Colon Intestine-Chip: A Microengineered Model to Study Human Colonic Epithelial Biology

Contributors: A. Apostolou[1,2], D. Manatakis[1], S.J. Kerns[1], M. Kasendra[1], R. Luc[1], M.D. Paraskevopoulou[3], J. Homsy[3], A. Manousopoulou[4], S.D. Garbis[4], E. Manolakos[5,6], A.J. Burns[3], G.A. Hamilton[1], C. Giallourakis[3], K. Karalis[1]

[1] Emulate Inc., USA, [2] Department of Medicine, National and Kapodistrian University of Athens, GR, [3] Takeda Pharmaceutical Company Ltd., USA, [4] Centre for Proteomics Research, University of Southampton, UK, [5] Department of Informatics and Telecommunications, National and Kapodistrian University of Athens, GR, [6] Northeastern University, USA

Background and Aims

In the effort to increase efficacy and specificity on newly developed drugs for intestinal disorders, human-based systems provide a promising new alternative to the animal-based studies. The interface of epithelium with endothelium allows for nutrient exchange and oxygen diffusion, as well as a gateway for circulating immune cells in states of inflammation. Our knowledge of the contribution of the microvasculature during intestinal mucosa development is still limited. We use Organ-Chips and our proprietary Human Emulation System to develop a platform where organoids are able to expand and differentiate to monolayers that consist of the three major intestinal epithelial cell types.

Here, we report the development of a colonoid-based platform for disease modeling, biomarkers identification, and drug testing on a patient-specific approach.

Methods

Human crypt-derived colonic organoids, derived from adult male individuals, are seeded in the Colon Intestine-Chip and interface with colonic human intestinal microvascular endothelial cells (cHIMECs). The Intestine-Chip is maintained on Zoë, an instrument that cultures the Intestine-Chip. Colonic organoids were cultured in the chip for 10 days in the presence of Wnt3a, Noggin, and Rspo1, under physiologically-relevant mechanical forces and shear stress, and were expanded to epithelial monolayers (IECs). Epithelial barrier function was assessed over time by the apparent permeability (P_{app}) of 3kDa Dextran. 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 from the fluidic culture and analyzed accordingly. Differential Gene Expression Analysis compared these samples to our *in vivo* control, which is 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). In order to further delineate the closeness between Colon Intestine-Chip and epithelial cells isolated from colon tissue (cIECs) gene expression, we referred to the strict transcriptomic signature (166 genes) of the colon tissue as per the Human Protein Atlas project (https://www.proteinatlas.org/humanproteome/tissue/colon). [Figure 1]

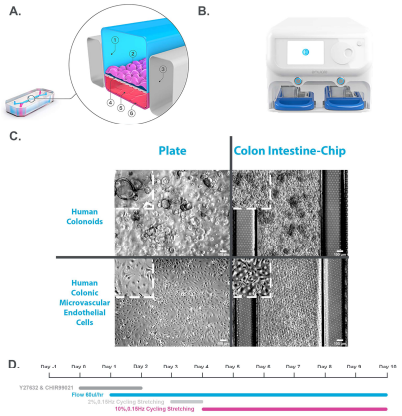

Figure 1. Methods and Experimental Methodology
[A] Cross section of the Colon Intestine-Chip, 1: Epithelial Channel, 2: Human Colonoids derived Epithelial Monolayer, 3: Vacuum Chamber, 4: Porous PDMS Membrane, 5: cHIMECs, 6: Vascular Channel [B] The automated instrument Zoë [C] Representative contrast 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

The establishment of a confluent monolayer on the Colon Intestine-Chip was confirmed by contrast phase microscopy, whereas barrier function was assessed by the apparent permeability of 3kDa Dextran, measured over the course of 10-days in fluidic culture. Development of an *in vivo* relevant cytoarchitecture, in the presence of applied physiologically relevant forces, was evident by F- actin staining. [Figure 2]

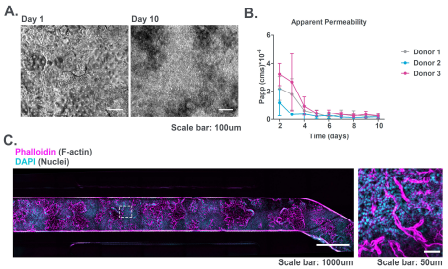

Figure 2. Epithelial Barrier Establishment
[A] Representative contrast phase microscopy images of the epithelial monolayer morphology, on Day 1 (left) and Day 10 (right) of the fluidic culture [B] Apparent Permeability of 3kDa Dextran across three different donors over 10- days in culture, *Mean ± SD* [C] Representative fluorescence image depicting F-actin staining in the colonic epithelial monolayer. *N= 3 chips/donor*

We confirmed the multilineage differentiation capacity of the Colon Intestine-Chip towards absorptive enterocytes, Goblet cells, and enteroendocrine cells, by qPCR and immunofluorescence against key genes and specific protein markers for each of the above- epithelial cell types, in three different donors.- [Figure 3]

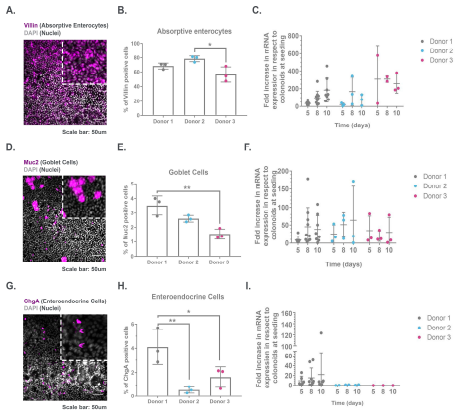

Figure 3. Differentiation State of the Colon Intestine-Chip
[A-D-G] Representative immunofluorescence images of Villin, Mucin 2 (Muc2) and Chromogranin A (ChgA) staining depicting the populations of absorptive enterocytes, Goblet and enteroendocrine cells respectively, on day 8 of the fluidic culture [B-E-H] Abundance of each epithelial cell type, -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/donor, Mean±SD, One-way ANOVA, Tukey's test, *: p<0.05, **: p<0.01* [C-F-I] Identification of each epithelial cell type by qPCR for the Alkaline Phosphatase (*Alk1*), Mucin 2 (*Muc2*) and Chromogranin A (*ChgA*) genes respectively. *1-4 experiments, n=2-10 chips/donor, Mean±SD*

The Differential Gene Expression Analysis, revealed significant decrease in the Differentially Expressed Genes (DEGs) on cells cultured on the Colon Intestine-Chip, in the presence of endothelium on day 5 of the fluidic culture. [Figure 4]

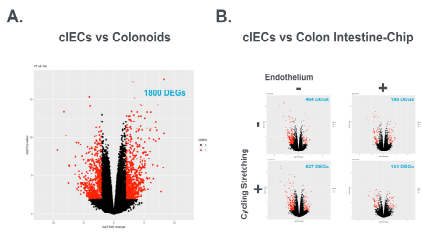

Figure 4. Differential Expression in the Colon Intestine-Chip
[A] Volcano plot depicting the Differential Gene Expression Analysis between colonic Intestinal Epithelial Cells (cIECs, public data) and Colonoids [B] Volcano plot of the Differential Gene Expression Analysis between cIECS and colonic epithelial cells on day 5 in the Colon Intestine-Chip under four different culture conditions. *Adjusted p-value <0.05, |log2FoldChange|≥2*

Spearman correlation of the ranking of the average expression of the 166 genes- mentioned as the strict transcriptomic profile of the colon tissue in Atlas- between cIECs, Colonoids or cells in the Colon Intestine-Chip, from different culture conditions, demonstrated the closeness between the Colon Intestine-Chip and the colonic epithelium directly derived from the tissue (*in vivo*). [Figure 5]

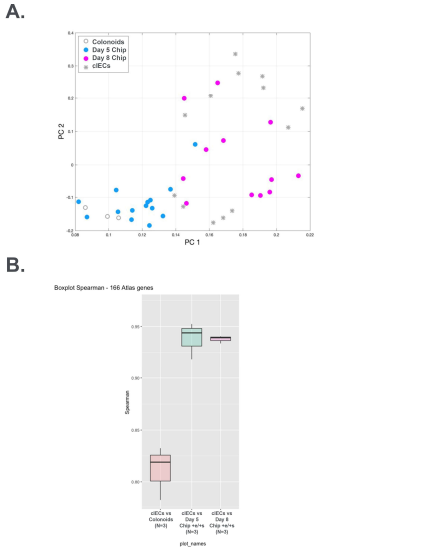

Figure 5. Comparison of the Transcriptomic Profile of the Colon Intestine-Chip and the Colon Tissue
[A] PCA analysis based on the 166 Atlas colon-specific genes in Colonoids, colonic epithelial cells cultured in the Colon Intestine-Chip on day 5 and 8 of the fluidic culture, and cIECs. PC1: 93.84% of variance, PC2: 3.59% of variance [B] Spearman correlation of the ranking of the average expression of the 166 Atlas genes between the cIECs and the Colonoids or cells from the Colon Intestine-Chip cultured in the presence of endothelial cells (+e) and stretching (+s).

Summary

- We report the development of a new microengineered Colon Intestine-Chip made of a transparent elastomeric polymer, poly-dimethylsiloxane (PDMS).
- Colon Intestine-Chip contains two parallel microchannels separated by a thin porous flexible membrane coated with tissue specific extracellular matrix, seeded with human crypts-derived colonic epithelial cells and human colonic microvascular endothelial cells respectively.
- The cells in the Colon Intestine-Chip are maintained under low shear stress, recreated by continuous flow at physiological rates, and mechanical forces recapitulating the *in vivo* intestinal peristalsis.
- Colon Intestine-Chip demonstrates a tight barrier over 10 days in culture, as shown across multiple donors.
- Colon Intestine-Chip allows for a multilineage epithelial differentiation in the presence of the stemness factors Wnt3a, Rspo1 and Noggin, as per published, standardized protocols.
- The Colon Intestine-Chip indicated the significance of endothelium and mechanical stretching for the functional maturation of human intestinal epithelial progenitor cells.
- The transcriptomic signature of the colonic epithelial cells in the Colon Intestine-Chip shows a great similarity to that of the human tissue, even in the hierarchical ranking of the colon-specific genes.

Conclusions

We developed a robust human Colon Intestine-Chip that recapitulates significant aspects of the human tissue biology including gene expression profiles. Our Colon Intestine-Chip platform provides a much needed experimental system to be used to assess human efficacy and safety of novel drugs, as well as support mechanistic studies of gastrointestinal diseases.