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

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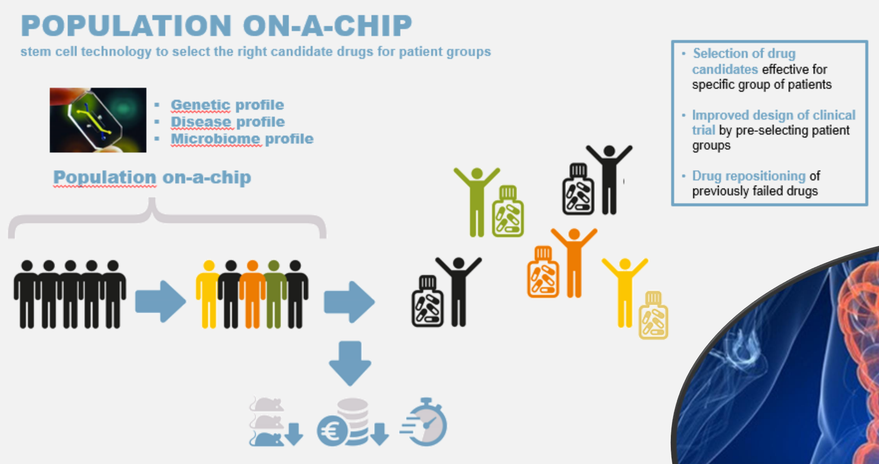
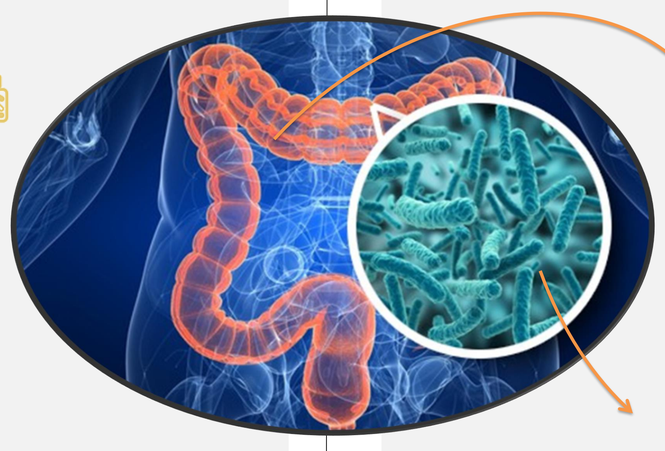
Introduction

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 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.





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**:

- 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 differences in microbiota composition of different age groups (Fig. 3)

Sustained functionality of human intestinal tissue in microfluidic model

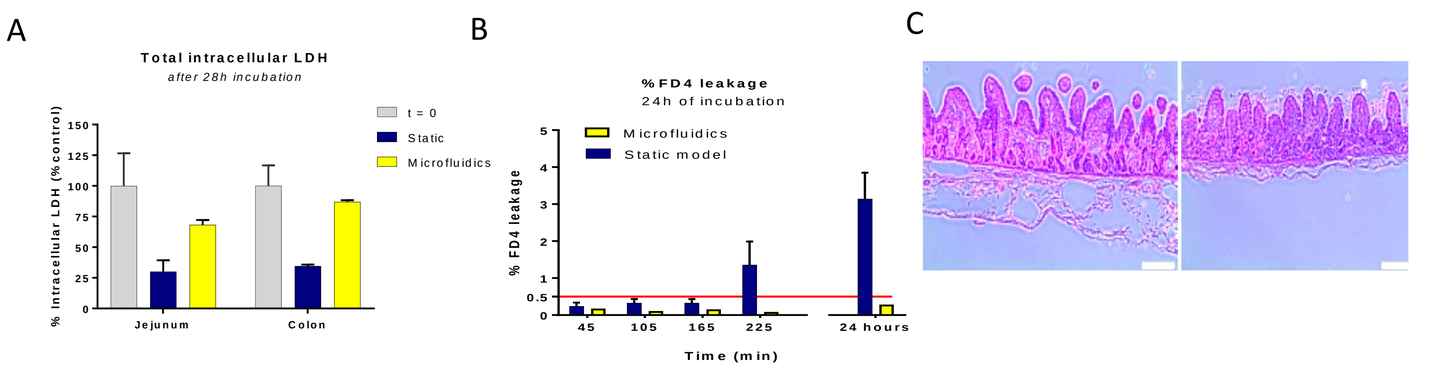


Fig 1. Improved functionality of human intestinal tissue. **A**) Intracellular LDH content showing the addition of an apical and basolateral flow significantly extends the viability of the tissue. **B**) Integrity of intestinal barrier determined by the leakage of FD4 in each incubation. Data represent 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

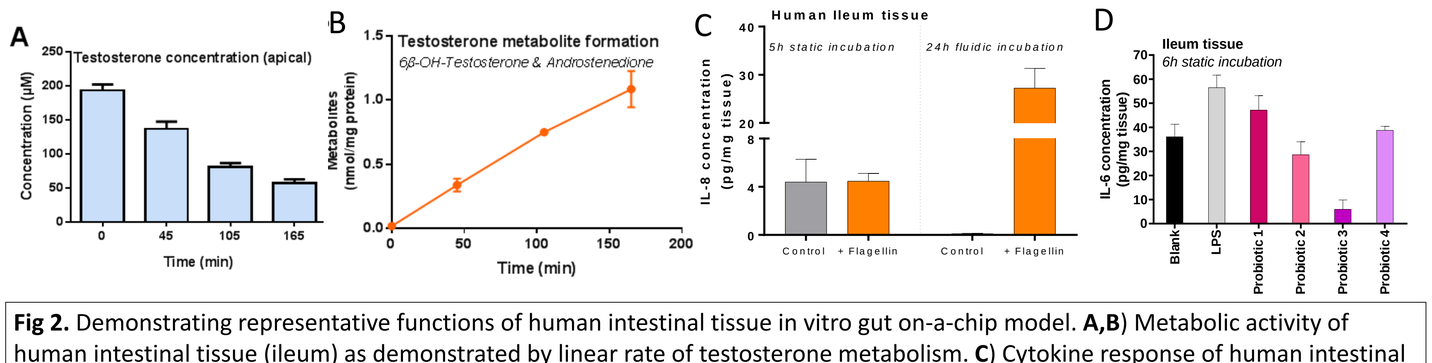
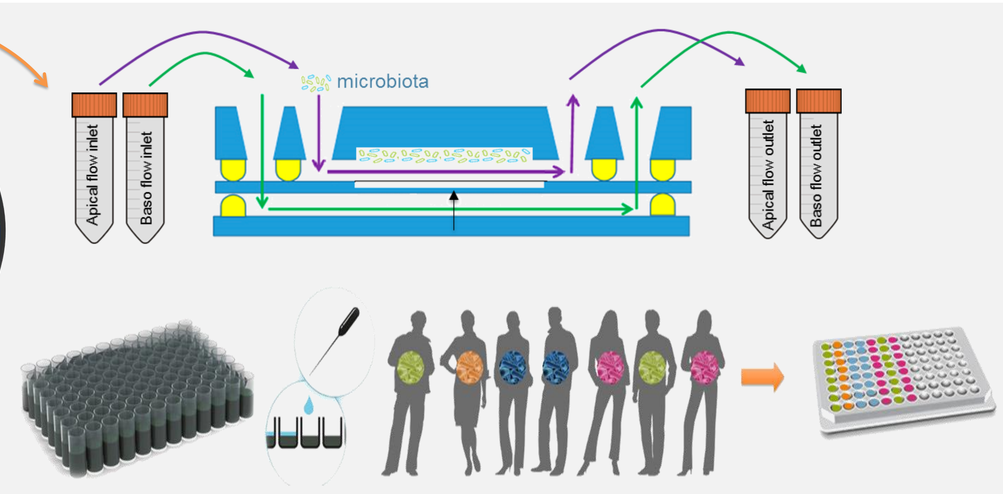


Fig 2. Demonstrating representative functions of human intestinal tissue in vitro gut on-a-chip model. **A,B**) Metabolic activity of human intestinal tissue (ileum) as demonstrated by linear rate of testosterone metabolism. **C**) Cytokine response of human intestinal tissue under static control and stimulated with flagellin (5h) and microfluidic conditions of control and after stimulation with flagellin for 24h) **D**) Ileum 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



Metabolism of drugs by intestinal microbiota

A

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	No decline of parent, no metabolites detectable	
Simvastatin		
Levodopa		
Acetaminophen		
Zonisamide		
Dapsone		

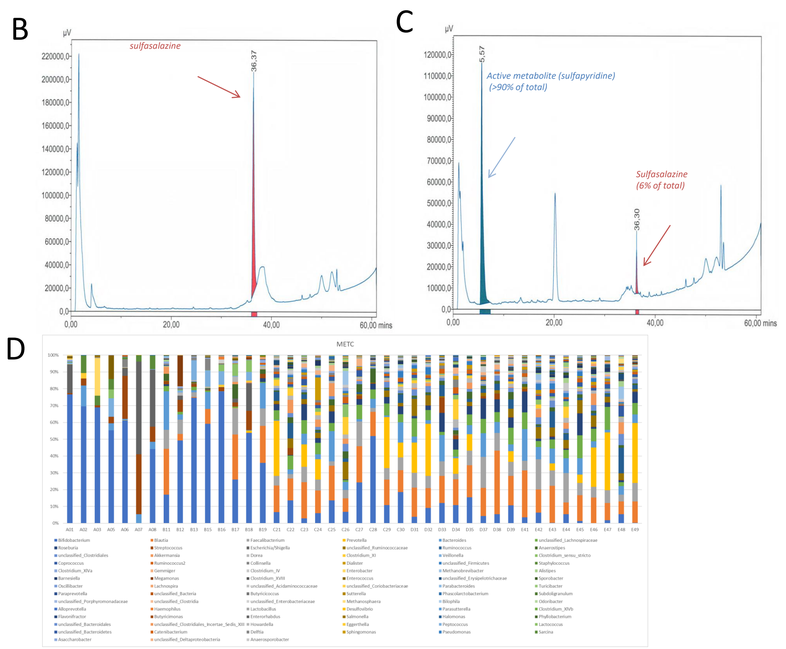


Fig 3. Demonstrating the microbiota-based drug metabolism **A**) List of 12 investigated drugs potentially susceptible to microbial metabolism in I-screen **B**) Sulfasalazine as a quality reference drug in all experiments to screen for anaerobic metabolism. **C**)Sulfasalazine is metabolized into 5-ASA 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.