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GUT ON-A-CHIP: TISSUE BASED HUMAN INTESTINAL BARRIER MODEL FOR STUDYING HOST MICROBE-IMMUNE RESPONSES

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INTRODUCTION

The majority of screening and predictive models do not reflect the physiology of the human intestinal tract since they show major limitations to include the processes that determine the oral bioavailability[1,2]. A major drawback of current intestinal models is the use of single cell lines and the static environment, which is in contrast with the dynamic processes *in vivo*[2]. Here we overcome these shortcomings by combining *ex vivo* models and organ on a chip technology[3-5].

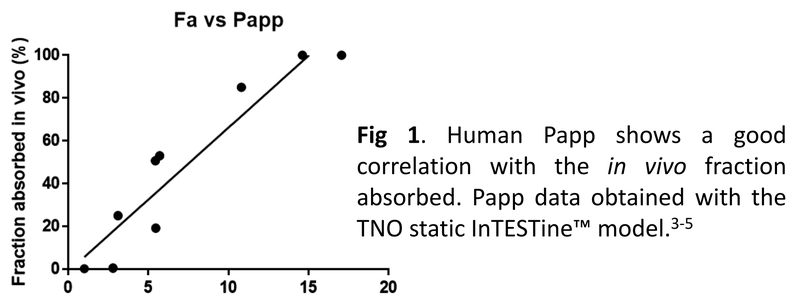


Fig 1. Human Papp shows a good correlation with the *in vivo* fraction absorbed. Papp data obtained with the TNO static InTESTine™ model.[3-5]

GOAL

The goal of this project was to use a 3D printed organ-on-a-chip device, developed at TNO, that integrates small sizes of intestinal tissues between two microchannels, allowing us to study processes that determine (human) intestinal permeability as well as (anaerobic) host-microbe-immune responses.

APPROACH

In this study, we developed a microfluidic chip (Fig 2(A)) that:

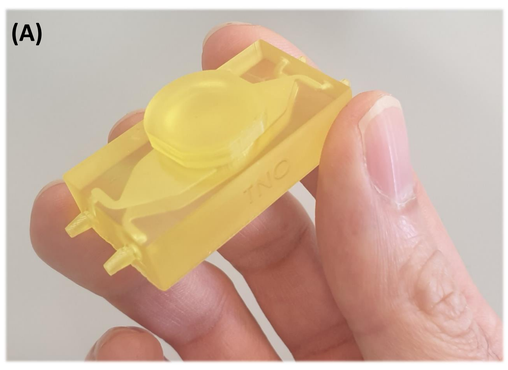
- ✓ integrates **small sizes** of fresh **intestinal tissue**,
- ✓ uses **standard** mechanisms and protocols to **increase** the **throughput**,
- ✓ has very **low/no drug adsorption** (Table 1), and
- ✓ can be **opened** and **closed** on demand (**resealable**).

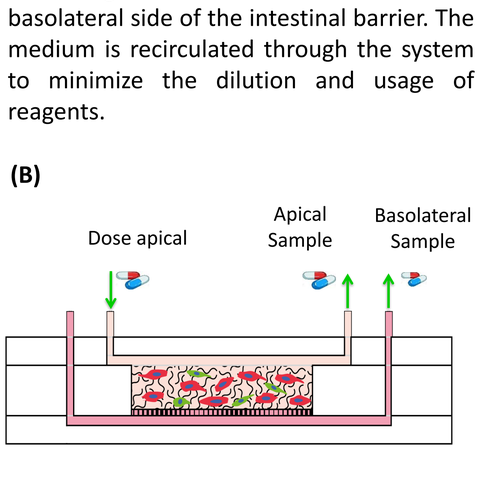
Table 1. Adsorption of model drugs inside the inTESTine Chip. The compounds were introduced from the inlets of the system and retrieved from the outlets. The inTESTine Chip has a very low adsorption of the compound.

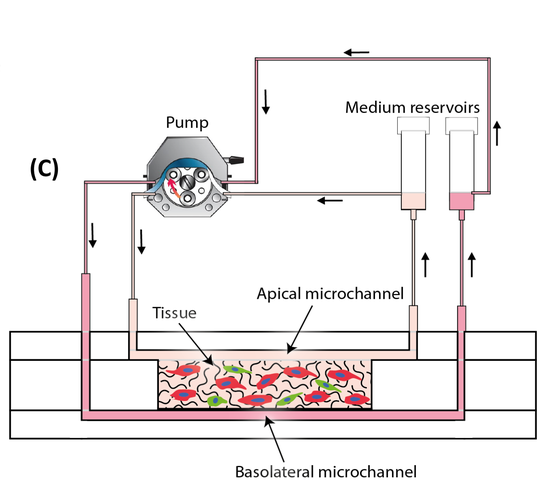
Drug	Recovery
Atenolol	100%
Antipyrine	85%
Caffeine	95%
Mannitol	95%
Digoxin	95%
Acyclovir	100%

The microfluidic chip has 2 compartments, one representing the lumen of the intestine and the other the basolateral side (Fig 2(B)).

Fig 2. **(A)** 3D printed inTESTine Chip that can integrate small samples of intestinal biopsies (5.6 mm in diameter) between two microfluidic channels. The chip can be opened and closed easily to insert or retrieve tissues or cells repetitively.
(B) Schematic of the dosing and measurement method in the inTESTine Chip. Markers for tissue integrity and absorption were added to the apical (luminal) side and measured in both apical and basolateral outlets.
(C) Schematic of the fluidic system. It consists of two microfluidic circuits, one mimicking the lumen and the other the basolateral side of the intestinal barrier. The medium is recirculated through the system to minimize the dilution and usage of reagents.







METHOD

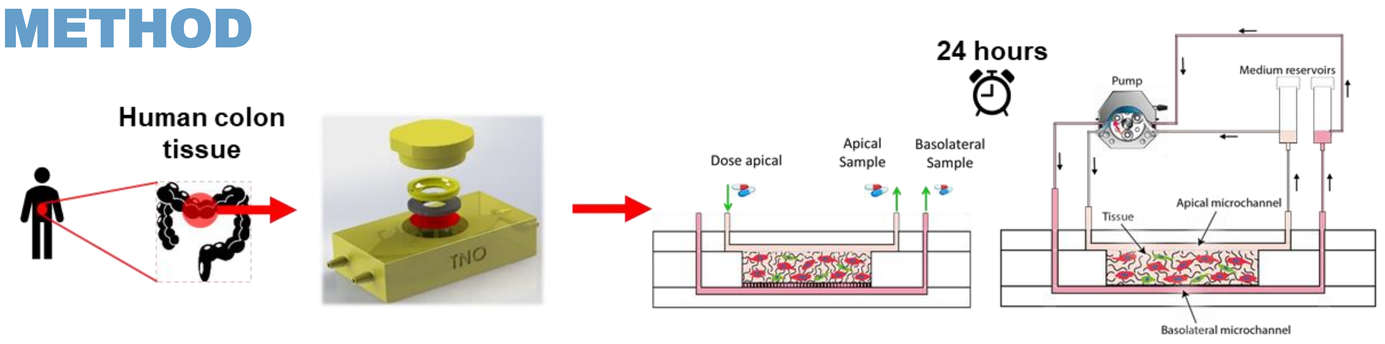


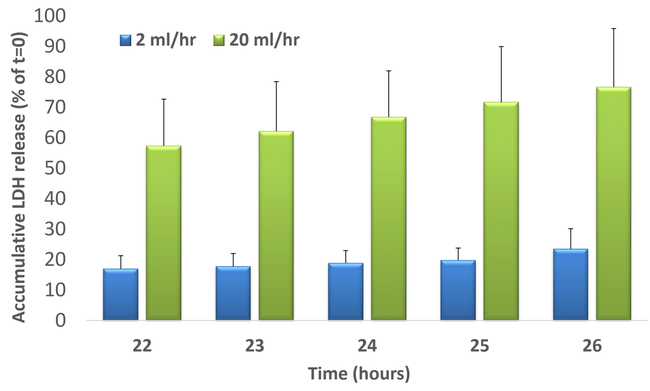
Fig 3. Schematic of the experimental design used in this study. We inserted human intestinal tissue (colon) inside the microfluidic chip, maintained it by constant perfusion for 26 hours at two different flow rates (2 and 20 ml/hr).

RESULTS

Human colon tissue was applied in the InTESTine Chip and studied for 26 h (Figure 3). Fluorescent dextran was added to the apical microchannel and the amount of the dextran in the basolateral microchannel in time was measured. The results showed very small amount of dextran leaks through the tissue (<0.1 %/h).

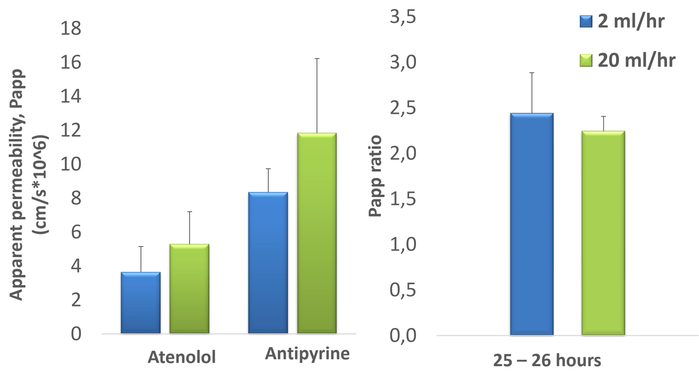
Tissue viability

Fig 4. Release of Lactate dehydrogenase (LDH) as a measure for the viability of the tissue expressed in percentage of the initial LDH content in the tissue. Although higher flow rate shows a clear sharp increase in LDH release, the level of intracellular LDH remains the same in both conditions (around 150% of the initial intracellular LDH). The experiments were performed at two different flow rates, i.e. 2 ml/hr and 20 ml/hr; N=3.



Tissue permeability

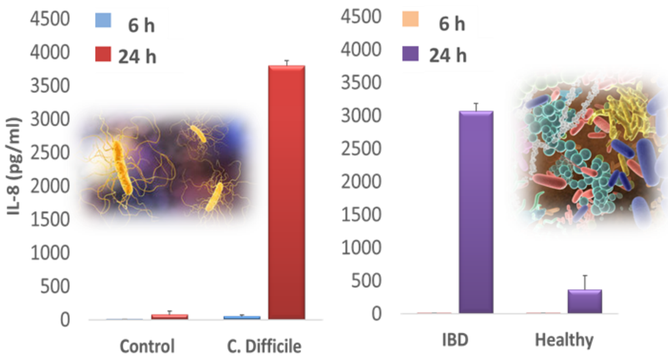
Fig 5. Permeability of Atenolol and Antipyrine through the tissue and their ratio between 25 h and 26 h. Atenolol can only diffuse through the space between the cells whereas antipyrine can be actively transported by the cells. Although slightly increased transport was observed under high flow conditions, the ratio stayed at the same level and were comparable to the beginning of the experiment (data not shown). The experiments were performed at two different flow rates, i.e. 2 ml/hr and 20 ml/hr; N=3.



Induced immune response

In order to induce inflammatory response, human colon tissue in the InTESTine Chip was exposed to fecal water of Inflammatory Bowel Disease (IBD) patients (pool of 10 IBD patients) or Clostridium Difficile toxin (10 µg/mL).

Fig 6. Secretion of IL-8 in the basolateral side of tissue after 6 and 24 hours incubated with healthy or IBD microbial supernatant or Cl. Difficile toxin. A 10-fold increase in the immune response was observed when the tissue was maintained with IBD microbial medium compared to healthy, comparable to the inflammation we observe when human colon tissue was exposed to Cl. Difficile toxin. Flow of 2 ml/hr was used for this experiment; N=4 (N=2 for C. Difficile).



CONCLUSIONS / NEXT STEPS:

- **We have developed a novel microfluidic platform with an enhanced throughput to be able to study (drug) absorption, and impact of drugs, nutrition and microbial environment on gut health**
- **InTESTine Chip can be applied to human intestinal tissue biopsies with remained tissue viability and functionality for 26 hours**
- **We are currently using the InTESTine Chip to study in combination with microbial components to study the influence of host-microbe interactions on drug absorption and immune response**

[1] Hur, Sun Jin, et al. "In vitro human digestion models for food applications." Food Chemistry 125.1 (2011): 1-12.
[2] Guerra, Aurélie, et al. Trends in biotechnology 30.11 (2012): 591-600
[3] Westerhout et al., Eur J Pharm Sci. 2014 Oct 15;63:167-77
[4] Stevens et al. Eur J Pharm Sci. 2019 Sep1; 137:104989
[5] Vaessen et al., Drug Metab Dispos. 2017 Apr;45(4):353-360.