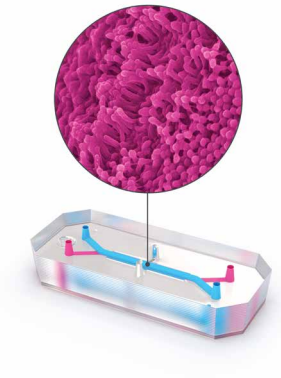
Characterization of the Colon Intestine-Chip



Introduction

The colon is the last region of the gastrointestinal (GI) tract, which plays an important role in digestion, absorption of water and electrolytes, secretion, and motility and is involved in the production and absorption of vitamins.[1,2] The colon has a simple columnar epithelium with a thin brush border and secreting cells. Various cell types compose the intestinal epithelium—including absorptive enterocytes, goblet cells (secrete mucin), enteroendocrine cells (secrete hormones), tuft cells, M cells and stem cells—that are responsible for the constant renewal of the epithelium.[3] The intestinal epithelium is also responsible for secreting cytokines and sensing luminal contents and microbes via toll-like receptors (TLRs) and NOD-like receptors (NLRs).[3]

A key component of the intestinal epithelium is its barrier property, which is critical in maintaining homeostasis in the body. The colonic epithelial barrier is composed of a monolayer of epithelial cells covered by mucus. Intestinal epithelial cells (IECs) aid in intestinal homeostasis through the regulation of host-microbial interactions (with commensal bacteria but not pathogens) and with secretory IECs that secrete mucins and antimicrobial peptides.[5] Mucus is needed to protect against mechanical damage, stabilize the luminal microenvironment, and trap material for mucociliary clearance.[6] Any disruption of this process can lead to decreased intestinal barrier function, nutrient malabsorption/diarrhea, infection, sepsis, and/or auto-immune disorders like inflammatory bowel syndrome (IBS) and inflammatory bowel disease (IBD).[7–9] New experimental models of the colon are needed to better understand the complex function of the intestinal barrier and physiology, and the effects of novel drug candidates. Intestinal disease models such as IBD would support the investigation into new therapeutics and treatments. Over 1.5 million North Americans and 2 million Europeans have IBD, and rates of incidence are rising in newly industrialized countries. As therapeutics are usually long-term treatments resulting in higher and durable medical costs, the development of curative treatments would reduce cost and benefit patient health.

Preclinical *in vitro* models of the colon commonly utilize established cell lines cultured in Transwells due to their ease of use, low cost, and accessibility.[11] Common cell lines utilized for assessing drug properties such as transport kinetics or epithelial barrier

Key Highlights

- Colon Intestine-Chip combines organoids and Organs-on-Chips technology to improve physiological relevance.
- Model forms tight barrier with low permeability, highly polarized epithelium, and mature epithelial phenotypes.
- Endothelial co-culture enhances barrier function, tight junction formation, morphology, polarization, and gene expression.
- Model can be applied to study cytokine-mediated barrier disruption.

function in the large intestine are Caco-2 cell lines which are derived from human colorectal cancer, T-84 cell line derived from human colon cancer, and HT-29, a human colorectal carcinoma cell line. These traditional *in vitro* models have limitations as they lack the 3D cytoarchitecture needed for cell-cell interactions, do not include mechanical forces to emulate peristaltic motion, do not accurately represent *in vivo* cell populations, and do not have high transcriptomic similarity to *in vivo* tissue due to their cancerous origin.[3] This limited physiological relevance limits the applicability of these models in studying intestinal barrier homeostasis beyond routine drug absorption studies.[3,17]

Intestinal organoids overcome 2D culture limitations by providing 3D cytoarchitecture, long term viability, spatial organization, and representative cellular diversity.[17,18] Unfortunately, organoids are not without their limitations. Organoids have reversed polarity, with the apical side in the middle of the organoid, rendering it difficult to access to experimentation.[3] Additional limitations of organoid cultures include variability between batches which affects reproducibility, maintenance cost, the lack of mechanical stimuli and multicellular composition—all while not fully recreating *in vivo* responses.[18]

Preclinical *in vivo* animal models of the large intestine are limited by species differences that can complicate translation to human responses. Mouse models are commonly used to investigate the gut microbiome and its relation to human diseases including Type 2 diabetes, obesity, and IBD.[23–26] However, anatomical differences of the intestinal tract are apparent—smooth with no division in mice, while clearly divided into ascending, transverse, and descending colon in humans.[27] Furthermore, increased permeability occurs in the mid-colon in mice and left colon in humans,[28] cellular distribution varies along the intestinal tract between mice and humans, humans are more susceptible to some infections such as E. coli, and mice lack the genetic variability observed in humans due to inbreeding.[23,29] Organs-on-Chips technology addresses

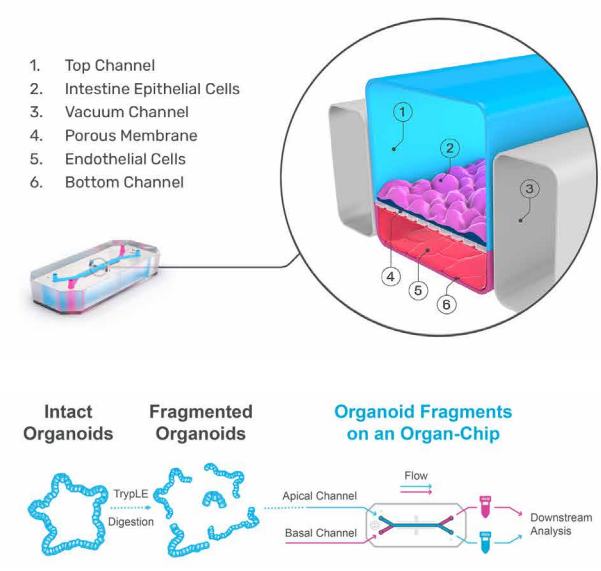
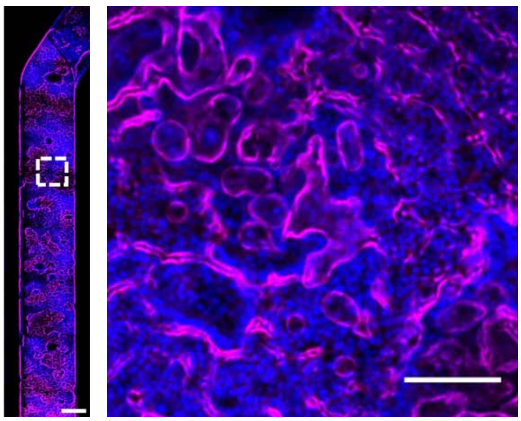


Figure 1. Colon Intestine-Chip Schematic



Phalloidin (F-actin)
Nuclei

Figure 2. Cytoarchitecture. *Confocal image of the epithelial monolayer stained with phalloidin.*

current experimental challenges by providing mechanical forces to support a physiologically relevant environment, enabling reproducible results and increased translation to *in vivo*.[3] Several different Organ-Chip models have been created such as the Liver-Chip, Kidney-Chip, and Duodenum Intestine-Chip. The human Colon Intestine-Chip combines organoid and Organs-on-Chips technology, with endothelial co-culture, flow, and stretch that improve functionality, resulting in higher transcriptomic similarity to human tissue than organoids in suspension (confirmed by omics analysis). Here we describe the development and characterization of the Colon Intestine-Chip with the ability to evaluate biochemical, genetic, and cellular responses that can be applied to drug development and disease models.

Goal

To develop and characterize a Colon Intestine-Chip that emulates *in vivo* like physiology for use across the preclinical drug discovery and development process.

Results

The Colon Intestine-Chip as seen in **Figure 1**, features two parallel channels divided by a porous polydimethylsiloxane (PDMS) membrane and two lateral channels for vacuum-driven stretch. Fragmented biopsy-derived colonic organoids were seeded in the top channel, while colonic microvascular endothelial cells were seeded in the bottom channel, creating a cell interface between the apical and luminal channels. Media was perfused at a flow rate of 60 µL/hour, and after three days of culture, mechanical stretch of 2% strain and 0.15 Hz frequency was applied, increased to 10% strain on day four. After five to eight days, confocal imaging of the Colon Intestine-Chip revealed the cytoarchitecture of the epithelial monolayer acquired *in vivo* relevant structure (**Figure 2**).

Confocal fluorescent images captured strong epithelial tight junctions established by day five of the Colon Intestine-Chip culture in the presence of endothelium (**Figure 3A**). Tight junctions were stained with anti-ZO-1 and anti-

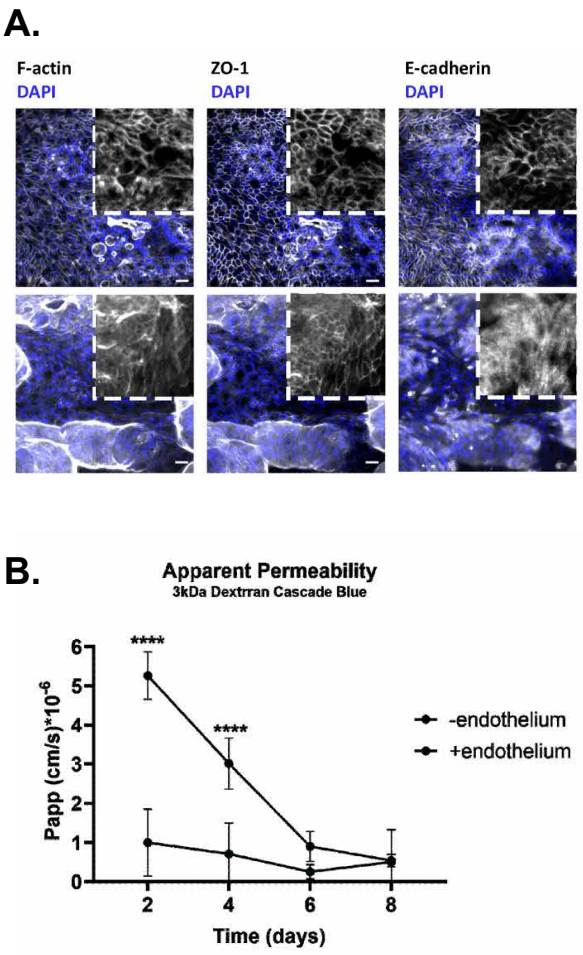


Figure 3. Effect of endothelium on the establishment of the epithelial layer of the Colon Intestine-Chip. *A. Representative confocal image of epithelial tight junctions on day five in culture.* ***B.*** *Apparent permeability (P_{app})*

E-cadherin (both important in tight junction formation), cell nuclei were stained blue with DAPI (4',6-diamidino-2-phenylindole), and the cytoskeleton was stained with phalloidin. Dextran (3kDa) cascade blue was utilized to test epithelial barrier function in the presence or absence of endothelium (**Figure 3B**). After 100 µg/mL of dextran cascade blue was administered to the apical channel, results demonstrated that the presence of the endothelium increased the formation of tight junctions and established a tight epithelial barrier, as measured by apparent permeability (P_{app}). Taken together, this demonstrates the importance of the endothelium in the differentiation of the epithelium in the Colon Intestine-Chip.

Phase contrast and scanning electron microscopy (SEM) images of the Colon Intestine-Chip at day five in culture demonstrate the enhanced maturation of the epithelial brush border in the presence of endothelial cells (**Figure 4A**). The high polarization of the epithelial monolayer is observed only in the presence of endothelium co-culture, as measured by confocal fluorescent imaging (**Figure 4B**). DRA (Down Regulated in Adenoma), or SLC26A3 transporter, is for chloride absorption and important in maintaining intestinal barrier function.[32] The Na+K+ATPase transporter is responsible for maintaining the Na+ gradient in the basolateral membrane and is important in formation of apical-basal polarity.[33,34] Fluorescent intensity of the apical and basolateral ion transporters DRA and Na+K+ATPase was quantified along the basal-apical axis of the epithelial cells (**Figure 4C**) and demonstrated that the endothelium is necessary for proper distribution of the indicative apical and basolateral ion transporters.

The differentiation state of the Colon Intestine-Chip was established by measuring the expression of cell-specific markers (**Figure 5**). Intestinal alkaline phosphatase (Alpi) is a brush border protein used as a marker for crypt villus differentiation and is only found in villus-associated enterocytes.[36] Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) is a marker for adult human stem cells.[37] Muc2 is a

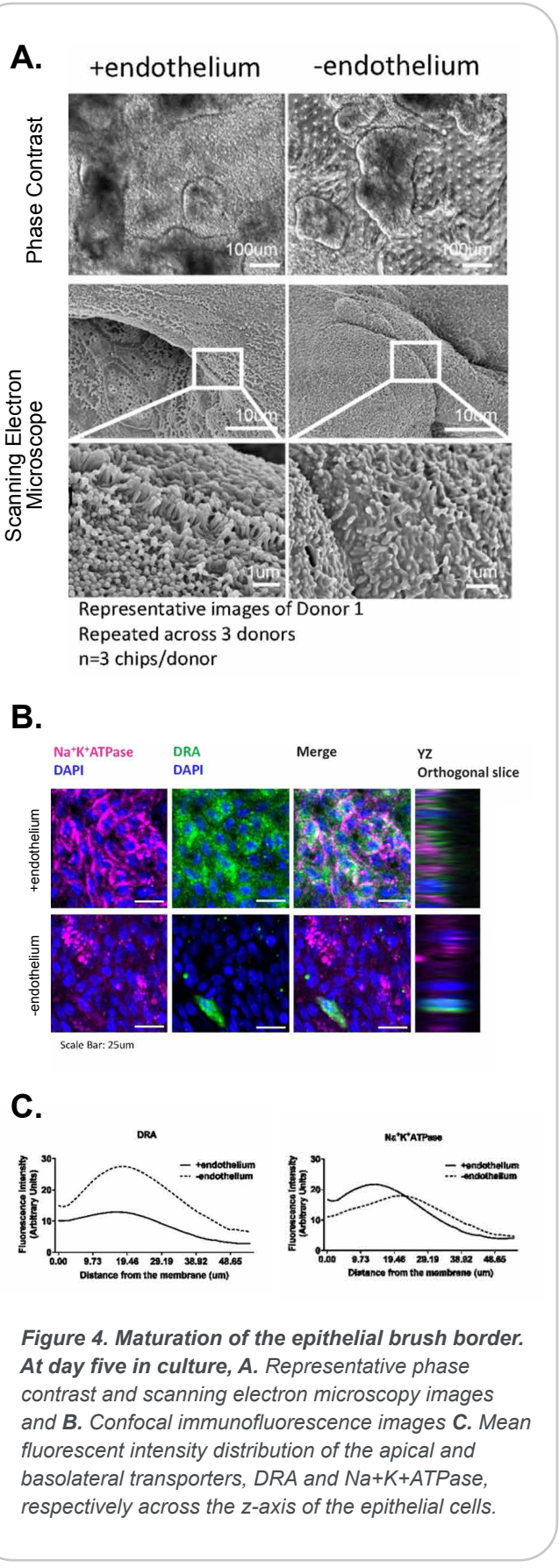


Figure 4. Maturation of the epithelial brush border. At day five in culture, A. *Representative phase contrast and scanning electron microscopy images and* ***B.*** *Confocal immunofluorescence images* ***C.*** *Mean fluorescent intensity distribution of the apical and basolateral transporters, DRA and Na+K+ATPase, respectively across the z-axis of the epithelial cells.*

highly-specific marker for goblet cells. Quantification by qPCR demonstrated that endothelial co-culture enhances differentiation through gene expression of absorptive enterocyte marker Alpi (**Figure 5A**) while not affecting the differentiation of the secretory lines as shown by Muc2 expression (**Figure 5B**), and decreasing expression of the cycling stem cell driving gene Lgr5 (**Figure 5C**).

Conclusion

The human Colon Intestine-Chip emulates human *in vivo* function by combining biopsy-derived organoids with colonic endothelial cells in a dynamic microenvironment, causing cells to differentiate into the appropriate cell populations and structures including absorptive enterocytes and mucus-producing goblet cells, to create a highly-polarized intestinal epithelial barrier with tight junctions, low permeability, and a continuous brush border with densely packed microvilli. Experiments demonstrated that the endothelium is critical in in the establishment and functional maturation of the intestinal epithelial barrier on the chip. Other benefits include the ease of data gathering through effluent collection, mucus secretion, and the ability to control mechanical forces caused by flow and stretch.

Applying the Colon Intestine-Chip rather than working in animal systems or tissues has the potential to reduce animal burden as well as clinical translation challenges caused by species differences. The model has been applied to study cytokine-mediated barrier disruption, and given the reproducible results obtained here, there is potential for use in pharmaceutical assessments of toxicity, efficacy, and testing of combinatorial therapeutics. Taken together, the human Colon Intestine-Chip supports the ability to evaluate biochemical, genetic, and cellular responses and can be used in developmental and regenerative medicine, preclinical studies during drug development, and disease modeling. Future work will include the incorporation of additional cell types such as immune cells and microbial coculture.

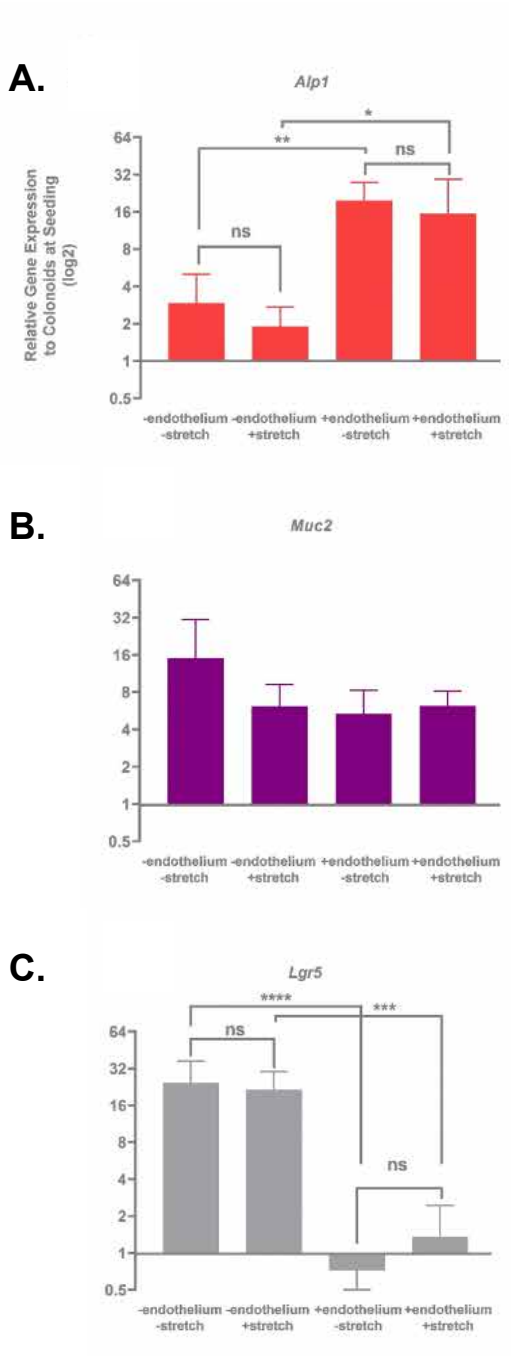


Figure 5. Differentiation State of the Colon Intestine-Chip. Gene expression of A. *Alp1,* ***B.*** *Muc2, and* ***C.*** *Lgr5 on Day five of culture in the presence of stretch and/or endothelium.*

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