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Gut-on-chip devices as intestinal inflammation models and their future for studying multifactorial diseases

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Inflammatory bowel disease (IBD), celiac disease, and other inflammatory conditions of the gastrointestinal tract are highly prevalent in society. Due to the complexity of factors involved, detailed pathogenesis is difficult to determine and thus remains elusive in many cases. The advent of gut-on-chip devices has permitted more accurate modeling of the gut microenvironment with the inclusion of fluid flow and mechanical cues that are true to intestinal architecture and physiology. This review outlines the evolution of gut-on-chip platforms towards greater physiological relevance and elucidates how these devices have been used to model and study intestinal inflammation in humans. In addition, we identify key elements from both technological and disease standpoints that are integral for accurate gut-on-chip models of IBD and celiac disease.

KEYWORDS

gut-on-a-chip, celiac disease, IBD, tissue engineering, intestinal epithelium

1 Introduction

The small and large intestines, which make up a considerable part of the gastrointestinal tract, each have distinct mucosal structures and microenvironments. Both anatomical parts contribute diversely to physiological functions such as digestion, nutrient absorption, microbial interactions, and immune responses. Throughout life, the cellular and molecular composition of the epithelium that lines the mucosa changes in response to evolving functional requirements and environmental exposures (Rodríguez et al., 2015; Elmentaite et al., 2021). Some of these environmental factors, along with diet and microbiota, are thought to contribute to the development of inflammatory bowel disease (IBD), celiac disease and other inflammatory conditions in genetically predisposed individuals (Liu et al., 2015; Huang et al., 2017; Guan, 2019; Krishnareddy, 2019). Accurately modeling the *in vivo* gut environment, as well as aberrations resulting in inflammation, is therefore crucial for further understanding the pathogenesis of chronic gastrointestinal conditions and for investigating effective treatments.

Conventional methods for recreating the intestinal environment for research purposes comprise immortalized cell lines or primary cell culture in traditional culture wells and Transwell inserts, as well as everted gut sacs (Alam et al., 2012; Yamaura et al., 2016; Altay et al., 2019). While these models have been vital for studying, for example, barrier function

and drug metabolism, they do not completely recapitulate the three-dimensional architecture and dynamic microenvironment of the small and large intestines. Although significant developments have been made to incorporate stem cells and organoids, many functional properties and supporting cell populations are still unaccounted for in static cultures (Spence et al., 2011; Gómez and Boudreau, 2021). In terms of inflammatory conditions, conventional methods are largely unable to depict features that are essential for modeling their multifactorial nature, such as the mechanical forces acting on cells and immune and microbe components. These limitations have led to the development of gut-on-chip devices.

In general, organ-on-chip models are microfluidic devices predominantly made of clear, flexible polymers into which several hollow channels or chambers are molded. Specific organs can be mimicked by culturing relevant cell types in the device, which is designed to allow control over parameters such as concentration gradients, mechanical forces, cell interactions, and tissue morphology (Wu et al., 2020). Both single-organ and multi-organ systems have been successfully established, where the latter are typically simpler biologically and prioritize the systemic interactivity between organs. Single-organ organ-on-chips have a greater focus on the detailed function of an organ. For example, by combining biomaterials, microfluidics, and cell culture, gut-on-chip devices enable the recreation of an epithelial barrier of polarized cells with high integrity and functionality. Moreover, incorporation of fluid flow and mechanical deformation supports cell differentiation and facilitates coculture, such as with microbial symbionts, without the overgrowth that is common in static models (Kim et al., 2012). The constantly evolving organ-on-chip technology therefore provides an opportunity for studying the multiple aspects at play in the development and progression of intestinal inflammatory conditions.

2 Human gut microenvironment in normal and inflammatory states

The gut lumen is a hollow, tubular passage lined with a protective barrier composed of a diverse range of intestinal epithelial cells (IECs). In the small intestine, the monolayer of IECs forms a crypt-villus axis that affects fluid flow and significantly increases the surface area for the absorption of nutrients (Clevers, 2013). Imaging of the different regions of the small intestine has revealed that villi are taller and more prevalent proximally in the duodenum and jejunum than distally in the ileum (Holmes et al., 1961). Absorptive enterocytes, which largely enable digestive and metabolic functions, make up the majority of the cell population, while several types of secretory cells perform more specialized roles within the epithelium. For instance, goblet cells secrete mucins to produce a physical mucous barrier that prevents large particles from directly contacting the epithelium and enteroendocrine cells produce and release hormones in response to different stimuli (Kim and Khan, 2013; Gribble and Reimann, 2019). Other secretory cells include immune response-mediating tuft cells and Paneth cells, which secrete antimicrobial molecules into the lumen for regulating the gut microbiota (Bevins and Salzman, 2011). In addition, Paneth cells are located adjacent to intestinal stem cells (ISCs) in the intestinal crypts, where they support the rapid renewal of the epithelium (Clevers and Bevins, 2013). Self-renewal and regeneration in response to injury are driven by the production of daughter progenitors by ISCs in the crypts (Clevers, 2013). Subsequently, these progenitor cells migrate towards the luminal surface and undergo multilineage differentiation to generate the different types of IECs.

Longitudinally, the large intestine can also be divided into parts encompassing the cecum, colon, rectum, and anus. In contrast to the small intestine, IECs in the large intestine are arranged into inner crypts which open into a flat luminal surface. Although the cellular compositions are largely similar, cell analyses have revealed that the large intestine houses a greater number of goblet cells and less Paneth cells (Specian and Oliver, 1991; Tanaka et al., 2001). These differences support a shift in function towards the absorption of water, electrolytes, and vitamins, as well as the formation of feces from indigestible and unabsorbed residues. Despite the distinctions between the small and large intestines, IECs collectively establish and maintain both a physical and biochemical barrier, offering protection from harmful pathogens and various other inflammatory stimuli. When the epithelium is intact, mucosal permeability is determined by the dynamic proteins that compose tight junctions, which regulate the paracellular transport of water, ions, and other molecules (Rescigno, 2011; Suzuki, 2012).

IECs also provide an interface for epithelial-microbe-immune interactions to further support a finely tuned system that maintains a delicate balance between the host and its microbial inhabitants. Thus, another important regulator of the gut microenvironment is the microbiota, which mostly comprises non-pathogenic bacteria aiding in metabolism, barrier function, and immune response stimulation (Hillman et al., 2017). Similar to cell composition, microbiota have also been found to have spatial variability throughout the intestines due to differences in flow rate and pH in the lumen (McHardy et al., 2013). Additionally, the distribution of microorganisms is largely dependent on diet, age, and the presence of an oxygen gradient in the mucosa (Rinninella et al., 2019). Luminal microbiota and mucosa-associated microbiota located in the mucus layer can affect the integrity of the gut barrier through activation of signaling pathways in IECs. Conversely, the composition of the microbiota can be modulated by signaling molecules and alternative energy sources provided by IECs. Similar communication occurs between IECs and immune cells located in the lamina propria underlying the IEC layer, which also contains blood vessels, lymphatic vessels, and nerves that further regulate intestinal motility and nutrient transport. IECs mediate the crosstalk between microbiota and immunologically active cells (T cells, B cells, dendritic cells, macrophages) as well as other luminal contents, often resulting in the adjustment of cytokine secretion to maintain intestinal homeostasis.

Furthermore, luminal flow, and peristalsis, which make up the intestinal mechanical microenvironment, are responsible for gut motility and in part inhibit microbial overgrowth. Peristalsis is the result of layers of smooth muscle contracting to propel food through the intestines and as the luminal contents move, shear forces result from the friction against the intestinal wall. These mechanical forces also act on IECs, contributing to normal epithelial differentiation, mucus production, and the establishment of stable symbiosis between the epithelium and resident gut microbiome (Kim et al., 2016; Jeon et al., 2022). In summary, establishing an *in vitro*

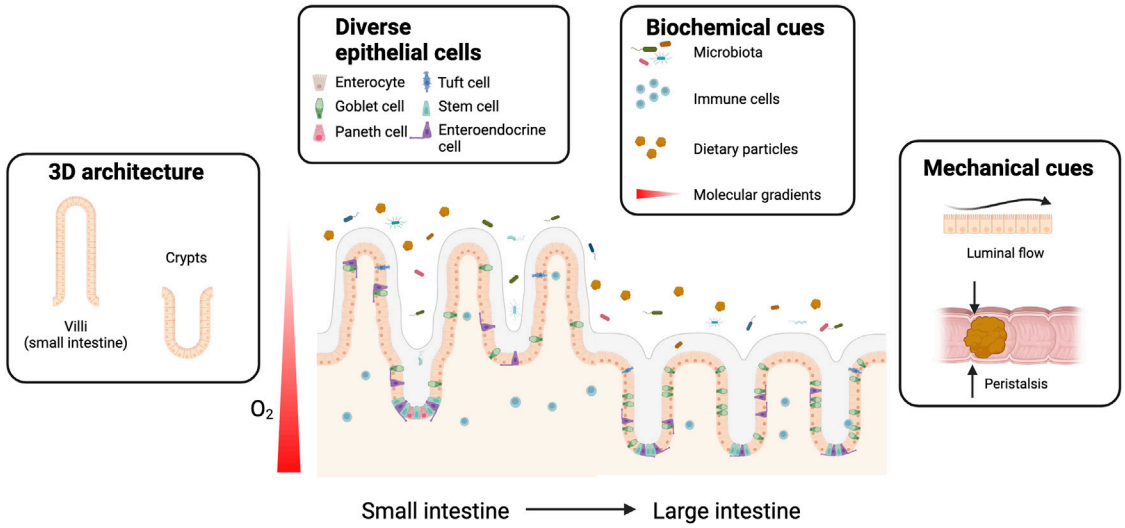

FIGURE 1
Gut-on-chip platforms enable mimicking of the human gut microenvironment. The microenvironment of the human intestines is variable depending on the anatomical region, but several common elements can be pinpointed as integral for normal functioning. These elements include three-dimensional (3D) tissue architecture, epithelial cellular diversity and various biochemical, and mechanical cues. Organ-on-chip technology can facilitate the generation of a more accurate *in vitro* intestinal model consisting of a combination of these features.

intestinal model that more closely resembles the native tissue requires incorporation of the diverse range of IECs and supporting cells, correct epithelial barrier morphology, mechanical forces, and the gut microbiota (Figure 1).

Intestinal diseases like IBD (Crohn's disease, ulcerative colitis) and celiac disease are characterized by chronic inflammation that affects various regions of the small and large bowels. Although there is some variation between patients, ulcerative colitis typically manifests within the colon, celiac disease in the small intestine, and Crohn's disease throughout the gastrointestinal tract. Many factors are thought to cause changes in the gut microenvironment and in turn contribute to the development and perpetuation of a dysregulated inflammatory response in these conditions. The proposed mechanisms of IBD suggest an interplay between genetic susceptibility, immune abnormalities, microbiota, and environmental factors (Strober et al., 2007; Lee et al., 2018). The sequence of pathogenetic events is unknown, but include microbial dysbiosis, compromised intestinal epithelium integrity, aberrant immune reactivity against antigens, and increased production of proinflammatory cytokines (Zeissig et al., 2007; Kamada et al., 2013; de Souza and Fiocchi, 2016; Parker et al., 2019). Eventually, a sustained inflammatory state can result in structural damage of the mucosa and increased IEC death coupled with decreased cell turnover (Parker et al., 2019). In celiac disease, small-bowel mucosal inflammation, crypt hyperplasia, and villous atrophy are a result of inappropriate immune responses to certain gluten-derived peptides from wheat, rye, and barley. Repetitive glutamine- and proline-rich regions in gluten resist proteolytic degradation and initiate both adaptive and innate immune responses, eventually leading to mucosal inflammation and IEC destruction (Hausch et al., 2002; Lindfors et al., 2019). Although some of the hallmarks of IBD and celiac disease have been identified, further understanding of the pathogenesis and development of possible novel treatments for these conditions relies on the generation of more accurate *in vitro* models.

3 Conventional models

To an extent, the compositions of the mouse small and large intestines are comparable to those of humans, with similar cell types and distributions. Thus, chemically induced, genetically engineered, spontaneous and transgenic murine models have been commonly employed to study different aspects involved in intestinal inflammatory diseases (Prattis and Jurjus, 2015; Mizoguchi et al., 2016; Wirtz et al., 2017). Despite the similarities, the translation of results to humans requires the consideration of significant differences in areas such as microbiota, diet, anatomical structure, and size. Particularly, while mouse models can mimic the phenotypes of inflammatory diseases, they are not as suitable for determining underlying mechanisms and etiology of multifactorial diseases in humans (Jiminez et al., 2015).

The need for more cost-effective, ethically acceptable, and high-throughput research models hasled to the widespread use of cell-based systems. Intestinal barrier permeability and toxicity studies are often performed with colorectal adenocarcinoma-derived Caco-2 cells cultured either on standard well plates or permeable inserts. Caco-2 cells are able to form differentiated and polarized monolayers, exhibiting characteristics similar to intestinal enterocytes (Darling et al., 2020). Though undoubtedly valuable, these static systems fail to model the complexity of the human intestinal tissue because they lack interactions with surrounding cell types and the extracellular matrix (ECM), which are necessary for proper epithelial formation. Compared to the normal human intestine, Caco-2 monolayers can have altered expression of transporters and enzymes affecting overall permeability (Sun et al., 2008). Without incorporation of the mechanical microenvironment, Caco-2 are also unable to adequately portray crypt-villus structures of the small intestine, and coculture with microbiota is difficult due to bacterial overgrowth. The addition of cell culture substrates and additional cell types, such as HT29-MTX

cells to increase mucus production, has resulted in the formation of an enhanced epithelial layer. However, the cancerous origin of Caco-2 cells means that possible gene mutations detract from their ability to portray normal epithelium (Bourgine et al., 2012; Hoffmann et al., 2021).

For a more advanced model, intestinal organoids can be derived from primary intestinal crypt stem cells, iPSCs or intact intestinal crypts isolated from biopsy samples (Kasendra et al., 2018; Altay et al., 2019; Yoshida et al., 2020). Unlike Caco-2 cell cultures, organoids represent the stem cell lineage, which further differentiate to encompass the heterogeneous population of the *in vivo* tissue. The self-organizing capability of intestinal crypt-like units, in combination with the addition of factors such as epidermal growth factor (EGF), Noggin, and R-Spondin-1, allow the crypts to develop into a closed spherical structure (Dignass and Sturm, 2001; Sato and Clevers, 2013). The main drawback of such systems is the inability to access both sides of the epithelium, but they are able to retain correct cellular spatial organization and cellular interactions (Beumer et al., 2020). Biopsy-derived organoids have been successfully cultured as two-dimensional monolayers with exposed apical surfaces. However, expansion is difficult and cannot be done through simple resuspension and passaging (Braverman and Yilmaz, 2018; Thorne et al., 2018). To date, organoids have been valuable in studying human cell mechanisms and pathways despite the lack of functional immune cells and vascularization in most current systems. Other intestinal models include the everted gut sac and Ussing chamber, which are used largely for absorption studies (Thomson et al., 2019). Although highly physiologically relevant, both typically utilize *ex vivo* tissue, leading to limited viability after approximately 2 h and the speed of absorption is often not comparable to that *in vivo*.

4 Evolution of gut-on-chip models

A majority of gut-on-chip models are composed of polydimethylsiloxane (PDMS) and contain two adjacent hollow channels that are separated by an ECM-coated porous membrane Table 1. Culturing IECs on the upper surface of the porous membrane allows access and manipulation of the conditions in contact with both the apical and basolateral sides of the epithelium. In contrast to conventional static models, the gut-on-chip platform enables continuous exchange of the culture medium, providing adequate oxygenation and nutrients, while also imparting physiological shear stress on cells. For instance, Jalili-Firoozinez et al. fabricated a two-channel PDMS microfluidic device with Caco-2 cells representing the IECs and human intestinal microvascular endothelial cells (HIMECs) representing the vascular endothelium (Figure 2A) (Jalili-Firoozinezhad et al., 2019). Under perfusion flow, Caco-2 cells formed an epithelium of polarized cells connected via tight junctions in the top channel and HIMECs formed a confluent monolayer in the bottom channel (Jalili-Firoozinezhad et al., 2019). In the presence of an oxygen gradient, the microenvironment of the gut-on-chip was also able to support a higher level of microbial diversity when compared to models without oxygen modulation (Jalili-Firoozinezhad et al., 2019). Additionally, IECs isolated from small intestinal biopsy-derived organoids have been cultured in the same two-channel design, resulting in multilineage differentiation, more accurate villus formation, and mucus production (Kasendra et al., 2018).

The porous membrane between the channels allows for apical-basal access to the epithelia, but the presence of an artificial membrane could have unwanted effects on permeability and cell morphology. As a solution, Beaurivage et al. utilized a gut-on-chip device (OrganoPlate) where the artificial membrane is replaced by an ECM gel that is patterned so that epithelial cells seeded into the perfusion channel formed a tubule structure (Figure 2D) (Beaurivage et al., 2019). The confluent tubule was defined by tight junction and brush border formation as well as increased expression of glucose and MRP2 transporters, suggesting that the ECM plays an important role in cell differentiation and protein expression (Trietsch et al., 2017).

Although some evidence exists supporting the spontaneous formation of villus-like structures by Caco-2 cells under perfusion flow and mechanical deformation, using a scaffold-based approach (Figure 2C) increases the reproducibility of the small intestinal crypt-villus axis (Sung et al., 2011; Kim and Kim, 2018; Castaño et al., 2019; Creff et al., 2019; Kim and Kim, 2020; Verhulsel et al., 2021). For example, Shim et al. fabricated a collagen scaffold using photolithography on which cultured Caco-2 cells formed intestinal structures resembling human intestinal villi (Shim et al., 2017). The combination of perfusion flow and three-dimensional morphology improved metabolic activity and influenced drug absorption, which were largely attributed to a larger absorptive surface and alterations in the expression of tight junctions and transporter proteins (Shim et al., 2017). The importance of cell-matrix interactions and spatial microstructure has also been noted with similar villus scaffolds made from other materials such as poly-lactic-glycolic acid (PLGA) (Costello et al., 2017; Wang et al., 2017).

Normal gut function also involves the involuntary contraction of smooth muscle underlying the mucosa and submucosa. This process, called peristalsis, has been recently incorporated into gut-on-chip models by attaching the central porous membrane to vacuum chambers (Figure 2B). Compared to static Transwell cultures, application of cyclic strain to cause peristaltic motion of the porous membrane enhanced Caco-2 barrier formation and absorptive functions (Jing et al., 2020). Kim et al. also found that cyclic strain increased the expression of proteins in lipid and carbohydrate metabolism, as well as enzyme activity related to intestinal differentiation (Kim et al., 2012). More advanced gut-on-chip models implement a combination of luminal flow and peristalsis-like movement, supporting the formation of villus architecture and coculture with microbiota and immune cells.

5 Gut-on-chip inflammation models

IBD and celiac disease are characterized by chronic inflammation affecting various parts of the GI tract. Existing gut-on-chip models utilize a variety of cell sources and methods of inducing inflammation. In most cases, a healthy model of the gut epithelium is first established, consisting of a polarized monolayer of IECs with tight junction formation. Then, to mimic the loss of barrier integrity, different combinations of inflammatory cytokines or endotoxins are introduced to the microchannels of the device.

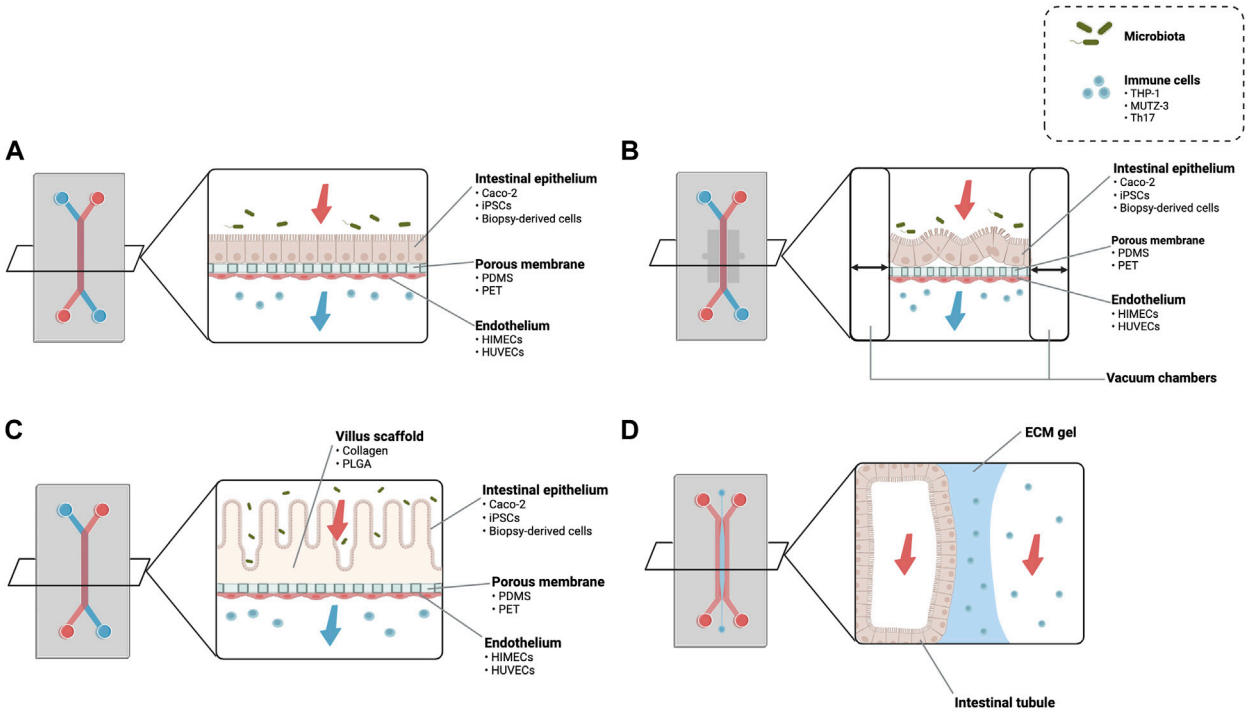

FIGURE 2
Schematic of different types of gut-on-chip devices. **(A)** A two-channel gut-on-chip with a porous membrane separating microchannels. The upper microfluidic channel contains the intestinal epithelium while the bottom channel can be used to incorporate other cell types, typically the vascular endothelium. Apical and basolateral medium flow enables coculture with factors such as microbiota and immune cells. **(B)** The application of cyclic suction through the integration of vacuum chambers attached to the porous membrane has been linked to the formation of villus-like structures. **(C)** Culturing intestinal epithelial cells on engineered villus scaffolds increases the reproducibility of the intestinal three-dimensional architecture. **(D)** A three-channel gut-on-chip design with an ECM gel in the center microchannel which guides the formation of an intestinal tubule. iPSCs, induced pluripotent stem cells; PDMS, polydimethylsiloxane; PET, polyethylene terephthalate; HIMECs, human intestinal microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; PLGA, poly (lactic-co-glycolic acid); ECM, extracellular matrix. Images based on (Kim et al., 2012; Shim et al., 2017; Jalili-Firoozinezhad et al., 2019; Beaurivage et al., 2020).

Several cytokine superfamilies have been identified as playing a key role in IBD pathogenesis, including interleukin (IL), tumor necrosis factor (TNF), and interferon (IFN) families (Neurath, 2014). During IBD-related inflammation, IECs produce and are exposed to these factors which have profound effects on tight junction formation, apoptotic activity, and mucosal healing (Andrews et al., 2018; Friedrich et al., 2019).

By culturing Caco-2 cells in a microenvironment with perfusion flow and peristalsis-like movement, Kim et al. were able to induce the spontaneous formation of *in vivo*-like intestinal villi with highly polarized cells connected by tight junctions (Kim et al., 2016). Introduction of lipopolysaccharide (LPS) or a combination of nonpathogenic bacteria and immune cells to the Caco-2 villi led to increased production of TNF-α, IL-1β, IL-6, and IL-8 by epithelial cells (Kim et al., 2016). Subsequently, as long as IL-8 was also present, each of these proinflammatory cytokines alone was able to elicit the destruction and shortening of villi and decreased barrier function that is characteristic for IBD patients (Kim et al., 2016). In a separate study with similar chip design by Min et al., proinflammatory cytokines TNF-α and IL-1β successfully impaired the Caco-2 barrier and led to intestinal inflammation (Min et al., 2022). Similarly, a combination of IL-1β, TNF-α and IFN-γ successfully triggered increased cell activation and loss of barrier integrity in Caco-2 tubules formed in the OrganoPlate platform, as supported by increased cytokine release by IECS and a drop in transepithelial electrical resistance (TEER) values (Beaurivage et al., 2019). In all three studies, as wells as a study by Liu et al., anti-inflammatory compounds or probiotic therapies were able to suppress the IEC destruction (Liu et al., 2023).

Some gut-on-chip models have also incorporated other intestinal cell types alongside Caco-2 cells to account for *in vivo* IEC diversity. In the OrganoPlate, Caco-2 enterocytes and HT29-MTX-E12 goblet cells were co-cultured in one channel and THP-1 and MUTZ-3 immune cells in the other, with the channels being separated by an ECM gel (Gijzen et al., 2020). The epithelial layer exhibited decreased barrier function and inflammation with the introduction of TNF-α and IL-1β (Gijzen et al., 2020). The inflammatory state was prevented with the application of TPCA-1, an anti-inflammatory drug. Gjorevski et al. used a similar experimental setup to study neutrophil infiltration and neutrophil-mediated epithelial damage after triggering inflammation in the Caco-2 tubule using LPS in the presence of THP-1 immune cells (Gjorevski et al., 2020). The addition of pro-inflammatory mediators resulted in a leaky epithelial barrier and the activation of macrophages, which in turn attracted and allowed the migration of neutrophils through the ECM gel to further promote tissue inflammation and damage (Gjorevski et al., 2020). More recently, Jeon et al. also reported similar results using the same

device design further enhanced with microelectrode arrays and endothelial cells cultured on the opposite side of the ECM gel (Jeon et al., 2022).

In an effort to improve the *in vivo* accuracy of the inflammation model, Beaurivage et al. cultured biopsy-derived human intestinal organoids in the OrganoPlate platform and induced IBD hallmarks using LPS and IFN-γ. As a result, the intestinal epithelium displayed an inflammatory state as well as increased cytokine production, attributed to an overrepresentation of cytokine regulation and bacterial response pathways that are commonly aberrant in IBD patients (Beaurivage et al., 2020). They further assessed the interplay between inflammation and the immune system by incorporating monocyte-derived macrophages that, upon cytokine treatment, differentiated into TNF-α and IL-6 secreting M1 inflammatory macrophages (Beaurivage et al., 2020). Shin et al. also created a gut-on-chip model by culturing intestinal organoids derived from patients with Crohn's disease and ulcerative colitis, showing that the diseased epithelial pathology and protein expression is retained *in vitro* (Shin et al., 2020). Although biopsy is a somewhat limited and invasive source of tissue, these results hold the promise of patient-specific disease modeling.

Since the causes and effects of IBD-related inflammation are not solely limited to the intestines, Trapecar et al. developed a gut-liver model for studying the effects of short-chain fatty acids (SCFAs) on intestinal inflammation. Again, patient-specific ulcerative colitis organoids were able to recapitulate the diseased condition and the addition of SCFAs led to enrichment of hepatic metabolic pathways and reduction of inflammatory pathways (Trapecar et al., 2020). Addition of regulatory cells (Treg) and T helper 17 (Th17) immune cells resulted in T cell-mediated inflammation and autoimmune hepatitis (Trapecar et al., 2020). Other multiorgan chips with an intestinal component have explored interactions with additional organs such as the brain and kidney (Lee et al., 2021; Trapecar et al., 2021).

Alternatively, dextran sodium sulfate (DSS) has been adapted from murine studies to induce ulcerative colitis-like pathologies in human gut-on-chip models for testing probiotic therapies (Shin and Kim, 2018). Similar to cytokines and LPS, DSS was capable of decreasing barrier integrity, decreasing villus height, and disrupting the mucus layer of a Caco-2 epithelium without cytotoxic effects (Shin and Kim, 2018). The barrier dysfunction contributed to the onset of inflammation by enhancing inflammatory cytokine production and immune cell recruitment, but if the intestinal barrier remained intact, the negative effects of LPS or nonpathogenic bacteria were suppressed. Probiotics were successful in maintaining the intestinal barrier when administered as pretreatment, but not when administered following DSS (Shin and Kim, 2018).

6 Considerations for modeling inflammatory intestinal disorders

The advancement of gut-on-chip devices with applicable physiological conditions and genetic and environmental factors enables more accurate modeling of IBD and celiac disease compared with standard cell cultures. However, as of yet, there is no singular, established gut-on-chip device that is used to model IBD and none have been developed to model celiac disease. Adapting current microfluidic intestinal inflammation models for more disease-specific purposes requires careful consideration of relevant technological and pathological aspects. Implementation of these features each come with their own limitations (Table 2).

Current state-of-the-art gut-on-chip models strive to incorporate as many physiological features of the intestines as possible, within the confines of engineering limitations and without hindering usability. As previously mentioned, to accurately recreate the mechanical microenvironment, a gut-on-chip device must be equipped with both luminal flow and peristaltic movement. Technologically, this requires perfusion of culture medium using pumps and the application of cyclic strain. Previous studies have sought to achieve a constant flow rate (30–60 μL/h) that imparted a shear stress of 0.02 dyne/cm2, which has been linked to proper cell organization and mucin production (Lindner et al., 2021). As the cells of the intestinal epithelium do not experience constant shear stress *in vivo*, dynamic flow rates have also been explored, largely with no additional effects on villus formation or protein expression (Shin et al., 2020; Fois et al., 2021). Nevertheless, dynamic flow rates could be capable of representing the transport of particles through the intestines more accurately. To mimic peristalsis, cyclic strain (5%–10% strain, 0.15–0.2 Hz) has typically been implemented by stretching the membrane under the IEC layer using vacuum suction (Kim et al., 2012). In order to study the complex anaerobic and aerobic microbes that are present in the intestines, a gut-on-chip device must also mimic the low oxygen environment of the intestinal lumen and the comparatively well-oxygenated intestinal tissue. To do so, PDMS gut-on-chip devices, due to their gas permeability, have been placed in anaerobic chambers continuously supplied with CO_2 (Jalili-Firoozinezhad et al., 2019; Grant et al., 2022). Alternatively, Shah et al. used polycarbonate to segregate the culture area and perfused anoxic medium to control the oxygen concentration (Shah et al., 2016). Due to the small size of microfluidic devices, precisely quantifying properties such as oxygen concentration, pH, and TEER requires integration of different sensing systems. For example, the barrier function of the intestinal epithelium can be monitored with TEER electrodes, or the amount of dissolved oxygen can be measured using optical sensors (Shah et al., 2016; Henry et al., 2017; Lesher-Pérez et al., 2017; Jalili-Firoozinezhad et al., 2018; Jalili-Firoozinezhad et al., 2019; Soucy et al., 2019; Okkelman et al., 2020).

The structural differences between the small and large intestines, as well as regional differences within each, should also be taken into consideration when creating an *in vitro* model. To date, most gut inflammation models seek to recreate the general crypt-villus architecture of the small intestine, which disregards the distinct regions of the intestines which can have variable responses to inflammation. Already, specific gut-on-chip devices have been constructed using biopsy samples to represent the duodenum and ileum as well as a colon model using human colonic mucosal tissue (Kasendra et al., 2018; Beaurivage et al., 2020; Kasendra et al., 2020; Sontheimer-Phelps et al., 2020). The use of region-specific gut-on-chip devices should be further expanded to study inflammation and disease progression in applicable areas of the GI tract. Similarly, the cellular components of the epithelium must be accurately represented. Caco-2 cells cultured in microfluidic devices have

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TABLE 1 Features of human gut-on-chip models. PDMS, polydimethylsiloxane; HIMECs, human intestinal microvascular endothelial cells; iPSC, induced pluripotent stem cells; HMVECs, human microvascular endothelial cells; PBMCs, peripheral blood mononuclear cells; PMMA, poly (methyl methacrylate); HUVECs, human umbilical endothelial cells; ECM, extracellular matrix; hiPSC, human induced pluripotent stem cells.

Model	Device material	Channel specifications	Membrane material	Peristalsis	Fluid flow	Oxygen modulation	Epithelial cell type	Epithelium morphology	Endothelium	Mucus production	Microbes	Immune cells	Application	
Peristaltic	PDMS	2 microchannels	PDMS	yes	yes	yes	Caco-2, intestinal biopsy-derived organoids	spontaneous villus-like structure	HIMECs	yes	yes	no	Coculture of anaerobic and aerobic commensal	Jalili-Firoozinezhad et al. (2019)
	PDMS	2 microchannels	PDMS	yes	yes	yes	Caco-2	spontaneous villus-like structure	no	yes	yes	no	Host-microbe coculture	Shin et al. (2019)
	PDMS	2 microchannels	PDMS	yes	yes	no	intestinal biopsy-derived organoids	spontaneous villus-like structure	HIMECs	yes	no	no	Preclinical drug assessment	Kasendra et al. (2018), Kasendra et al. (2020)
	PDMS	2 microchannels	PDMS	yes	yes	no	iPSC-derived organoids	spontaneous villus-like structure	no	yes	no	no	Modeling intestinal physiology	Workman et al. (2018)
	PDMS	2 convoluted microchannels	PDMS	yes	yes	no	Caco-2, intestinal biopsy-derived organoids	3D epithelial microarchitectures	no	yes	yes	no	Formation of patient-derived intestinal organoid epithelium	Shin et al. (2020)
	PDMS	3 microchannels	PDMS	yes	yes	no	Caco-2	spontaneous villus-like structure	HMVECs	yes	yes	PBMCs	Gut inflammation	Kim et al. (2016)
	PDMS, PMMA	3 microchannels	PDMS	yes	yes	no	Caco-2	spontaneous villus-like structure	HUVECs	yes	yes	human macrophage U937	host-microbial interactions	Jing et al. (2020)
	PDMS	2 microchannels	PDMS	yes	yes	no	Caco-2	spontaneous villus-like structure	no	yes	yes	no	Host-microbe coculture	Kim et al. (2012), Villenave et al. (2017), Shin and Kim (2018)
	PDMS	2 microchannels	PDMS	yes	yes	no	Caco-2	spontaneous villus-like structure	HUVECs	yes	no	no	Disease modeling, countermeasure drug screening	Jalili-Firoozinezhad et al. (2018)
	PDMS	2 microchannels	PDMS	yes	yes	no	Caco-2	spontaneous villus-like structure	no	yes	no	no	Recreation of gut microenvironment	Kim and Ingber (2013)
Scaffold-based	PDMS	2 microchannels	Polyethylene terephthalate	no	yes	no	Caco-2	Villus-like on collagen scaffold	no	N/A	no	no	Intestintal absorptive function	Shim et al. (2017)
	VeroClear RGD810	2 chambers	N/A	no	yes	no	Caco-2	Villus-like on poly-ethylene-co-vinyl-acetate scaffold	no	yes	no	no	Characterization of intestinal physiology	Costello et al. (2017)
	Polystyrene (OrganoPlate)	3 microchannels	ECM gel	no	yes	no	intestinal biopsy-derived organoids	Intestinal-like epithelial tubules	no	yes	no	monocyte-derived macrophages	Model inflammatory processes	Beaurivage et al. (2020)
	Polystyrene (OrganoPlate)	3 microchannels	ECM gel	no	yes	no	hiPSCs	Intestinal-like epithelial tubules	no	yes	no	no	Differentiation of intestinal tubules	Naumovska et al. (2020)
	Polystyrene (OrganoPlate)	3 microchannels	ECM gel	no	yes	no	Caco-2, HT29	Intestinal-like epithelial tubules	no	yes	no	THP-1, MUTZ-3	Study inflammatory processes	Gijzen et al. (2020),

(Continued on following page)

TABLE 1 (*Continued*) Features of human gut-on-chip models. PDMS, polydimethylsiloxane; HIMECs, human intestinal microvascular endothelial cells; iPSC, induced pluripotent stem cells; HMVECs, human microvascular endothelial cells; PBMCs, peripheral blood mononuclear cells; PMMA, poly (methyl methacrylate); HUVECs, human umbilical endothelial cells; ECM, extracellular matrix; hiPSC, human induced pluripotent stem cells.

Model	Device material	Channel specifications	Membrane material	Peristalsis	Fluid flow	Oxygen modulation	Epithelial cell type	Epithelium morphology	Endothelium	Mucus production	Microbes	Immune cells	Application	
														Gjorevski et al. (2020)
	Polystyrene (OrganoPlate)	3 microchannels	ECM gel	no	yes	no	Caco-2	Intestinal-like epithelial tubules	no	N/A	no	no	Modeling inflammatory state and drug discovery, barrier integrity assessment	Trietsch et al. (2017), Beaurivage et al. (2019)
Perfusion-only	PDMS	2 microchannels	PDMS	no	yes	yes	intestinal biopsy-derived organoids	spontaneous villus-like structure	no	N/A	no	no	Establishing oxygen gradients	Grant et al. (2022)
	PDMS	2 microchannels	PDMS	no	yes	no	intestinal biopsy-derived organoids	spontaneous villus-like structure	no	yes	no	no	Analysis of mucus layer	Sontheimer-Phelps et al. (2020)
	Polystyrol	2 microchannels	Polyethylene terephthalate	no	yes	no	Caco-2	spontaneous villus-like structure	HUVECs	yes	yes	PBMCs	Microbial interactions	Maurer et al. (2019)
	PDMS	3 microchannels	ECM gel	no	yes	no	Caco-2	monolayer with villus-like structures	HUVECs	yes	yes	no	Microbial contribution to intestinal inflammation	Jeon et al. (2022)
	Polysulfone	6 culture modules	Transwell insert	no	yes	yes	intestinalbiopsy-derived organoids	flat monolayer	no	yes	yes	no	Super oxygen-sensitive microbial interactions	Zhang et al. (2021)
	Polycarbonate, silicone	3 microchannels	Polycarbonate	no	yes	yes	Caco-2	flat monolayer	no	N/A	yes	primary $CD4^+$ T cells	Host-microbe molecular interactions	Shah et al. (2016)
	PDMS	2 microchannels	polyethylene terephthalate	no	yes	no	Caco-2	flat monolayer	no	N/A	no	no	Intestinal absorptivefunctionality	Imura et al. (2009)
	PDMS	2 microchannels	polycarbonate	no	yes	no	Caco-2	flat monolayer	no	N/A	no	no	Drug permeability	Gao et al. (2013), Pocock et al. (2017)
	PDMS, PMMA, polystyrene	2 microchannels	polyethylene terephthalate	no	yes	no	Caco-2	flat monolayer	no	no	no	human macrophage U937	Nutrition metabolism, immunomodulatory function	Ramadan and Jing (2016)
	PDMS	1 microchannel	nitrocellulose	no	yes	no	Caco-2	flat monolayer	no	yes	no	no	Modeling drug metabolism	Guo et al. (2018)
Multi-organ	Polysulfone, acrylic	gut module brain module liver module	polyurethane	no	yes	no	intestinal biopsy-derived organoids	flat monolayer	no	N/A	microbe metabolites	primary $CD4^+$ T cells	Studying neurodegenerative diseases, inflammation model	Edington et al. (2018), Trapecar et al. (2020), Trapecar et al. (2021)
	PDMS	2 microchannels	Transwell insert	no	yes	no	Caco-2	Villus-like on collagen scaffold	no	yes	no	no	*in vitro* hepatic steatosis model	Jeon et al. (2021)
	PDMS	gut microchannel liver microchannel	polyester	no	yes	no	Caco-2	flat monolayer	no	no	no	no	First pass metabolism of drugs	Choe et al. (2017)
	PDMS	gut module BBB module	polyester	no	yes	no	Caco-2	flat monolayer	no	no	no	no	Transport across epithelial and endothelial barriers	Kim et al. (2021)

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TABLE 2 Advantages and limitations to gut-on-chip features.

Gut-on-chip features	Advantage	Implementation limitations
Diverse cell population	Ability to model epithelial, endothelial, and immune components	•Different growth media requirements
		•Different microenvironments
Complexity of microbiome	Ability to model metabolic functions, pathogen protection, and immune modulation	•Bacterial overgrowth
		•Different microenvironments (pH, gas composition, water activity)
Fluid flow	Mimics intestinal nutrient transport and physiological forces acting on cells	•No standardized flow rate
		•Possible cell layer damage
		•Response time and pressure control depend on pump type
Persitalsis	Mimics digestion, nutrient transport, and physiological forces acting on cells	•No standardized strain
		•Requires stretchable membrane under cell layer, typically PDMS
Inflammatory triggers	Ability to induce inflammation	•No standardized method
		•Many different inflammatory pathways
Oxygen modulation	Recreates oxygen gradient along the crypt-villus axis	•Requires integration of oxygen sensors
		•Oxygen gradients difficult to maintain

been reported to spontaneously form structures similar to the small intestine, but genomic analysis has revealed lower expression of physiologically relevant genes compared to gut-on-chip cultures of biopsy-derived organoids (Yin et al., 2020). Biopsy samples and iPSC-derived organoids share the same genetic features and develop similar cellular architecture as intestinal epithelium *in vivo* but with a closed configuration, making them inaccessible from the apical side. A further development for gut-on-chip models would be to use intestinal organoids to generate the epithelial monolayer, or to differentiate and mature iPSCs towards intestinal epithelial cells in 2D (Negoro et al., 2018; Thorne et al., 2018).

Inflammatory conditions of the intestines involve a combination of immune and microbial factors, the coculture of which has been facilitated by microfluidic devices. The multichannel composition of gut-on-chip devices permits the application of different factors to the apical or basolateral surface of the intestinal epithelium to study their effects. In a homeostatic state, most models contain an intact epithelial barrier which separates microbial populations on the apical (lumen) side and immune components on the basal side. PBMCs, THP-1, Th17, and MUTZ-3 immune cells have already been incorporated into gut-on-chip models, specifically into microfluidic channels adjacent to the intestinal epithelium (Shin et al., 2020; Trapecar et al., 2020; Lee et al., 2021; Jeon et al., 2022). An immunocompetent model is especially important with regard to the development of a celiac disease gut-on-chip, where $CD4^+$ T cells have an important role in creating an inflammatory milieu enabling the development of tissue damage (Christophersen et al., 2019; Lindfors et al., 2019). The intestinal microbiota also has several proposed roles in IBD and celiac disease. For example, dysbiosis of the microbiota could alter the permeability of the intestinal barrier, allowing for the translocation of the luminal contents into the lamina propria and subsequently triggering immune responses (Levy et al., 2017). Since the exact role and interplay between immune cells, the intestinal microbiota, and other cell types are largely unknown, further study requires their integration into future gut-on-chip disease models. Unlike most conventional intestinal models, organ-on-chip technology allows for evaluating the relative contributions of the different factors to inflammatory diseases.

Along with immune cells, the lamina propria also contains the intestinal blood supply and lymphatic vasculature. Blood and lymphatic vessels are mainly lined by endothelial cells that can be maladaptive in diseased conditions, playing a role in areas such as cell infiltration, cytokine production, and immunological reactivity (Myrsky et al., 2009; Cromer et al., 2011). In existing two-channel gut-on-chip models it is relatively common to include HUVECs or HIMECs with the result that they form an endothelial monolayer adjacent to the intestinal epithelium. Other cells that are thought to play a role in the progression of mucosal barrier destruction are intraepithelial lymphocytes (IELs) that represent a population of T cells interspersed between IECs. Generally, IELs participate in the protection of the intestinal epithelial layer. However, in celiac disease, these cells are found to be cytotoxic in nature and capable of inducing IEC apoptosis upon the onset of an immune response (Lindfors et al., 2019). The central role of IELs in promoting atrophy of small intestinal villi conveys the need for their representation in an accurate *in vitro* celiac disease model.

In terms of pathogenesis, ulcerative colitis and Crohn's disease have different inflammatory mediator profiles, the former being driven by infiltration of Th2 cell-associated cytokines and the latter by Th1 cell-associated cytokines (Nemeth et al., 2017). Some overlap also exists, as both forms of IBD show increased expression of factors such as Th17 cytokine IL-17 and TNF (Atreya et al., 2011; Monteleone et al., 2011). The existence of specific cytokine networks that lead to mucosal inflammation is further supported by the key cytokines linked to the perpetuation of celiac disease, including IFN-γ, IL-15, and IL-21 (Garrote et al., 2008). Thus,

especially when using Caco-2 cells, the right combination of triggers is necessary to result in gut-on-chip inflammation that has both structural changes and levels of cytokine release comparable to the specific disease. Utilizing a cell source that retains the disease pathology without the use of an external stimulant could be a way to surpass this challenge.

It is also important to consider whether disease severity can be manipulated within gut-on-chip models, as different forms of IBD and celiac disease present differently in individual patients. Beaurivage et al. compared rapid and prolonged exposure of inflammatory factors to Caco-2 cells, finding that there was no significant effect on cytokine production, TEER values, or E-cadherin localization (Beaurivage et al., 2019). Though there was no change in the severity of the inflammatory state, it was proposed that further optimizing the concentration of cytokines could more accurately depict different disease phenotypes.

Finally, there are currently no standardized methods to trigger inflammation in gut-on-chip models. Delivering inflammatory modulators to apical, basal, or both sides of the intestinal epithelium has resulted in the secretion of different concentrations of pro-inflammatory cytokines. In a study using LPS and IFN-γ as triggers, there was no apparent difference in cytokine levels regardless of whether LPS was applied to the apical or basal compartment, as long as IFN-γ was only applied to the basal side (Beaurivage et al., 2020). When LPS and IFN-γ were simultaneously applied to both sides, there were fluctuations in the secretion of multiple cytokines both apically and basally. In this case, the conclusion was to apply the inflammatory trigger to both sides to promote the complete activation and differentiation of macrophages, but many other studies apply triggers to only one side of the epithelium (Beaurivage et al., 2020). No clear answer exists to how inflammation should be triggered, but the results from different approaches illuminate how even slight variability can have an impact on the overall accuracy of a disease model.

7 Conclusion

This review outlines the general construction of gut-on-chip devices as well as how they have been adapted to model intestinal inflammation. An ideal device should provide an intact epithelial barrier with mucus production, peristaltic motion, luminal flow, and interactions between IECs and immune and microbial components. Many existing models have been able to incorporate a combination of these features, but limitations still exist as the field is fairly new. Whether these models can be further adapted for modeling multifactorial diseases such as IBD and celiac disease relies on careful consideration of aspects such as inflammatory mediator profiles, cell type, tissue architecture and the application of microbial and immune factors in an *in vivo*-like manner. Gut-on-chip models are progressively getting closer to mimicking the intestines in both architecture and physiology, providing more opportunities to create accurate models to study complex inflammatory intestinal conditions. While gaining new insights into the pathogenesis and treatment of these diseases can be achieved with a gut-on-chip device, complete modeling most likely requires the integration of multiple different approaches. All in all, organ-on-chip technology provides an excellent platform for accurately modeling intestinal inflammation, with the promise of further development into IBD and celiac disease models.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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