Development of a Human Colon Intestine-Chip to Study Colonic Mucosa Development and Functionality

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Methods

Human crypt-derived colonic organoids, derived from adult male individuals, were seeded in the Colon Intestine-Chip interfacing with colonic human intestinal microvascular endothelial cells (cHIMECs). Intestine-Chip was maintained on Zoë, an instrument that supports the culture of the chips, e.g. provides and controls flow and stretch. Colonic organoids were fragmented and introduced in the apical channel of the chip. They were cultured in the chip for up to 10 days in the presence of Wnt3a, Noggin, and Rspo1, under physiologically-relevant mechanical and shear stress and expanded to epithelial monolayers in the chip. Beginning on day 5 of culture, the apical channel of the Colon Intestine-Chip, was periodically exposed to air and nutrients on a daily basis. Epithelial barrier establishment and function was assessed by immunofluorescence for tight junction proteins and over time by the apparent permeability (P_{app}) of 3kDa Dextran, respectively. The relative abundance of the main epithelial cell subtypes was assessed by qPCR and immunofluorescence staining. In depth transcriptomic analysis was performed using bulk RNAseq. Specifically, colonoids either in suspension or expanded to monolayers on Intestine-Chip, in the presence or absence of endothelium (HIMECs) and/ or cycling stretching, were harvested on days 5 and 8 of the fluidic culture and analyzed accordingly. Differential Gene Expression Analysis of these samples as compared to publicly available bulk RNAseq data from human colonic IECs (cIECs) (Kraiczy J, et al. Gut 2017; 0:1–13. doi:10.1136/gutjnl-2017-314817, Howell KJ et al. Gastroenterology 2018; 154:585–598. doi: 10.1053/j.gastro.2017.10.007). IL-1β, TNFα and IFNγ were applied at different concentrations, on the basolateral side of the chip, to challenge the integrity of the epithelial barrier.

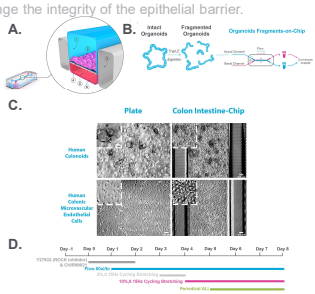

Figure 1. Methods and Experimental Methodology

[A] Cross section of the Colon Intestine-Chip, 1: Epithelial Channel, 2: Human Colonoids derived Epithelial monolayers, 3: Vacuum channel, 4: Porous PDMS Membrane, 5: cHIMECs, 6: Vascular Channel [B] Steps for the colonoids introduction in the apical channel of the chip [C] Representative contrast phase microscopy images of each cell type morphology , on a conventional plate culture (left) and right after their introduction in the Colon Intestine-Chip (right) [D] Experimental timeline

Results

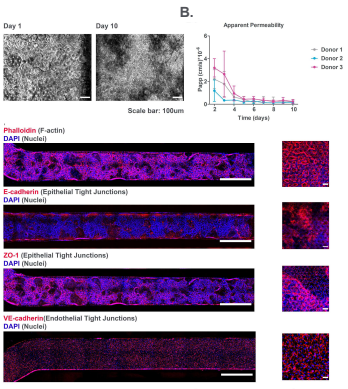

Figure 2. Epithelial Barrier Establishment

The apparent permeability of 3kDa Dextran and immunostaining for E-cadherin and ZO-1 confirmed the establishment of the epithelial barrier. The *in vivo* relevant cytoarchitecture, in the presence of physiologically relevant mechanical forces, was evidenced by F- actin staining.

[A] Representative contrast phase microscopy images of the epithelial monolayer morphology, on Day 1 (left) and Day 10 (right) of the fluidic culture [B] Apparent permeability of 3kDa Dextran across three different donors over 10 days in culture, *Mean ± SD* [C-D-E-F] Representative fluorescence images depicting F-actin, E-cadherin and ZO-1, and VE-cadherin staining in the colonic epithelial, and endothelial monolayer respectively. *N= 3 chips/donor*

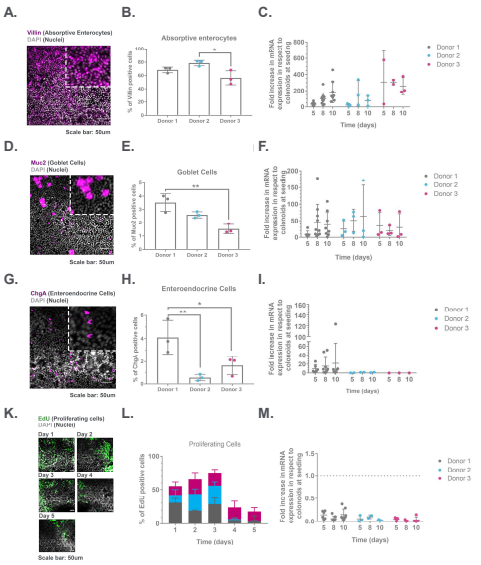

Figure 3. Differentiation State of the Colon Intestine-Chip

We applied qPCR and immunofluorescence analysis against key genes and specific proteins, established markers for each of the epithelial cell types, in different donors.

[A-D-G-K] Representative immunofluorescence images from staining with Villin, Mucin 2 (Muc2) Chromogranin A (ChgA), and EdU, depicting the populations of absorptive enterocytes, Goblet, enteroendocrine, and proliferating cells respectively, done on day 8 of the fluidic culture [B-E-H-L] Abundance of each epithelial cell type, is expressed as a percentage over the total number of nuclei. Quantification is done by counting positive cells in 5 different fields of view per chip. *1 experiment, n= 3 chips/donor, Mean±SD, One-way ANOVA, Tukey's test, *: p<0.05, **: p<0.01* [C-F-I-M] Identification of each epithelial cell type by qPCR for the Alkaline Phosphatase (*Alk1*), Mucin 2 (*Muc2*), Chromogranin A (*ChgA*) and Leukine-Rich Repeat-Containing G-Protein Coupled Receptor (*Lgr5*) genes respectively. *1-4 experiments, n=2-10chips/donor, Mean±SD*

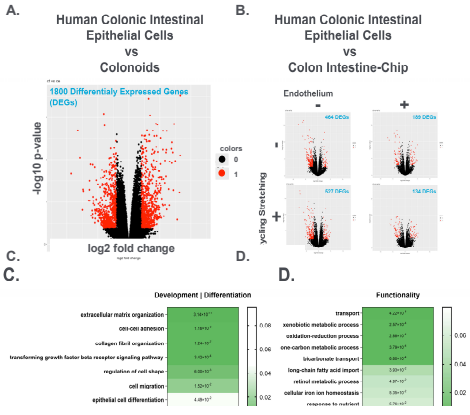

Figure 4. Transcriptomic profile of the Colon Intestine-Chip

Gene Ontology pathway analysis, on day 8 of the culture, indicated the upregulation of pathways related to the development and functional maturation of intestinal epithelium.

[A] Volcano plot depicting the Differential Gene Expression Analysis between colonic Intestinal Epithelial Cells (cIECs, public data) and Colonoids [B] Volcano plot of the Differential Gene Expression Analysis between cIECS and colonic epithelial cells on day 5 in the Colon Intestine-Chip under four different culture conditions. *Adjusted p-value <0.05, |log2FoldChange|≥2* [C-D] Gene Ontology pathway analysis, based on the differential expressed genes between colonoids and the Colon Intestine-Chip on day 8 of culture. Heat map intensity represents the Fischer Exact Test values

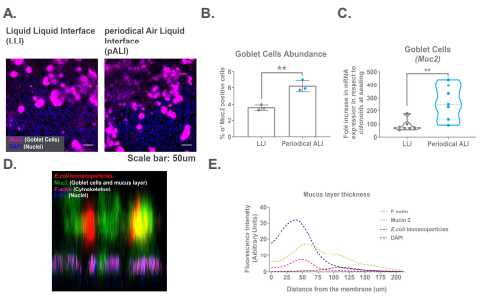

Figure 5. Effect of periodical ALI on the maturation of Goblet cells

Alternating exposure of the epithelium to nutrients and air, reminiscent of the periodic transition of food chyme from the human colon, resulted in increased population of Goblet cells, as assessed by qPCR and immunofluorescence for Mucin2, while it allowed for formation of mucus layer, of thickness similar to that found in the colon tissue.

[A] Representative immunofluorescence images of Mucin 2 (Muc2) depicting the population of Goblet cells, on day 8 of the fluidic culture [B] Abundance of Goblet cells, expressed as a percentage over the total number of nuclei. Quantification was performed, in 5 different fields of view per chip. *1 experiment, n= 3 chips/group, Mean±SD, Unpaired T-test, **: p<0.01* [C] Identification of Goblet cells by qPCR for the Mucin 2 (*Muc2*) gene. *2 experiments, n=7chips/group, Mean±SD, Unpaired T-test, **: p<0.01* [D] Representative orthoslice image of immunostaining against Mucin 2, F-actin, DAPI and E.coli bio nanoparticles for assessment of the mucus layer thickness. [E] Representative plot of the mean fluorescent intensity per z-stack, for each one of the aforementioned markers

A.

Barrier Disrupting Agent	Day of Stimulation	Time of Stimulation	Results
1ng/ml IL-1β + 20ng/ml TNFα - Basal	Day 5	24h	No effect
10ng/ml IL-1β + 20ng/ml TNFα - Basal	Day 5	24h	No effect
100ng/ml IL-1β + 20ng/ml TNFα - Basal	Day 5	24h	No effect
3% DSS - Apical	Day 4	72h	No effect
10nM Lantruculin A – Apical	Day 4	72h	No effect
25ng/ml IFNγ - Basal	Day 4	48h	2.9 fold increase on day 6
100ng/ml IFNγ - Basal	Day 4	48h	3.9 fold increase on day 6

B.

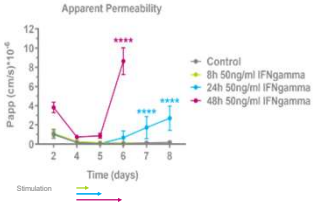

Figure 6. Disruption of the intestinal epithelial barrier in the Colon Intestine-Chip

IFNgamma was a potent inflammatory stimulus for disruption of the epithelial barrier, acting in a time-dependent manner, as shown by gradual increase in the epithelial permeability from 24h to 48h post-stimulation.

[A] Index of barrier disrupting agents tested in the Colon Intestine-Chip [B] Apparent Permeability of 3kDa Dextran depicting the time dependent response of the Colon Intestine-Chip to stimulation with 50ng/ml IFNgamma, *2 experiments, n= 4-8 chips/group, Mean±SD, Two-way Anova, Tukey's test, ****p<0.0001*

Conclusions

- We developed a robust human Colon Intestine-Chip model that emulates significant aspects of the human tissue biology as measured by:
  a. Morphology and cytoarchitecture
  b. Cell population and ratios
  c. Functional endpoints – e.g. barrier function
  d. Gene expression profiles
- Our Colon Intestine-Chip demonstrates the ability for *in vivo*-relevant cytokine-mediated barrier disruption.
- Our Colon Intestine-Chip provides a human relevant model that could enable applications in efficacy and safety assessment of new therapeutics, disease modeling and mechanistic studies in gastrointestinal diseases, for identification of biomarkers and novel therapeutic targets .

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