CellPress

Trends in Biotechnology

Review

In Vitro Models of Intestine Innate Immunity

Terrence T. Roh,[1] Ying Chen,[1] Sara Rudolph,[1] Michelle Gee,[1] and David L. Kaplan[1,*]

Animal models have delivered critical insights into mechanisms underlying the intestinal innate immune system; however, inherent differences exist between human and animal systems. To further understand the intestine innate immune system, there is a growing need for *in vitro* tissue model systems using human cells. A critical feature of *in vitro* cell and tissue models is the subepithelial environment, which contains additional cell types and includes 2D, microfluidic, organoid, and 3D tissue models. Where mouse models for the study of intestinal innate immune systems fall short, developments from *in vitro* models continue to grow in importance to aid efforts to understand this system in the context of disease and potential treatments.

Highlights

Studies using cocultures with multiple human cell types and microbial populations show interactions (cell signaling) between cell types, enhancing model fidelity to native human intestine tissue.

Nontransformed human intestinal organoids are replacing cell lines in *in vitro* model platforms, improving the physiological relevance of models, including patient-specific characteristics.

3D tissue culture systems provide attachment sites for cell migration and stromal-like contents under an epithelial layer to better mimic the complex structural organization of intestinal tissue.

Fluid flow across the intestinal epithelium contributes to improved epithelial barrier function with impact on *in vitro* microbial interactions with the epithelium.

3D tissue models support inflammation components (e.g., macrophages) and innervation, as key components of a human intestinal model to study diseases.

Rationale for *In Vitro* Models of the Intestine Innate Immune System

The intestinal innate immune system is the first line of host defense against intestinal pathogens. This system encompasses barrier defenses maintained by the epithelial cell layer, as well as bactericidal immune cell populations consisting of macrophages and neutrophils (Box 1). Animal models have delivered critical insights into mechanisms underlying the intestinal innate immune system, but there is a growing need for new *in vitro* tissue model systems using human cells to further understand this system, due to the differences between animal and human innate immunity, especially in the intestine. Mice, which form the basis of immunology research, have evolved to handle short lifespans in environments closer to the ground, leading to different immunologic challenges. Inherent differences between human and mouse intestines include more types of **α-defensins** (see Glossary) secreted by Paneth cells [1], a lower neutrophil to lymphocyte ratios [2], and altered microbiome compositions [3] (Table 1). Recent advances in the development of *in vitro* tissue models include more cell types, particularly through the use of intestinal organoids, which have enabled the improved recapitulation of human intestinal epithelial complexity [4,5]. Furthermore, different cell and tissue model formats (e.g., 2D cell culture, microfluidic chips, organoids, 3D tissues with scaffolds) have enhanced the utility of *in vitro* models (Figure 1). These advances now enable studies of intestinal innate immunity, including those related to **inflammatory bowel disease (IBD)**, by investigating fundamental cell and tissue level interactions (Figure 2).

The structure of the subepithelial space, where immune cell types can be cultivated, is a critical feature impacting intestinal innate immunity and provides a basis for grouping these *in vitro* cell and tissue models. For example, 2D models are static cultures typically with a planar epithelial space with or without subepithelial culture space on the lower side of Transwell (Figure 1A). Microfluidic models similarly have a planar epithelial space but with a dynamic (fluid flow) culture subepithelial space, though a subepithelial space comprised of an encapsulated matrix is possible (Figure 1B). Organoids that have a hollow **lumen** enclosed with an epithelium have a **Matrigel** subepithelial space with limited access to apical and basal cell surfaces (Figure 1C). 3D tissue models that tend to have a lumen space on top of an epithelium cultured on a 3D matrix have a 3D scaffold-based subepithelial space (Figure 1D). 3D tissue models with bioreactor perfusion also provide long-term culture and peristaltic stimulation, enabling the study of both acute and chronic inflammation. There is a need to clarify how well the various cell and tissue model systems mimic the native system, including the strengths and limitations of each of these options (Table 2,

[1]Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, MA 02155, USA

*Correspondence: david.kaplan@tufts.edu (D.L. Kaplan).

Box 1. Barrier Defenses of the Intestinal Innate Immune System

The intestinal epithelium has several key features that are part of its function as a selective barrier that allows nutrient uptake while barring pathogens from entering the body. Goblet cells secrete a transparent layer of mucus and acts as a barrier between epithelial cells and gut microorganisms (Figure IA). The small intestine epithelium tends to have a thinner mucus layer, though a thick mucus layer can be induced [67]. In the large intestine, two distinct layers of mucus are present: an inner layer that is firmly attached to the epithelium and is void of microorganisms and a loose outer layer populated with bacteria [67]. Due to the barrier functions of the mucus layer, this is a crucial part of the intestine's innate immune system. Epithelial barrier permeability is controlled by tight junctions, which regulate the translocation of water, nutrients, and other solutes across the microscale spaces between enterocytes and other epithelial cells, including enteroendocrine cells (Figure IB) [68]. Hormone-secreting enteroendocrine cells and the enteric nervous system work in conjunction to sense the luminal environment, then regulate intestinal barrier function and the immune response [69,70]. Paneth cells secrete α-defensin and other antimicrobial peptides into the lumen, which prevents colonization of epithelial surfaces by pathogens (Figure IC) [1]. Epithelial barrier disruption by bacteria triggers an inflammatory response involving cytokine release, which results in localized tissue responses such as infiltration of neutrophils and macrophages (Figure ID). Neutrophils provide defenses against microbes and recruit other cells, including macrophages, which are responsible for phagocytizing pathogens [71]. During and after inflammation, fibroblasts and myofibroblasts secrete factors involved with inflammatory response and ECM remodeling for tissue repair [72]. Tissue renewal to re-establish intestinal barrier function is driven by LGR5+ stem cells, which reside in intestinal crypts and are capable of recreating all cells types in the intestinal epithelial layer [4].

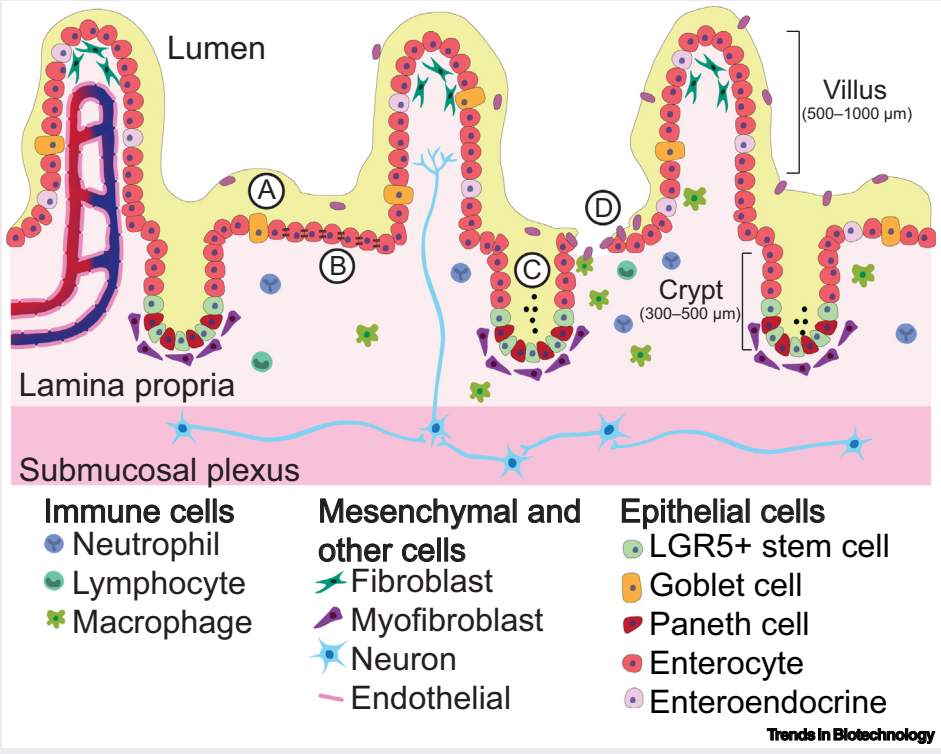

Figure I. Illustration of Intestinal Barrier Cells.

Key Table), the subject of this review. Synergies between cell culture, tissue engineering, and immunology continue to improve the relevance of *in vitro* tissue models for investigating disease mechanisms and treatments.

2D Models: Tissue Culture Plastic, Transwells, Ussing Chambers

The 2D models comprise cells grown on a flat substrate, often tissue culture plastic (Figure 1A), in a static culture environment and are commonly used to investigate cell interactions *in vitro* due to

Glossary

α-Defensins: antimicrobial peptide produced by Paneth cells and human neutrophils.
Butyrate: a short chain fatty acid, a major energy source for epithelial cells.
Caco-2, HT-29, T-84: three commonly used human colon cell lines for modeling the intestinal epithelium.
Card15/Nod2-deficient: mice with little Card15 and Nod2 expression.
Casp8 $^{\Delta IEC}$: mice that do not express caspase-8 (casp8, critically involved in mediating cell death) in the intestinal epithelium.
Celiac disease: characterized by intestinal inflammation against gluten.
Colonoids, enteroids, induced pluripotent stem cell (iPSC): derived from the large intestine, small intestine, and iPSCs, respectively.
Crohn's disease: type of inflammatory bowel disease, typically affecting small intestine epithelia.
Cyclic GMP: cyclic guanosine 3′,5′-cyclic monophosphate, a signaling molecule that modulates a range of intestine functions, including proliferation and barrier functions.
Cystic fibrosis: hereditary disease, characterized by thick mucus.
Dextran sulfate sodium (DSS): induces colitis in mice by disrupting epithelial lining.
Extracellular matrix (ECM): network of macromolecules that provide structural support and cell signals.
Gliadin: storage protein responsible for gluten intolerance.
H19$^{-/-}$: mice without H19 expression.
IFNβ: interferon beta, a type 1 interferon that protects epithelial cells under viral infections.
IFNγ: interferon gamma, proinflammatory cytokine.
IL-17: proinflammatory cytokine.
IL17A$^{-/-}$: mice without IL17A expression.
Inflammatory bowel disease (IBD): uncontrolled immune reaction of the intestinal epithelial lining; Crohn's disease and ulcerative colitis are types of IBD.
LGR5: leucine-rich repeat-containing G-protein coupled receptor 5, marker for intestinal epithelial stem cells.
Lipopolysaccharide (LPS): proinflammatory molecule derived from bacteria.
Lumen: interior of tubular structure.
Matrigel: gelatinous protein mixture derived from mouse tumor cells.

Table 1. Features in the Human Intestine That Are Different Than in Mice

Location	Human intestine	Mouse intestine	Refs
General differences in intestine	• Higher Firmicutes:Bacteroidetes ratio • Actinobacteria present • Enteroendocrine populations are sparsely populated • L-cells express more *GIP*, *CHGA*, *ASIC5*, *GIPR*, *GPR142*, *SCTR*, *PTH2R*, *CHRNA5*, and *OPRK1* • Neutrophils produce defensins • Blood composition is 30–50% lymphocytes and 50–70% neutrophils • Only 60% of dendritic cells express CD103 in ileum and colon	• Higher Bacteroidetes:Firmicutes ratio • Actinobacteria absent • Enteroendocrine populations are densely populated • L-cells express more *Gpr174*, *Gpr171*, *Ghr*, *Grpr*, *Ptger1*, *Cnr1*, *Insl5*, *Gpr22*, and *Ghrl* • Neutrophils do not produce defensins • Blood composition is 70–90% lymphocytes and only 10–30% neutrophils • Almost all dendritic cells express CD103 in ileum and colon	[3,73–76]
Small intestine	• Shorter villi with mucosal folds • Enteroendocrine populations secrete motilin • Paneth cells secrete two α-defensins	• Taller villi with no mucosal folds • Enteroendocrine populations do not secrete motilin • Paneth cells secrete over 20 α-defensins	[74,75,77]
Large intestine	• Susceptible to *E. coli* infection at low doses • Paneth cells present in cecum and proximal colon • Goblet cells present from cecum to rectum • Small pouches segmenting the large intestine, known as haustra, are present • Increased permeability in left colon	• Susceptible to *E. coli* infection at much higher doses • Paneth cells present only in cecum • Goblet cells abundant in proximal colon but diminish in distal colon and rectum • Haustra absent • Increased permeability in midcolon	[23,31,77]

Peripheral blood mononuclear cells: a mixed population of lymphocytes and monocytes.
Short chain fatty acid: produced by fermentation and the primary energy source of colonic epithelium.
STAT1: signal transducer and activator of transcription 1, involved in gene regulation under interferon stimulation.
THP-1: human monocyte-like cell line, used to model monocytes and macrophages.
TNFα: tumor necrosis factor alpha; proinflammatory cytokine.
TNF$^{\Delta ARE}$: mice with uncontrolled TNFα expression.
Transepithelial electrical resistance (TEER): quantifies barrier integrity.
Ulcerative colitis: inflammatory bowel disease, typically affecting large intestine epithelia.

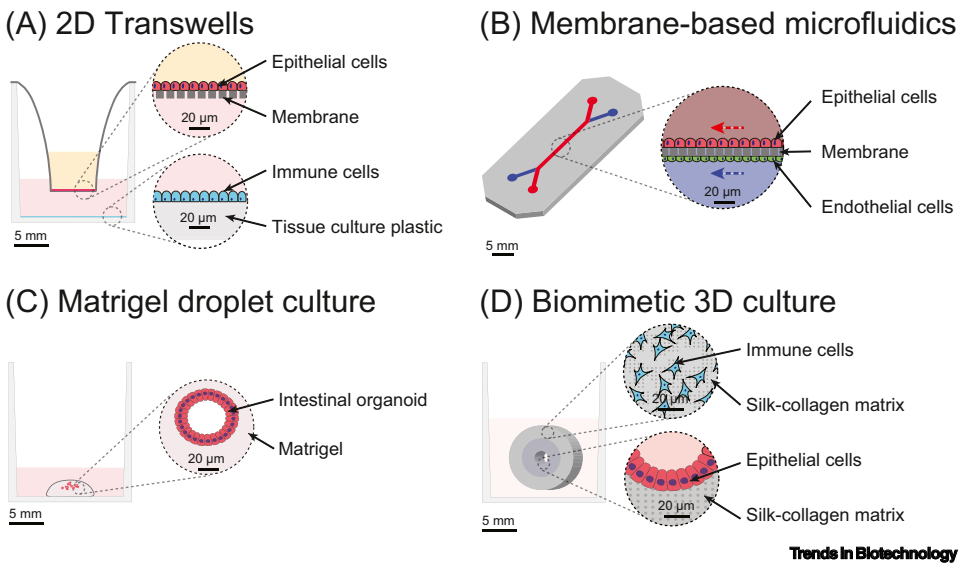

Figure 1. *In Vitro* Models of Intestinal Innate Immune Functions. (A) The 2D models present a flat cell culture surface with static media conditions. Transwell membranes enable coculture with multiple cell types and can provide each cell type with optimized media [16–19]. (B) Microfluidics enable dynamic culture conditions. The mechanical microenvironment of the of cell is known to influence cell function [27]. By continuously streaming in media, cells experience shear stress, which improved epithelial barrier functions and modulated microbial–epithelial interactions [10,26,27]. Membrane-based microfluidics enable cocultures of different cell types separated by a porous membrane and can expose cells and microbes to shear stress [25–32]. (C) LGR5+ intestinal organoids form hollow multicellular structures in Matrigel droplets. Organoids derived from small and large intestinal epithelia of patients can be stably propagated in Matrigel culture. The epithelial cells are typically polarized with microvilli protrusions facing the inside, mimicking the intestinal epithelium. (D) Epithelial cells can be cultured on a 3D substrate embedded with immune cells; these 3D substrates present a microenvironment differing significantly from 2D, resulting in altered cell morphology and gene expression [79]. A spongey matrix with collagen gel can be used to form a tubular structure, with epithelial cells lining the inside surface and immune cells embedded within the spongey matrix to mimic intestinal architecture [65].

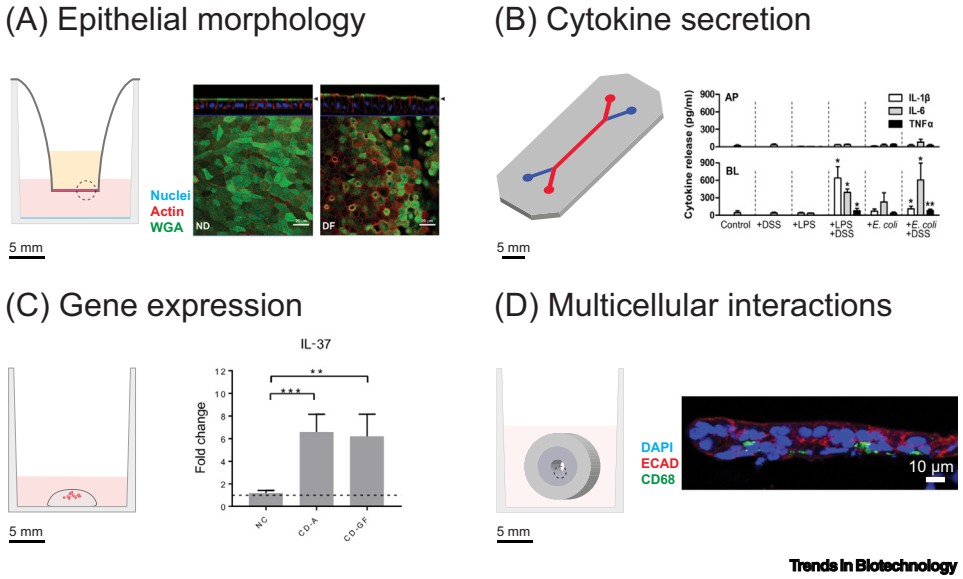

Figure 2. How *In Vitro* Models Are Used to Study Diseases of the Intestine Innate Immune System. (A) Epithelial proteins such as wheat germ agglutinin (WGA) and actin may be labeled via immunohistochemistry on non-differentiated (ND) and differentiated (DF) epithelial cells to confirm epithelial organization and polarity (scale bars: 20 µm) [16]. Reproduced from [16] under a Creative Commons license. (B) Secretion of cytokines such as interleukin (IL)-1β, IL-6, and TNFα can be quantified using ELISAs in either apical (AP) or basal (BL) compartments of microfluidics-based systems inflamed by dextran sulfate sodium (DSS), lipopolysaccharide (LPS), or *Escherichia coli* [36]. Reproduced from [36] under the PNAS license. (C) Expression of genes associated with innate immune responses, such as IL-37, can be assessed via qRT-PCR in organoids derived from non-celiac patients (NC), celiac patients with active disease (CD-A), and celiac patients in remission (CD-GF) [19]. $^{**}P < 0.01$, $^{***}P < 0.001$ based on the Mann–Whitney test. Reproduced from [19] under a Creative Commons license. (D) Histological sections of 3D biomimetic tissue models can be immunostained for e-cadherin and CD68 to label epithelial cells and macrophages, respectively, to observe immune interactions with the epithelium [65]. Reprinted, with permission, from [65].

cost effectiveness, ease of use, and speed (Table 2). While 2D models are convenient, they present a cell environment unlike that of the native tissue as cells are confined to a monolayer. Strategies to improve the physiological relevance of 2D models include adding multiple cell types [6,7], stratifying cells, incorporating **extracellular matrix (ECM)** components, and dynamic elements [8,9].

Key Table

Table 2. Overview of *In Vitro* Models of the Intestine Innate Immune System

	Transwell	Microfluidic chips	Organoids	3D tissue cultures
Spatial architecture	Planar	Planar	3D	Planar & 3D
Compartmentalization	✓	✓		✓
Anaerobic lumen		✓		✓
Microbiome	✓	✓	✓	✓
Peristalsis		✓		✓
Long-term cultivation (>1 month)				✓
Immune coculture (>1 week)				✓
3D innervation			✓	✓
Epithelial growth area	0.33 cm^2	<0.33 cm^2	N/A	≥0.33 cm^2
Refs	[9,16–18,20]	[25,27,29–31,36]	[42,45,57,79]	[61,64–66,78,80]

Common cell sources for *in vitro* models of the intestinal epithelial barrier include immortalized cell lines such as **Caco-2**, **HT-29**, or **T84** due to ease of use and relatively low cost [6,7,10,11]. Coculturing enterocyte-like Caco-2 with goblet-like HT-29 cell lines, as opposed to monocultures of either of these lines, provided a better mimic of intestinal nutrient absorption and mucus secretion [6]. However, T84 cell lines were better *in vitro* mimics of colonocytes than Caco-2 cells due to their shorter microvilli and their response to **short chain fatty acids**, features that are prominent when moving from the small to the large intestine [11]. A synthetic mucus layer overlaid on a Caco-2 monolayer improved viability and barrier functions with a fed state simulated intestinal fluid over single and cocultures of Caco-2 and HT-29 [7]. Studies also used intestinal organoids that are intestinal stem cells derived directly from the native intestine epithelium without transformation for 2D studies. Unlike organoid studies, 2D models enable easy access to the apical cell surface for feeding and treatment [9,12]. Additionally, 2D models reduce variability in cell morphology so that other variables, such as proliferation, differentiation, self-renewal, and polarization, can be studied to optimize culture conditions [9].

Inflammatory molecules, ECM components, and bacterial cultures can be added to 2D intestinal epithelial barrier models to study tissue response [13–15]. Addition of collagen peptides on the apical side of a Transwell insert counteracted increases in barrier permeability in Caco-2 cells induced by the addition of **TNFα** to the basolateral side of the insert [13]. Furthermore, **lipopolysaccharide (LPS)**-induced inflammation in Caco-2 and **THP-1** cell lines affected the levels of anti-inflammatory cytokine, interleukin 10 (IL-10), and proinflammatory cytokines, IL-1β and TNFα [14]. *Bifidobacterium dentium* enhanced goblet cell function in cocultures with T84 cells, providing a protective advantage for the host [15].

To study interactions between the intestinal epithelium and underlying immune cell populations, 2D Transwell membrane systems with an epithelial monolayer seeded on the top side of the Transwell membrane and a macrophage layer in the bottom compartment are common (Figure 1A) [16,17]. Transwell-based systems can be used to study cell interactions since both anchorage-dependent and anchorage-independent cell lines can be cultured in separate media. This is due to a porous membrane that separates the different cell types and media allowing for migration, invasion, and permeability studies. The porous membrane enables measures of **transepithelial electrical resistance (TEER)**, which provides quantitation of the electrical, ohmic resistance of the membrane bound cell layer. TEER provides a measure of barrier functions and therefore allows for increased physiological relevance of *in vitro* epithelial cell monolayers [18,19]. In one study, TEER was used to investigate the contribution of the intestinal permeability to **celiac disease** [19]. Organoids from celiac disease patients and controls were seeded onto Transwell plates, challenged with **gliadin**, and finally given microbiota-derived byproducts. TEER measurements showed decreased barrier function upon addition of gliadin and improved barrier function upon addition of microbial byproducts [19]. Oxygen perfusion was also used to generate a dynamic Transwell system using affordable and commercially available materials. This setup is especially useful for studies involving coculture with anaerobic microbes that typically only last up to 8 hours in the absence of fluid flow. The addition of perfused oxygen from the basolateral side of the epithelial monolayer increased the TEER of the monolayer and also supported longer-term cultures of over 24 hours in the absence of fluid flow [20].

A coating of ECM protein on transwell membranes or plastic can increase cell viability, differentiation, migration, and proliferation. Common ECM protein coatings include collagen, fibronectin, poly-L-Lysine, and Matrigel [8,9]. Many studies use a collagen coating, as this is a well-established, reliable coating method that mimics in part the native composition of the intestinal stroma [21,22]. Matrigel coatings improved organoid monolayer survival compared with poly-L-

Lysine or collagen coatings, however, this study was conducted using monolayers derived from mouse enteroids and further studies would be needed to confirm this outcome with human enteroids [9]. ECM coated models are important in culturing organoid as these cells have more complex needs and require more remodeling than immortalized cell lines.

Ussing chambers provide a system for studying the transport of nutrients, ions, and drugs across epithelial tissue. An Ussing chamber can be used to take electrophysiological measurements of active transport across a membrane, such as an intestinal epithelium, by eliminating forces like hydrostatic pressure, osmotic pressure, and membrane potential. Ussing chambers are therefore useful for modeling and measuring gut integrity and innate immunity. Ussing chamber models are also used to study *in vitro* constructed epithelial tissues as well as harvested *ex vivo* epithelial tissues [23,24]. While Ussing systems present advantages in limiting outside forces and allowing for *ex vivo* tissue permeability analysis, they are seldom used for *in vitro* studies due to the challenges involved in equipment setup and methodology [23].

The standard for *in vitro* modeling has long been 2D tissue culture and even as newer models emerge that better represent the complexities found *in vivo*, 2D culture remains important. It is fast, straightforward, and cost-effective and consequently imperative to retrieving preliminary data and serving as a control group when testing more complicated *in vitro* modeling platforms.

Intestinal Barrier Models in Microfluidic Systems

Membrane-based microfluidics are comprised of an ECM coated membrane with micro-level fluid flow on either side of the membrane, mimicking the dynamic microenvironment of epithelial tissue (Figure 1B). Multiple studies have investigated the relevance of features such as fluid flow [25–28], oxygen content control [29,30], cyclic membrane stretch [25–27], and whole membrane imaging [31,32] in these systems. Coupled with the capability to culture multiple cell types and microbial species, these systems enable investigation of host–microbe interactions for insights into the intestine's innate immune functions, especially in epithelial barrier defense. Recent advances in organoid cultures have yielded intestine chips using human **colonoids**, **enteroids**, and **induced pluripotent stem cell (iPSC)** organoid cells, although Caco-2 cell lines continue to reveal insights. However, like 2D cultures, cells in microfluidics systems are often seeded in a monolayer, which restricts cell proliferation to a planar growth area (Table 2).

Human colonoids are used to model the large intestine epithelium in membrane-based microfluidic systems to leverage imaging of the whole epithelium in assessing epithelial response to enterohemorrhagic *Escherichia coli* and metabolites [31]. Changes in colonoid mucus production were also studied and showed that colon mucus thickness could be modulated by prostaglandin E2 [32]. While luminal flow was used in these studies, contributions of cyclic stretch and oxygen content were not explored.

Human enteroids are used on membranes in microfluidic systems to model the small intestine epithelium and to investigate the relevance of flow and cyclic stretch [25] on the functions, with comparisons to Caco-2 systems [10,28,33]. In one study, human jejunal enteroids cocultured with heat-stable enterotoxin from enterotoxigenic *E. coli* showed that fluid flow increased cell heights and **cyclic GMP** secretion [25]. In another study, multiple patient-derived enteroids cultured with human intestinal microvascular endothelial cells had more transcriptomic similarities with the native tissue than enteroids in Matrigel culture [33]. Lastly, human enteroids derived from iPSCs cultured in the membrane-based microfluidics showed the formation of villi-like projections and response to **IFNγ** and TNFα, two inflammatory cytokines present in IBD [28].

The lumen of the intestine is largely oxygen-free space and many intestinal microbes are obligate or facultative anaerobes; as a result, controlling the oxygen content is necessary for physiologically relevant cocultures of microbiomes and intestine cells. Oxygen control has been explored in membrane-based microfluidics using Caco-2 cell lines to coculture microbial species [29,30,34]. Anoxic media in the top compartment with an oxygenated bottom compartment resulted in comparable Caco-2 epithelial viabilities between anoxic and oxygenated conditions when cocultured with bacteria [30]. Moreover, low oxygen conditions sustained diversity of a mixed population of bacteria in an oxygen-sensing intestine chip with Caco-2 cells [29]. In the HuMiX microfluidics system, membranes separated anoxic bacterial cultures from the oxygenated epithelial layer, allowing transcription and metabolic and immunologic analyses of host–microbe interactions [34].

Fully oxygenated membrane-based microfluidics systems with Caco-2 cells also contributed important insights into modeling intestinal barrier functions and microbe interactions [26,27,35–39]. Luminal flow and cyclic strain increased intraepithelial cell growth of *Shigella* [26]; however, those mechanical forces also suppressed *E. coli* overgrowth in an earlier study [27]. In one study, multi-organ tissue flow (MOTiF) membrane-based microfluidic chips with dual flow were adapted for a successful triculture of Caco-2, human vascular endothelial cells, and monocyte-derived macrophages with cultures of *Lactobacillus rhamnosus*, a probiotic bacteria, and *Candida albicans*, a pathogenic fungus [35]. The importance of maintaining epithelial barriers for studying the effects of probiotics was demonstrated and the adherence of **peripheral blood mononuclear cells** to the bottom side of the membrane was observed under **dextran sulfate sodium (DSS)** and LPS stimulation [36]. Viral infections were also supported and the apical and basal release of viral loads and cytokines was demonstrated [37]. Characterization of microfluidics with Caco-2 cells showed populations of enterocytes, goblet cells, Paneth, and enteroendocrine cells, as well as a mucus layer, establishing Caco-2 cells as relevant for studying epithelial barrier functions [38]. Moreover, coculture of the Caco-2 system with *L. rhamnosus GG* showed improved Caco-2 differentiation and the formation of villi-like structures [39]. Though organoid cultures have demonstrated improved immunologic responses over Caco-2 cell lines [28], important microfluidics studies using Caco-2s continue to be published.

Alternative microfluidic systems include whole organ and tissue biopsy culture systems [40,41]. In one study, whole mouse intestines were removed and perfused through the lumen, preserving the native cell populations and architecture of the organ for up to 40 hours [40]. This system was used to investigate innate immune and neuronal response to bacteria and demonstrated peristaltic activity. Human tissue biopsy samples of intestines have also been integrated into microfluidics systems with sustained culture for 72 hours and separation between luminal and bulk media flow [41]. Biopsies in the system from IBD patients showed sustained release of calprotectin, a marker for inflammation.

Dynamic culture conditions, cyclic stretch, and anaerobic sensing are unique features of microfluidic models. These models can highlight how mechanical influences such as shear stress and epithelial stretch impact the epithelial barrier and culture with microbes.

Innate Immunity in Intestinal Organoids via Matrigel Droplet Culture

Intestinal organoids are hollow multicellular structures derived from the small or large intestine **LGR5**+ epithelial cell populations and can be indefinitely propagated as a nontransformed and stable cell population in Matrigel droplets (Figure 1C). While organoids can display features of the derived intestine tissue, spatial control of organoids for coculture is difficult and compartmentalization of difference cell populations is not possible (Table 2).

Accessing the apical side of intestinal organoids, which mimics the lumen of the intestine, is challenging; however, techniques such as fragmentation and microinjection have supported cultures of pathogens, including *Toxoplasma gondii* [42], *Cryptosporidium parvum* [43], *Salmonella typhimurium* [44], *and Lactobacillus acidophilus* [44] within the lumen of mouse and human intestinal organoids. The successful infection of genetically modified *T. gondii* in mouse Intestinal organoids [42] and *C. parvum* in human enteroids enabled studies to explore these organisms. For *C. parvum*, the parasite completed its life cycle, validating organoids as a physiologically relevant platform to study parasitic infections [43]. *L. acidophilus* cultured within organoids via fragmentation prevented organoid disruption, while culturing *S. typhimurium* elevated TNFα secretion [44]. Basal cocultures of immune cells were also performed by mixing cell suspensions of bone marrow-derived dendritic cells (BMDCs) [45], or lamina propria leukocytes (LPLs) [45] with dissociated organoids in liquid Matrigel prior to droplet formation. Cocultures of LPLs or BMDCs with mice intestinal organoids showed that adhesion of LPLs or BMDCs to organoids resulted in large cystic morphologies with goblet cell depletion [45].

Monocultures of intestinal organoids were used to investigate intestinal immunity to derive mechanistic insight. Human intestinal organoids were propagated directly from patient intestinal biopsies to model innate immunity in IBD [46–48], very early onset IBD [49], **cystic fibrosis** [50], and celiac disease [19]. In a study using organoids established from inflamed intestinal epithelial regions of patients with **ulcerative colitis**, increased frequencies of mutations were detected compared with organoids from uninflamed regions [46]. Furthermore, **IL-17** was found to indirectly promote expansion of mutated cells [46]. Single cell gene expressions showed that stem cells from active **Crohn's disease** lesion sites had altered expression of stem cell markers and organoid reformation rates compared with healthy patients and those undergoing remission, suggesting the inflammatory environment of Crohn's modifies intestinal stem cell properties [47]. **Butyrate** consumption in organoids from IBD patients were found to remain similar to organoids from non-IBD controls [48]. Additionally, TNFα treatment lowered responsiveness to butyrate in differentiated organoids compared with organoids without TNFα treatment [48]. Investigations on epithelial cell death response in organoids derived from patients with very early onset IBD showed that caspase-8 deficient intestinal epithelial cells had impaired apoptotic functions [49]. Intestinal organoids derived from cystic fibrosis patient-iPSCs were cultured and their potential to model cystic fibrosis was demonstrated [50]. Gene expression analysis of celiac patient organoids showed differential gene expression profiles related to barrier function, innate immunity, and stem cell function compared with non-celiac controls [19].

Human intestinal organoids were also utilized to validate conclusions derived from mouse intestinal organoids [51,52] and cell lines [53]. Human enteroids and colonoids showed that mouse organoids recapitulated IFNγ-mediated apoptosis in human intestinal stem cells [51]. **IFNβ** stimulation in human and mice colonoids validated mouse intestinal organoids for investigating type 1 interferon responses [52]. IFNγ-mediated increases in permeability and changes in expression of claudin-2 in T84 and HT-29 cell lines were ameliorated with Janus kinase inhibition and was confirmed in human intestinal organoids [53].

Mouse intestinal organoids derived from genetically engineered mice such as the **Casp8** $^{\Delta IEC}$ [54], **Card15/Nod2-deficient** [55], **TNF**$^{\Delta ARE}$ [56], **IL17A**$^{-/-}$ [57], and **H19**$^{-/-}$ [58] mice were used to derive mechanistic insights into innate immunity responses in humans. Casp8 $^{\Delta IEC}$ mice were used to show that Paneth cell death was mediated by high levels of IFNγ. This relationship was observed in patient ileum tissue with severe inflammation; it was also observed that inhibiting **STAT1** ameliorated Paneth cell death [54]. In one study, muramyl dipeptide, a bacterial peptidoglycan, protected intestinal stem cells against autophagy by inducing destruction of the

mitochondria, resulting in lowered levels of reactive oxygen species under cell stress [55]. Intestinal organoids from $TNF^{\Delta ARE}$ mice showed that inflammation impaired the growth of organoids, suggesting that inflammation impaired the function and growth of LGR5+ intestinal stem cells and Paneth cells [56]. Investigation of CXCL10, a proinflammatory cytokine, found that while IL-17 decreased CXCL10 production in healthy mice colonoids, IL-17 had no effect on CXCL10 levels in DSS inflamed mice organoids [57]. Mouse organoids from $H19^{-/-}$ mice showed increased lysozyme and mucin 2 positive cells with an increased tolerance to LPS [58].

Intestinal organoids are derived from patient biopsies and recapitulate many features of the patient. However, functional characteristics are difficult to measure and control over spatial distribution of other cell populations is not possible. As a result, these organoids may be seeded on other *in vitro* model platforms to better characterize epithelial interactions with mechanical forces and other cell types.

3D Tissue Models of Intestine Innate Immune Functions

To recapitulate the complex architectural and cellular environments found *in vivo*, 3D bioengineered tissue models of the human intestine have been developed using scaffolds and multiple intestinal cell populations (Figure 1D). These share common features with other transwells, microfluidic chips, or organoids, such as spatial architecture, anaerobic conditions, microbiome colonization, peristalsis, and innervation (Table 2). However, 3D tissue models have new features that other systems cannot recapitulate, including long-term immune coculture and an increased epithelial growth area (Table 2).

A 3D intestinal coculture model involving an intestinal epithelial cell line, macrophages, and dendritic cells was used to assess the inflammatory responses to nanoparticles [59]. The model mimicked diseased conditions and therefore was suitable for the assessment of the inflammatory effects of engineered nanomaterials. However, the effects of proinflammatory cytokines and hypoxia conditions were not considered in the study. More recently, a poly(*N*-isopropylacrylamide) hydrogel scaffold was used to support the growth of a mixed population of intestinal cells under dynamic conditions to develop an *in vitro* intestinal epithelium model [60]. After treatment with proinflammatory cytokines IL-1β, TNFα, or varying hypoxic conditions, the model mimicked the inflammatory response observed during IBD. Many other studies have exploited 3D scaffolding and 3D printing technology to obtain *in vitro* models of the intestine using primary epithelial cells to study infection and inflammation [61–63]. Findings from those studies further demonstrated that 3D intestinal tissues can mediate injury responses to compound- or bacteria-induced toxicity and inflammation by releasing the proinflammatory cytokines, such as IL-1 and TNFα, which resembles human IBD. The implementation of these tissue engineering approaches may help to ensure drug safety and efficacy during drug development.

Human intestinal enteroids or colonoids can also be disassociated and grown as polarized epithelial monolayers on 3D cell culture platforms to generate the native human intestinal epithelium conditions. For example, human intestinal enteroid-derived epithelial cells can be grown in a 3D silk-collagen protein composite scaffold to resemble the human intestine [64]. Upon infection with *E. coli*, genes involved in the innate immune response were significantly upregulated in this 3D tissue model. In addition, many of these upregulated genes are associated with human IBD. These findings demonstrated that intestinal epithelial cells actively participate in immunological processes in the intestinal mucosa. More recently, a novel system using human colonoids and human primary monocyte-derived macrophages cultured in a bi-layered silk scaffold sponge was established [65]. This *in vitro* model exhibits different phenotypic responses to inflammation

through disruption of epithelial tight junctions, macrophage migration/invasion, and the secretion of cytokines associated with IBD. The results also confirmed that the immune cell component in intestinal epithelial models was important to better understand innate immunity in the small intestine. Additional systems with neuronal cultures have enabled investigation of interactions with the enteric nervous system, enabling studies of neuroinflammation [66].

In summary, the implementation of these tissue engineering approaches may help to ensure drug safety and efficacy during drug development and advance current IBD research practices.

Concluding Remarks and Future Perspectives

Current investigative methods into the intestine innate immune system for autoimmune diseases, including IBD and celiac disease, have yielded few clinical treatments, inconsistent patient responses, and high costs for these treatments. Current discovery relies heavily on mouse models, which have provided general insights in immunology but due to inherent differences in biology, drug targets are often unable to translate to human systems and thus the clinic. Furthermore, mouse models have resulted in the identification of the most obvious drug targets, while a new paradigm in drug discovery is critically needed to refine human system insights for the next generation of targeted and effective treatments for these diseases.

To accelerate and improve methods of identifying cellular pathways leading to effective clinical treatments, investigators have continued to develop various *in vitro* models, taking advantage of advances in human intestine biology, stem cell biology, and bioengineering. The *in vitro* platforms developed can be categorized into 2D cultures, microfluidic devices, organoids, and 3D biomimetic tissue cultures. The 2D cultures and microfluidic devices present planar cell culture surfaces to study cell interactions. However, microfluidics devices enable dynamic culture conditions, whereas 2D cultures are in static culture conditions. The 3D biomimetic tissue cultures and organoids present 3D cell culture environments. However, 3D cultures enable spatial control over cell populations and compartmentalization.

Successful development of model systems with high relevance to human biology is a necessary technological leap towards discovering future treatments for diseases of the intestinal innate immune system (see Outstanding Questions). Specific areas of development include multicellular interactions between bacterial, epithelial, immune, mesenchymal, and neural cell types. By focusing on increasing complexity of cell–cell interactions, *in vitro* models will be more physiologically relevant; 3D biomimetic tissue models are especially suitable for this challenge due to spatial control of multiple cell populations. Combined with perfusion capabilities, 3D models can recapitulate dynamic culture conditions found *in vivo*. Where mouse models fall short, developments from *in vitro* tissues models should aid efforts to understand these diseases, leading to improved treatments.

Acknowledgments

We thank Professor Ralph R. Isberg for helpful suggestions and edits. We also thank the NIH (U19AI131126, R21AI128093, P41EB027062) for support of this work.

References

1. Ouellette, A.J. (2006) Paneth cell α-defensin synthesis and function. In *Antimicrobial Peptides and Human Disease* (Shafer, W.M., ed.), pp. 1–25, Springer
2. Doeing, D.C. *et al.* (2003) Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin. Pathol.* 3, 3
3. Nagpal, R. *et al.* (2018) Comparative microbiome signatures and short-chain fatty acids in mouse, rat, non-human primate, and human feces. *Front. Microbiol.* 9, 2897
4. Sato, T. *et al.* (2009) Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche. *Nature* 459, 262

Outstanding Questions

Bacterial-epithelial cocultures tend to have short culture times (acute responses) due to destruction of the epithelium layer, but the intestine maintains a steady state with the microbiome. How can *in vitro* models enable coculture with microbial populations for longer than a couple of days, thus providing both acute and chronic studies relevant to various disease states?

The mucus layer in the large intestine is ~100 μm thick but *in vitro* models fail to achieve this level of mucus thickness. How can tissue models improve intestinal epithelial mucus secretion?

Intestinal fibroblasts play an important role in intestinal barrier maintenance and innate immune responses. How do intestinal fibroblast populations contribute to epithelial innate immune functions in intestinal innate immune tissue models?

How does the intestinal nervous system interact with epithelial barrier functions and inflammation responses in normal and disease states in the *in vitro* tissue models?

How does combining 3D tissue engineering techniques with flow perfusion affect *in vitro* modeling of the intestine mucosa?

Mouse models do not recapitulate aspects of intestine immunity; how do *in vitro* models with human cells compare against mouse models for drug discovery and mechanisms?

The intestine epithelium contains crypts, with the small intestine also having villi macrostructures. Does the topography of *in vitro* intestinal epithelium affect the innate immune defense?

The intestine is subject to variations in food consumption, digestion, and related differences. How can food processing, in combination with microbiomes and *in vitro* intestine models, be developed and utilized to assess the impact of nutrient content, food choices, and probiotics on tissue function in normal and disease states?

5. Sato, T. *et al.* (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 141, 1762–1772
6. Lozoya-Agullo, I. *et al.* (2017) Usefulness of Caco-2/HT29-MTX and Caco-2/HT29-MTX/Raji B coculture models to predict intestinal and colonic permeability compared to Caco-2 monoculture. *Mol. Pharm.* 14, 1264–1270
7. Birch, D. *et al.* (2018) Evaluation of drug permeation under fed state conditions using mucus-covered Caco-2 cell epithelium. *Eur. J. Pharm. Sci.* 118, 144–153
8. Liu, Y. *et al.* (2018) Monolayer culture of intestinal epithelium sustains Lgr5+ intestinal stem cells. *Cell Discov.* 4, 32
9. Thorne, C.A. *et al.* (2018) Enteroid monolayers reveal an autonomous WNT and BMP circuit controlling intestinal epithelial growth and organization. *Dev. Cell* 44, 624–633
10. Kasendra, M. *et al.* (2020) Duodenum intestine-chip for preclinical drug assessment in a human relevant model. *eLife* 9, e50135
11. Devriese, S. *et al.* (2017) T84 monolayers are superior to Caco-2 as a model system of colonocytes. *Histochem. Cell Biol.* 148, 85–93
12. Noel, G. *et al.* (2017) Enterotoxigenic *Escherichia coli* is phagocytosed by macrophages underlying villus-like intestinal epithelial cells: modeling *ex vivo* innate immune defenses of the human gut. *Gut Microbes* 9, 382–389
13. Chen, Q. *et al.* (2017) Collagen peptides ameliorate intestinal epithelial barrier dysfunction in immunostimulatory Caco-2 cell monolayers via enhancing tight junctions. *Food Funct.* 8, 1144–1151
14. Lopez, Z. *et al.* (2019) *In vitro* immunomodulatory effect of food supplement from *Aloe vera*. *Evid. Based Complement. Alternat. Med.* 2019, 5961742
15. Engevik, M.A. *et al.* (2019) *Bifidobacterium dentium* fortifies the intestinal mucus layer via autophagy and calcium signaling pathways. *mBio* 10, e01087-19
16. Noel, G. *et al.* (2017) A primary human macrophage-enteroid coculture model to investigate mucosal gut physiology and host-pathogen interactions. *Sci. Rep.* 7, 45270
17. Kämpfer, A.A.M. *et al.* (2017) Development of an *in vitro* coculture model to mimic the human intestine in healthy and diseased state. *Toxicol. In Vitro* 45, 31–43
18. Altay, G. *et al.* (2019) Self-organized intestinal epithelial monolayers in crypt and villus-like domains show effective barrier function. *Sci. Rep.* 9, 10140
19. Freire, R. *et al.* (2019) Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. *Sci. Rep.* 9, 7029
20. Fofanova, T. *et al.* (2019) A novel human enteroid-anaerobe coculture system to study microbial-host interaction under physiological hypoxia. *bioRxiv* Published online February 20, 2019. https://doi.org/10.1101/555755
21. Hosmillo, M. *et al.* (2020) Norovirus replication in human intestinal epithelial cells is restricted by the interferon-induced JAK/STAT signaling pathway and RNA polymerase II-mediated transcriptional responses. *mBio* 11, e00215-20
22. Anzai, S. *et al.* (2020) TGF-β promotes fetal gene expression and cell migration velocity in a wound repair model of untransformed intestinal epithelial cells. *Biochem. Biophys. Res. Commun.* 524, 533–541
23. Thomson, A. *et al.* (2019) The Ussing chamber system for measuring intestinal permeability in health and disease. *BMC Gastroenterol.* 19, 98
24. Lobo de Sá, F.D. *et al.* (2019) Curcumin mitigates immune-induced epithelial barrier dysfunction by *Campylobacter jejuni*. *Int. J. Mol. Sci.* 20, 4830
25. Sunuwar, L. *et al.* (2019) Mechanical stimuli affect *E. coli* heat stable enterotoxin (ST)-cyclic GMP signaling in a human enteroid intestine-chip model. *Infect. Immun.* 88, e00866-19
26. Grassart, A. *et al.* (2019) Bioengineered human organ-on-chip reveals intestinal microenvironment and mechanical forces impacting *Shigella* infection. *Cell Host Microbe* 26, 435–444
27. Kim, H.J. *et al.* (2016) Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc. Natl. Acad. Sci. U. S. A.* 113, E7
28. Workman, M.J. *et al.* (2017) Enhanced utilization of induced pluripotent stem cell-derived human intestinal organoids using microengineered chips. *Cell. Mol. Gastroenterol. Hepatol.* 5, 669–677
29. Jalili-Firoozinezhad, S. *et al.* (2019) A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat. Biomed. Eng.* 3, 520–531
30. Shin, W. *et al.* (2019) A robust longitudinal co-culture of obligate anaerobic gut microbiome with human intestinal epithelium in an anoxic-oxic interface-on-a-chip. *Front. Bioeng. Biotechnol.* 7, 13
31. Tovaglieri, A. *et al.* (2019) Species-specific enhancement of enterohemorrhagic *E. coli* pathogenesis mediated by microbiome metabolites. *Microbiome* 7, 43
32. Sontheimer-Phelps, A. *et al.* (2019) Human colon-on-a-chip enables continuous *in vitro* analysis of colon mucus layer accumulation and physiology. *Cell. Mol. Gastroenterol. Hepatol.* 9, 507–526
33. Kasendra, M. *et al.* (2018) Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. *Sci. Rep.* 8, 2871
34. Shah, P. *et al.* (2016) A microfluidics-based *in vitro* model of the gastrointestinal human–microbe interface. *Nat. Commun.* 7, 11535
35. Maurer, M. *et al.* (2019) A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies. *Biomaterials* 220, 119396
36. Shin, W. and Kim, H.J. (2018) Intestinal barrier dysfunction orchestrates the onset of inflammatory host–microbiome cross-talk in a human gut inflammation-on-a-chip. *Proc. Natl. Acad. Sci. U. S. A.* 115, E10539
37. Villenave, R. *et al.* (2017) Human gut-on-a-chip supports polarized infection of Coxsackie B1 virus *in vitro*. *PLoS One* 12, e0169412
38. Kim, H.J. and Ingber, D.E. (2013) Gut-on-a-chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integr. Biol.* 5, 1130–1140
39. Kim, H.J. *et al.* (2012) Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* 12, 2165–2174
40. Yissachar, N. *et al.* (2017) An intestinal organ culture system uncovers a role for the nervous system in microbe-immune crosstalk. *Cell* 168, 1135–1148
41. Dawson, A. *et al.* (2016) A microfluidic chip based model for the study of full thickness human intestinal tissue using dual flow. *Biomicrofluidics* 10, 064101
42. Luu, L. *et al.* (2019) An open-format enteroid culture system for interrogation of interactions between *Toxoplasma gondii* and the intestinal epithelium. *Front. Cell. Infect. Microbiol.* 9
43. Heo, I. *et al.* (2018) Modelling *Cryptosporidium* infection in human small intestinal and lung organoids. *Nat. Microbiol.* 3, 814–823
44. Lu, X. *et al.* (2020) *Lactobacillus* protects against *S. typhimurium*–induced intestinal inflammation by determining the fate of epithelial proliferation and differentiation. *Mol. Nutr. Food Res.* 64, 1900655
45. Ihara, S. *et al.* (2018) Adhesive interactions between mononuclear phagocytes and intestinal epithelium perturb normal epithelial differentiation and serve as a therapeutic target in inflammatory bowel disease. *J. Crohn's Colitis* 12, 1219–1231
46. Nanki, K. *et al.* (2020) Somatic inflammatory gene mutations in human ulcerative colitis epithelium. *Nature* 577, 254–259
47. Suzuki, K. *et al.* (2018) Single cell analysis of Crohn's disease patient-derived small intestinal organoids reveals disease activity-dependent modification of stem cell properties. *J. Gastroenterol.* 53, 1035–1047
48. Ferrer-Picón, E. *et al.* (2019) Intestinal inflammation modulates the epithelial response to butyrate in patients with inflammatory bowel disease. *Inflamm. Bowel Dis.* 26, 43–55
49. Lehle, A.S. *et al.* (2019) Intestinal inflammation and dysregulated immunity in patients with inherited caspase-8 deficiency. *Gastroenterology* 156, 275–278
50. Mithal, A. *et al.* (2020) Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells. *Nat. Commun.* 11, 215

Intestinal diseases and treatment efficiencies vary with sex, age, and many related factors. How can such variations be captured in these *in vitro* tissue models to better match disease state, patient specifics, and treatments?

51. Takashima, S. *et al.* (2019) T cell–derived interferon-γ programs stem cell death in immune-mediated intestinal damage. *Sci. Immunol.* 4, eaay8556
52. Neil, J.A. *et al.* (2019) IFN-I and IL-22 mediate protective effects of intestinal viral infection. *Nat. Microbiol.* 4, 1737–1749
53. Sayoc-Becerra, A. *et al.* (2019) The JAK-inhibitor tofacitinib rescues human intestinal epithelial cells and colonoids from cytokine-induced barrier dysfunction. *Inflamm. Bowel Dis.* 26, 407–422
54. Günther, C. *et al.* (2019) Interferon lambda promotes Paneth cell death via STAT1 signaling in mice and is increased in inflamed ileal tissues of patients with Crohn's disease. *Gastroenterology* 157, 1310–1322
55. Levy, A. *et al.* (2020) Innate immune receptor NOD2 mediates $LGR5^{+}$ intestinal stem cell protection against ROS cytotoxicity via mitophagy stimulation. *Proc. Natl. Acad. Sci. U. S. A.* 117, 1994
56. Khaloian, S. *et al.* (2020) Mitochondrial impairment drives intestinal stem cell transition into dysfunctional Paneth cells predicting Crohn's disease recurrence. *Gut* Published online February 28, 2020. https://doi.org/10.1136/gutjnl-2019-319514
57. Walrath, T. *et al.* (2019) IFN-γ and IL-17A regulate intestinal crypt production of CXCL10 in the healthy and inflamed colon. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318, G479–G489
58. Yu, T-X. *et al.* (2019) Long noncoding RNA H19 impairs the intestinal barrier by suppressing autophagy and lowering Paneth and goblet cell function. *Cell. Mol. Gastroenterol. Hepatol.* 9, 611–625
59. Susewind, J. *et al.* (2016) A 3D co-culture of three human cell lines to model the inflamed intestinal mucosa for safety testing of nanomaterials. *Nanotoxicology* 10, 53–62
60. Dosh, R.H. *et al.* (2019) Long-term *in vitro* 3D hydrogel co-culture model of inflammatory bowel disease. *Sci. Rep.* 9, 1812
61. Shaban, L. *et al.* (2018) A 3D intestinal tissue model supports *Clostridioides difficile* germination, colonization, toxin production and epithelial damage. *Anaerobe* 50, 85–92
62. Madden, L.R. *et al.* (2018) Bioprinted 3D primary human intestinal tissues model aspects of native physiology and ADME/Tox functions. *iScience* 2, 156–167
63. Giuffrida, P. *et al.* (2019) Decellularized human gut as a natural 3D platform for research in intestinal fibrosis. *Inflamm. Bowel Dis.* 25, 1740–1750
64. Chen, Y. *et al.* (2017) *In vitro* enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses. *PLoS One* 12, e0187880
65. Roh, T.T. *et al.* (2019) 3D bioengineered tissue model of the large intestine to study inflammatory bowel disease. *Biomaterials* 225, 119517
66. Manousiouthakis, E. *et al.* (2019) Bioengineered *in vitro* enteric nervous system. *J. Tissue Eng. Regen. Med.* 13, 1712–1723
67. Gustafsson, J.K. *et al.* (2012) An *ex vivo* method for studying mucus formation, properties, and thickness in human colonic biopsies and mouse small and large intestinal explants. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302, G430–G438
68. Förster, C. (2008) Tight junctions and the modulation of barrier function in disease. *Histochem. Cell Biol.* 130, 55–70
69. Gribble, F.M. and Reimann, F. (2019) Function and mechanisms of enteroendocrine cells and gut hormones in metabolism. *Nat. Rev. Endocrinol.* 15, 226–237
70. Nezami, B.G. and Srinivasan, S. (2010) Enteric nervous system in the small intestine: pathophysiology and clinical implications. *Curr. Gastroenterol. Rep.* 12, 358–365
71. Fournier, B.M. and Parkos, C.A. (2012) The role of neutrophils during intestinal inflammation. *Mucosal Immunol.* 5, 354–366
72. Roulis, M. and Flavell, R.A. (2016) Fibroblasts and myofibroblasts of the intestinal lamina propria in physiology and disease. *Differentiation* 92, 116–131
73. Artegiani, B. *et al.* (2020) Fast and efficient generation of knock-in human organoids using homology-independent CRISPR–Cas9 precision genome editing. *Nat. Cell Biol.* 22, 321–331
74. Roberts, G.P. *et al.* (2019) Comparison of human and murine enteroendocrine cells by transcriptomic and peptidomic profiling. *Diabetes* 68, 1062–1072
75. Gibbons, D.L. and Spencer, J. (2011) Mouse and human intestinal immunity: same ballpark, different players; different rules, same score. *Mucosal Immunol.* 4, 148–157
76. Roe, M.M. *et al.* (2017) Differential regulation of CD103 (αE integrin) expression in human dendritic cells by retinoic acid and Toll-like receptor ligands. *J. Leukoc. Biol.* 101, 1169–1180
77. Nguyen, T.L.A. *et al.* (2015) How informative is the mouse for human gut microbiota research? *Dis. Model. Mech.* 8, 1–16
78. Workman, M.J. *et al.* (2017) Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat. Med.* 23, 49–59
79. Chen, Y. *et al.* (2015) Robust bioengineered 3D functional human intestinal epithelium. *Sci. Rep.* 5, 13708
80. Zhou, W. *et al.* (2018) Multifunctional bioreactor system for human intestine tissues. *ACS Biomater. Sci. Eng.* 4, 231–239