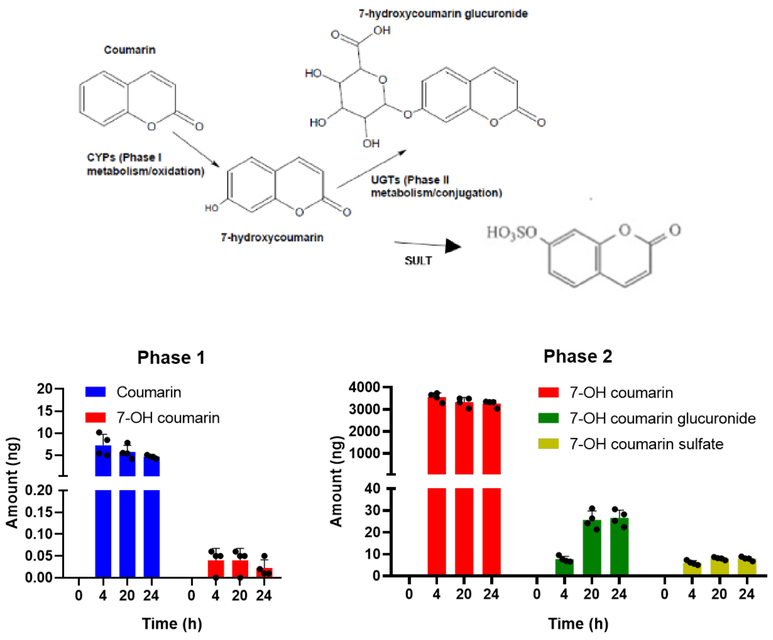

***Figure 4.** Human jejunum tissue explants were mounted in the IEBC and exposed to coumarin (3 µM) or 7-OH coumarin (333 µM) for 24 hours (n = 4). Parent compound and metabolites were measured by LC-MS/MS. Data are presented as mean + SD.*

***Table 1.** Transport rates of coumarin and its metabolites from the apical to basolateral side of the tissue*

Compound	Papp x 10^{-6} cm/s (3-24 h)
Coumarin	n.a.
7-OH coumarin	1.9 ± 0.6
7-OH coumarin glucuronide	41.1 ± 5.0
7-OH coumarin sulfate	24.6 ± 3.8

***Table 2.** Overview of metabolizing enzymes assessed in IEBC (and in InTESTine™)*

Metabolism	Parent compound	Measured metabolites	Enzymes involved
Phase I	Midazolam	1-OH midazolam, 4-OH midazolam	CYP3A4
	Diclofenac	4-OH diclofenac	CYP2C9
	Testosterone	6β-OH testosterone, androstenedione	CYP3A4/17β hydroxysteroid dehydrogenase
	Coumarin	7-OH coumarin	CYP2A6
Phase II	Irinotecan	SN38	CES2
	7-OH coumarin	7-OH coumarin glucuronide, 7-OH coumarin sulfate	UGTs, SULTs

RESULTS

Host-microbe interactions

- Inulin-treatment of microbiome stimulated short chain fatty acid (SCFA) production.
- Increased SCFA levels in i-screen spent medium improved tissue barrier integrity:
  - 1.7-3.5 fold reduction in FITC-dextran 4000 (FD4) leakage.
  - 1.4-2.1 lower transcellular and paracellular transport of antipyrine and atenolol, respectively.
- Furthermore, the release of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8 was (significantly) reduced.

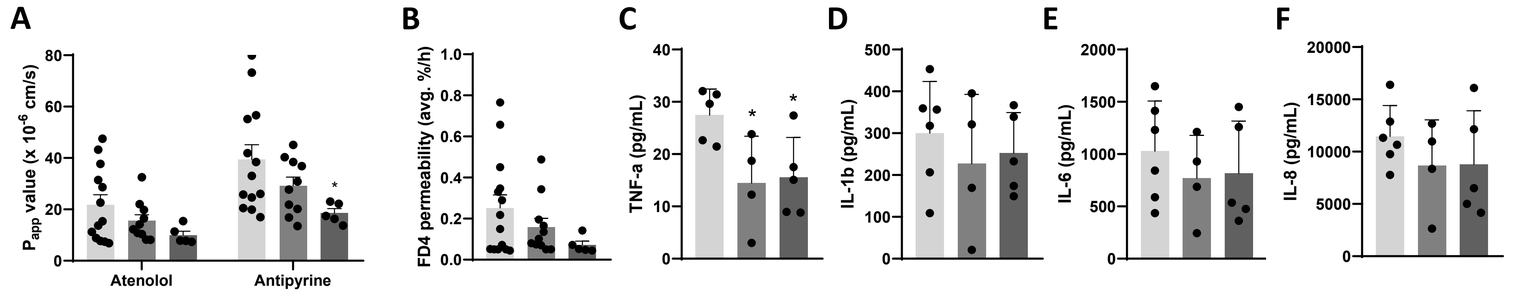

***Figure 5.** Human colon tissue explants in the IEBC were exposed to spent medium from i-screen (untreated control, inulin-treated, and untreated control with added SCFA) for 24 hours (n = 5–13/group. A) The average apparent permeability (Papp) of atenolol (10 µM) and antipyrine (10 µM) was calculated between 20-24 hours. B) Average FD4 permeability between 20-24 hours. C-F) TNF-α, IL-1ß, IL-6, and IL-8 secretion into the basolateral compartment after 24h*

- An aerobic-anaerobic interface was developed to co-culture living microbiome with *ex vivo* tissue explants (**patent WO2022055345** filed)

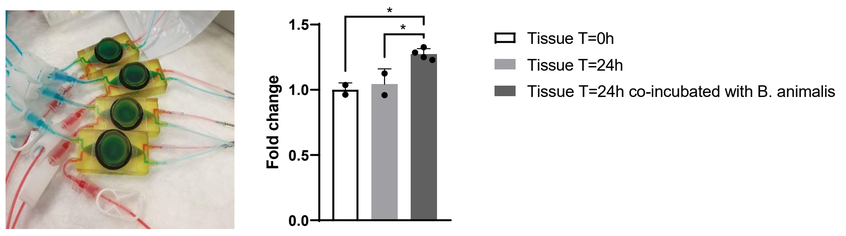

***Figure 6.** Aerobic-anaerobic interface of the IEBC. Strict anaerobic Bifidobacterium Animalis was co-cultured with porcine colon tissue. Attachment of B. animals to the tissue was demonstrated by qPCR.*

CONCLUSIONS

- The IEBC can successfully answer advanced drug absorption and metabolism questions.
- The IEBC is and advanced physiological relevant *in vitro* model to study and understand host-microbe effects on tissue functionality and local inflammation in the human intestine.