Human ex vivo model to study intestinal processes and microbiome induced metabolism

The innovation for life

Selection of drug

groups

andidates effective for

specific group of patients

Drug repositioning of

previously failed drugs

Improved design of clinical trial by pre-selecting patient

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Introduction

The majority of the preclinical intestinal screening models do not properly reflect the complex physiology of the human intestinal tract, resulting in low translational value to the clinical situation. A major drawback of the current intestinal models is the use of single cell lines and the

Sustained functionality of human intestinal tissue in microfluidic model



static environment, which is in contrast with the dynamic processes in vivo.

Goal

We aim to develop a physiological in vitro human intestinal model that can be used to study (drug) absorption and impact of drugs, nutrition and microbial environment on gut health. The ultimate goal is to develop a population-on-a-chip model to aid in the development of precision medicines by targeting patient variability using intestinal tissue and microbiota from various individuals reflecting populational variation.

Fig 1. Improved functionality of human intestinal tissue. A) Intracellular LDH content showing the addition of an apical and basolateral flow significantly extends the viability of the tissue. **B**) Integrity of intestinal barrier determined by the leakage of FD4 in each incubation. Data represent the mean ± SD (n=4). C) Viability of human intestinal tissue (ileum) before (A) and after (B) 24h incubation in microfluidic gut on-a-a chip model shown by H&E staining.

Representative functions of human intestinal tissue

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Fig 2. Demonstrating representative functions of human intestinal tissue in vitro gut on-a-chip model. A,B) Metabolic activity of human intestinal tissue (ileum) as demonstrated by linear rate of testosterone metabolism. C) Cytokine response of human intestinal tissue under static control and stimulated with flagellin (5h) and microfluidic conditions of control and after stimulation with flagellin for 24h) **D**) lleum tissue, stimulated with LPS showing increase in IL-6 response and the suppressing effect of different probiotics (n=4) All data represent the mean ± SD (n=4



POPULATION ON-A-CHIP

Population on-a-chip

stem cell technology to select the right candidate drugs for patient groups

microbiota





Approach

TNO has developed two ex vivo predictive models, the InTESTine-on-a-chip model in which chronic drug exposure effects can be studied using a (dual flow) microfluidic device, and the I-screen platform which is a screening multi-well platform simulating the human colonic microbiota conditions to study microbiota induced metabolic transformation of drugs using pooled human colonic microbiota under fully anaerobic conditions.

Results

First steps in development of the **gut on-a-chip model**:

Metabolism of drugs by intestinal microbiota

Compound	Molecular Transformation	Formed Metabolite
Risperidone	N-O reduction	Imine intermediate → Reduced metabolite, complete conversion
Sulindac	S-oxide reduction	Deoxysulindac, complete conversion
Sulfinpyrazone	S-oxide reduction	Deoxysulfinpyrazone
Nizatidine	N-O reduction	Deoxynizatidine
Sulfasalazine	Reduction	Reductively cleaved metabolite of sulfasalazine
Metronidazole	Decline of parent	No metabolites detectable
Omeprazole Simvastatin		
Levodopa Acetaminophen	No decline	of parent, no metabolites detectable
Zonisamide		
Dapsone		



- Sustained functionality of human intestinal tissue mounted within microfluidic model (Fig. 1)
- Representative functions of *ex vivo* human intestinal tissue when mounted in gut on-a-chip model (Fig. 2)

Demonstrating the microbiota-based drug metabolism using the **I-Screen model**

- Among the 12 drugs examined, five were demonstrated to generate metabolites. All metabolic transformations were known to occur in human *in vivo* (Fig. 3)
- Able to culture fecal samples and showing ulletdifferences in microbiota composition of different age groups (Fig. 3)

0% A01 A02 A03 A05 A06 A07 A08 B11 B12 B13 B15 B16 B17	B18 B19 C21 C22 C23 C24 C25	C26 C27 C28 C29 C30 D31 D32	D33 D34 D35 D37 D38 D39 E41	E42 E43 E44 E45 E46 E47 E48 E49
Bifidobacterium Blautia	■ Faecalibacterium	Prevotella	Bacteroides	unclassified_Lachnospiraceae
Roseburia Streptococcus	Escherichia/Shigella	unclassified_Ruminococcaceae	Ruminococcus	Anaerostipes
unclassified_Clostridiales Akkermansia	■ Dorea	Clostridium_XI	Veillonella	Clostridium_sensu_stricto
Coprococcus Ruminococcus2	Collinsella	Dialister	unclassified_Firmicutes	Staphylococcus
Clostridium_XIVa Gemmiger	III Clostridium_IV	Enterobacter	Methanobrevibacter	Alistipes
Barnesiella Megamonas	Clostridium_XVIII	Enterococcus	unclassified_Erysipelotrichaceae	Sporobacter
Oscillibacter Lachnospira	unclassified_Acidaminococcaceae	unclassified_Coriobacteriaceae	Parabacteroides	Turicibacter
Paraprevotella unclassified_Bacteria	Butyricicoccus	Sutterella	Phascolarctobacterium	Subdoligranulum
unclassified_Porphyromonadaceae	unclassified_Enterobacteriaceae	Methanosphaera	Bilophila	Odoribacter
Alloprevotella Haemophilus	■ Lactobacillus	Desulfovibrio	Parasutterella	Clostridium_XIVb
Flavonifractor Butyricimonas	Enterorhabdus	Salmonella	Halomonas	Phyllobacterium
unclassified_Bacteroidales unclassified_Clostridiales_Incertae_Sedis_XII	■ Howardella	Eggerthella	Peptococcus	Lactococcus
unclassified_Bacteroidetes Cate nibacterium	■ Delftia	Sphingomonas	Pseudomonas	Sarcina
Asaccharobacter unclassified_Deltaproteobacteria	■ Anaerosporobacter			

Fig 3. Demonstrating the microbiota-based drug metabolism A) List of 12 investigated drugs potentially susceptible to microbial metabolism in I-screen B) Sulfasalazine as a quality reference drug in all experiments to screen for anaerobic metabolism. **C**)Sulfasalazine is metabolized into 5-ASA and sulfapyridine by azoreductases of gut microbiota **D**) showing populational variation in microbiota composition between newborns, infants, toddlers, teenagers and adults

Conclusions / next steps:

- We have successfully developed an in vitro human intestinal model, based on human intestinal, which can be applied as a reliable tool for longer-term incubations
- We here present an in vitro tool (I-screen) containing human colonic microbiota to study microbiota driven metabolism of drugs. We identified different types of drug transformations that may occur in the human gut and are known to occur in vivo.
- Next, the two platforms can be combined in order to create a physiological representation of human intestinal processes including intestinal permeability as well as (anaerobic) host-microbe-immune responses.