



Gut-on-chip: Recreating human intestine in vitro

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Abstract

The human gut is important for food digestion and absorption, as well as a venue for a large number of microorganisms that coexist with the host. Although numerous in vitro models have been proposed to study intestinal pathology or interactions between intestinal microbes and host, they are far from recapitulating the real intestinal microenvironment in vivo. To assist researchers in further understanding gut physiology, the intestinal microbiome, and disease processes, a novel technology primarily based on microfluidics and cell biology, called “gut-on-chip,” was developed to simulate the structure, function, and microenvironment of the human gut. In this review, we first introduce various types of gut-on-chip systems, then highlight their applications in drug pharmacokinetics, host–gut microbiota crosstalk, and nutrition metabolism. Finally, we discuss challenges in this field and prospects for better understanding interactions between intestinal flora and human hosts, and then provide guidance for clinical treatment of related diseases.

Keywords

Microfluidics, gut-on-chip, host–microbiome interactions, drug pharmacokinetics, artificial gut

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Introduction

Because of its significant roles in digestion, absorption, disease regulation, and immune function, the human intestine has attracted great attention in recent years, particularly as research involving the human microbiome progresses. Most nutrients and oral drugs need to be absorbed in the intestinal tract. More than 70% of immune cells are located in the intestinal mucosa, and the number of lymphocytes is much higher than that of other lymphoid tissues.¹ Thus, intestine is a natural protective barrier to pathogens and other harmful microorganisms.² In addition, microbes in the human intestine are reportedly associated with a series of diseases, such as indigestion, obesity, and immune damage.^{3–5} Therefore, in vitro simulation of the intestinal microenvironment is of great significance for drug development and disease treatment.⁶ At present, mammalian animal models and static Transwell models are most commonly utilized to mimic the human gut system for investigations of human gut physiology and the microbiome.⁷ Mammals have been used extensively to test drug metabolism, toxicity, and efficacy in preclinical periods,^{8,9} and two-dimensional (2D) Transwell culture systems offer a simple in vitro approach to realize

short-term observations and detection of microbiome–host interactions through the growth of a monolayer intestinal epithelium with tight junctions and cellular polarity.^{10,11} However, deficiencies of these two models limit exploration within this field. On the one hand, animal models require a long experimental cycle, involve extensive labor, and have high costs. Furthermore, as there are large differences between animal and human physiology, the physiological response of the human intestinal tract may not be well

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predicted.^{12–14} On the other hand, the static model cannot simulate features of the intestinal microenvironment such as fluid flow, villi structures, and peristalsis. Thus, it is difficult to mimic the comprehensive functions of human intestinal tract and realize real-time observations of interactive dynamics.¹⁵ Up to date, organoid culture using induced pluripotent stem cells or primary intestinal cells is an emerging method to regenerate three-dimensional (3D) intestine architecture with fully differentiated intestinal epithelial cells.¹⁶ Nevertheless, organoid culture stays static that does not experience fluid flow and peristalsis existing in human intestine. Although intestine organoid can simulate gut physiology, it is hard to inject microbial cells or drug to the apical side of intestinal epithelium because the lumen of intestine organoid is enclosed.¹⁷ So this approach is still challenged by host–microbiome interactions and intestine physiology including absorption, drug pharmacokinetics and nutrition metabolism. As a suitable platform for *in vitro* cell culture, microfluidics has attracted a lot of attention for recreating diverse human organs, namely organ-on-chip technology.^{18,19} In particular, gut-on-chip is becoming a powerful tool to construct physiological models of the human gut, perform drug testing and development, and investigate host–microbiome interactions.^{17,20,21} Compared with conventional model systems, gut-on-chip offers the capabilities of real-time observation, simulation of the intestinal microenvironment, adjustable fluid flow shear stress and host–microbiome interface.^{22,23}

In this review, we first provide comprehensive information about the human intestinal microenvironment, including the structure, function, immune components, and microbiome, and demonstrate why gut-on-chip technology has been developed. Next, we classify the design of gut-on-chip platforms into 2D models with an epithelial layer and 3D models with intestinal villi, and introduce their establishment. Finally, we summarize emerging applications of gut-on-chip in drug pharmacokinetics and development, interactions between host and intestinal microorganisms, and nutrition metabolism, and further depict what gut-on-chip systems can explore. Furthermore, we provide viewpoints on the importance and current limitations of this technology, and envision future applications in biomedical fields.

Characteristics of the human gut microenvironment

The human intestine is not only the biggest digestion and absorption organ, but also home to a wide variety of microorganisms that inhabit the human intestinal tract, estimated at 100 trillion bacteria representing 500 to 1000 species.^{24,25} The gut microbiome is closely related to human health and essential for facilitating gut homeostasis, contributing to nutrition absorption, drug metabolism, maturation of the immune system, and protection against pathogens.^{26–28} Once the intestinal flora is disturbed,

changes in its structure and function (even breaking the immune balance) likely lead to a series of intestinal diseases, such as inflammatory bowel disease (IBD), and ileus or even colorectal cancer.^{29,30}

To support physiological function, intestine has a unique microenvironment, as shown in Figure 1(a). Normally, intestine consists of a closed inner cavity that folds into small finger-like projections, called villi, that tremendously increase the absorptive surface area compared with a flat surface. At the base of the intestinal glands, called crypts, proliferative stem cells produce new epithelial cells capable of differentiating into four cell types: absorptive enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. The differentiated cells then migrate upwards to form the epithelial layer.¹⁵ Integrity of the intestinal epithelium is mostly characterized by tight junctions and polarity, which ensure that digested nutrients can only enter the body from the intestinal cavity to capillaries surrounding each villous.³² As one of the largest bacterial habitats, balanced host–microbe interactions within the human intestine are necessary for maintaining homeostasis. The gut microbiota has multiple functions in human health. For instance, intestinal flora is helpful for the fermentation of non-digestible carbohydrates to produce short-chain fatty acids.³³ The gut microbiome provides protection against pathogens via competition for uptake of trace elements and ecological niches, and the strengthening of host defense mechanisms. When the composition of the microbiome changes, whether by dietary changes, antibiotic treatment, or incursive pathogen, disruptions in the balance of the microbiota can induce the occurrence of disease in the organism. The intestine has unique structures and functions for maintaining its homeostasis. The mucus layer covering the intestinal epithelium creates a boundary between the gut lumen and host tissue, which limits direct contact between intestinal or invasive microorganisms and human cells.³⁴ In the presence of mucus, antimicrobial molecules produced by the epithelium can accumulate at a high local concentration, which efficiently inhibits the detrimental effects of bacterial colonization and growth.³⁵ Even if some bacterial colonies can penetrate the mucus layer, enterocytes can further secrete responsive chemokines and cytokines to regulate the functionality of immune cells in the lamina propria.³⁶ These local immune responses are considered to be key for solving intestinal barrier infections and promoting mucosal repair after intestinal injury.⁵ The epithelium is encircled by smooth muscle layers, with the enteric neural system embedded within muscle to control intestinal peristalsis and move materials within the intestinal cavity in a constant forward direction.^{37–39} Variations in oxygen distribution are another feature of the intestinal microenvironment. From the mucosal layer to the cavity, the oxygen gradient gradually decreases to an anaerobic environment that determines the distribution of various microbes.^{35,40}

Recent studies have found that intestinal microorganisms play a very important role in maintaining intestinal

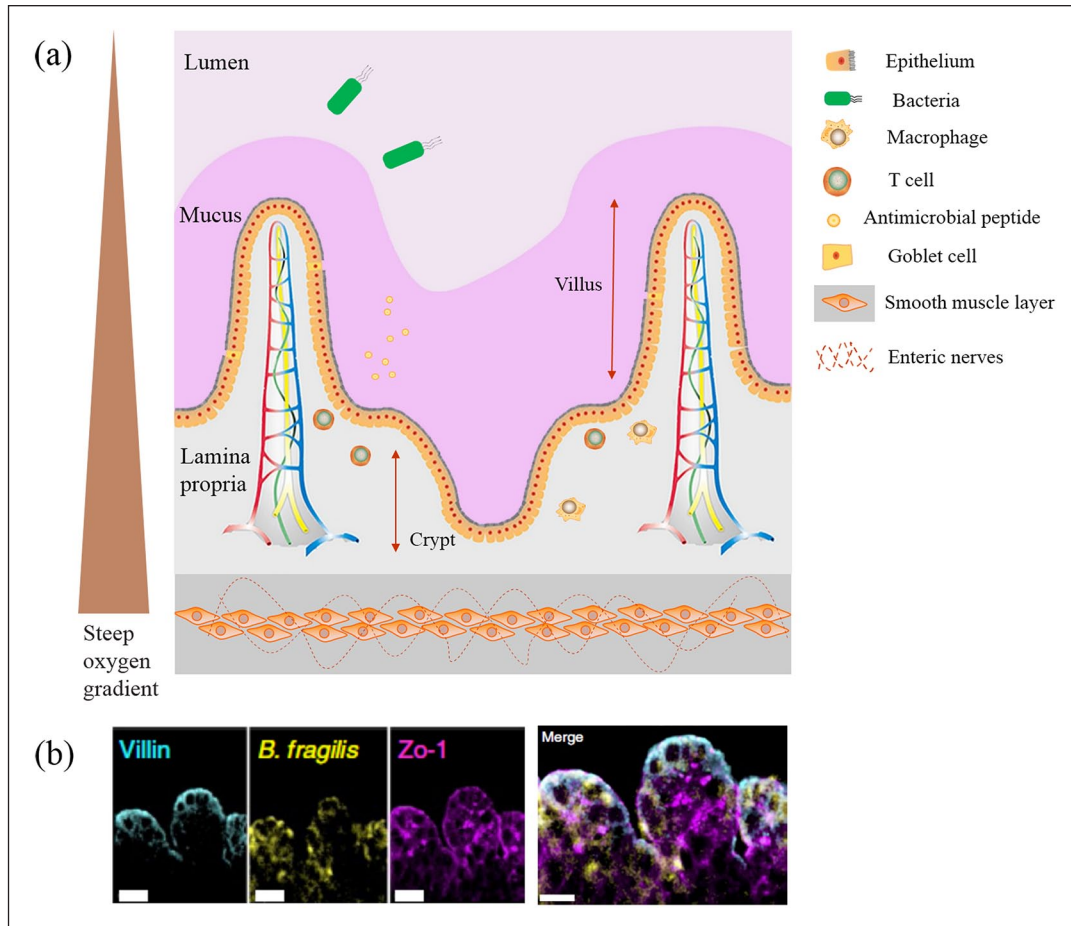


Figure 1. Microenvironment in human intestine and gut-on-chip. (a) Illustration of the human gut microenvironment. The main components of the intestinal microenvironment include the intestinal epithelial villi, mucus layer, lamina propria, and symbiotic microbial community. Capillaries and the blood vasculature, and immune cells within the lamina propria control transport of nutrients and immune responses, respectively. Three muscle layers encircle the epithelium to regulate intestinal movements in combination with neural plexus embedded within the muscle. (b) Vertical cross-sectional confocal views through the intestinal epithelial villi–microbiome interface stained for villin (cyan) and ZO-1 (magenta) in gut-on-chip co-cultured with HADA-labelled *B. fragilis* (yellow) under anaerobic condition, scale bar, 50 μm . Reprint permission was obtained from Jalili-Firoozinezhad et al.³¹

health and immune regulation. To decipher molecular mechanisms, most research thus far has employed traditional 2D models, such as Transwell technology.⁴¹ These 2D models cannot reproduce intestinal cell physiology or tissue morphology, and are unable to reconstruct key intestinal differentiation functions. Furthermore, such static models such as organoids are far removed from real physiological fluid environment, and are thus insufficient to support co-cultures of bacteria and intestinal cells. Therefore, a more realistic 3D model is imperative to simulate the core structures and key functions of intestine (Figure 1(b)).

Design of gut-on-chip

The rapid development of microfluidic technology in recent years has made it an effective way to emulate the human gastrointestinal tract. Typically called “gut-on-chip,” intrinsic features of this platform include low cost and consumption, flexible control of multiple system

parameters (including fluid flow and oxygen concentration), and compatibility with cell graphic culture and real-time microscopic imaging.^{42,43} Over the past 10 years, gut-on-chip platforms have evolved from simple 2D structures to include more comprehensive functionality, such as villi structures, intestinal peristalsis, oxygen gradients, and even immune systems. The development of gut-on-chip system is summarized in Table 1 and Figure 2 shows a brief diagram of these models.

Two-dimensional gut-on-chip model

Initially, the most common device structure contained two channels (upper and lower) separated by a semipermeable membrane, such as polycarbonate or polyester materials. Intestinal epithelial cells growing on the membrane form apical and basolateral sides of a monolayer epithelium (Figure 2(a)). This simple model has commonly been used to assess the pharmacokinetic properties of drug and

Table 1. Characteristics and applications of gut-on-chip models.

| Model | Cell type | Supporting materials for cell | Static or fluid flow | Oxygen modulation | Application |
|--|---|--|------------------------------|---|---|
| 2D model | Caco-2 | Polyethylene terephthalate (PET) porous membrane | Fluid flow (1 μ L/min) | No | Intestinal absorptive functionality ⁴⁴ |
| | Caco-2 | Polycarbonate (PC) porous membrane | Fluid flow (0.1 μ L/min) | No | Drug permeability ⁴⁵ |
| | Caco-2 | PC porous membrane | Fluid flow (25 μ L/min) | Yes | Host–microbe molecular interactions ⁴⁶ |
| | Caco-2, HepG2 | Polyester porous membrane | Fluid flow (96 μ L/h) | No | First pass metabolism of drugs ⁴⁷ |
| | Caco-2, U937 | PET porous membrane | Fluid flow (10–20 nL/s) | No | Nutrition metabolism and immunomodulatory function ⁴⁸ |
| Scaffold-based 3D model | Caco-2 | Poly (lactic-co-glycolic acid) scaffolds | Static | No | Interaction between pathogen and probiotic ⁴⁹ |
| | Caco-2 | Hydrogel scaffold | Fluid flow (100 μ L/min) | No | Intestinal absorptive functionality ⁵⁰ |
| | Caco-2 | Hydrogel scaffold | Static | No | Intestinal barrier function ⁵¹ |
| Stretching-based 3D model | Caco-2 | PDMS porous membrane | Fluid flow (30 μ L/h) | No | Host-microbe co-culture, ^{52,53} enteric virus infection ⁵⁴ |
| | Caco-2, PBMCs, capillary HMVECs, lymphatic HMVECs | PDMS porous membrane | Fluid flow (30 μ L/h) | No | Gut inflammation model ⁵⁵ |
| | Caco-2, PBMCs | PDMS porous membrane | Fluid flow (50 μ L/h) | No | Epithelial–immune interactions and host–microbiome cross-talk ⁵⁶ |
| | Intestinal biopsy-derived organoids | PDMS porous membrane | Fluid flow (60 μ L/h) | No | Modeling normal intestinal physiology ⁵⁷ |
| | Caco-2, HIMECs, intestinal organoids | PDMS porous membrane | Fluid flow (60 μ L/h) | Yes | Co-culture of anaerobic and aerobic commensal ³¹ |
| | Caco-2 | PDMS porous membrane | Fluid flow (50 μ L/h) | Yes | Co-culture of obligate anaerobic gut microbiome ⁵⁸ |
| Caco-2, human umbilical vein endothelial cells | PDMS porous membrane | Fluid flow (60 μ L/h) | No | Recapitulating disease model and countermeasure drugs screening ⁵⁹ | |

PDMS: polydimethylsiloxane; PBMCs: peripheral blood mononuclear cells; capillary HMVECs: human capillary microvascular endothelial cells; lymphatic HMVECs: human lymphatic microvascular endothelial cells; HIMECs: human intestinal microvascular endothelial cells.

nutrition absorption *in vitro*.²² For example, Kimura et al. developed a similar device with integrated on-chip pumping and optical detection. The two independent channels were vertically separated by a polyester semipermeable membrane, on which cells were inoculated and cultured.⁶⁰ Imura et al. designed a more complex system consisting of two-tiered channels divided by a collagen-coated semipermeable membrane and the absorptive functionality was assessed as permeation of the anticancer drug cyclophosphamide.⁴⁴ In addition, other gut-on-chip models attempted to integrate more advanced instruments to perform specific detection modalities.⁶¹ For instance, Gao et al. integrated an ESI-Q-TOF MS (electrospray ionization quadrupole time-of-flight mass spectrometer) to analyze molecules transported across the epithelial layer.⁴⁵ The microchip consisted of two units: one simulating cell culture on a polycarbonate membrane, the other for molecule purification and concentration via miniaturized solid-phase extraction. Shah et al. designed a HuMiX (human–microbial crosstalk) system allowing co-culture of human and microbial cells at the gastrointestinal human–microbe

interface; in addition, oxygen sensors were integrated to monitor gas conditions.⁴⁶ Their system comprised three microchannels for medium flow, cell culture, and embedded sensors, respectively.

As described above, the 2D gut-on-chip model can simulate fluid shear stress on intestinal cells to reduce the demand on cells and medium. Continuous flow maintains the nutrition supply and washes away metabolic waste over time.⁶² In most cases, the viability of intestinal epithelium is better than Transwell, thus allowing long-term co-culture of microbiome and host cells.⁶³ However, the villous microstructure, which is one of the most functional units within the intestine, fails to be simulated and investigated.

Three-dimensional gut-on-chip model with villi microstructures

The villi microstructures within the intestinal tract not only act as a physiological barrier for the epithelial layer, but more importantly increase the absorption area of the intestinal surface.⁶⁴ Various types of villi-like structures

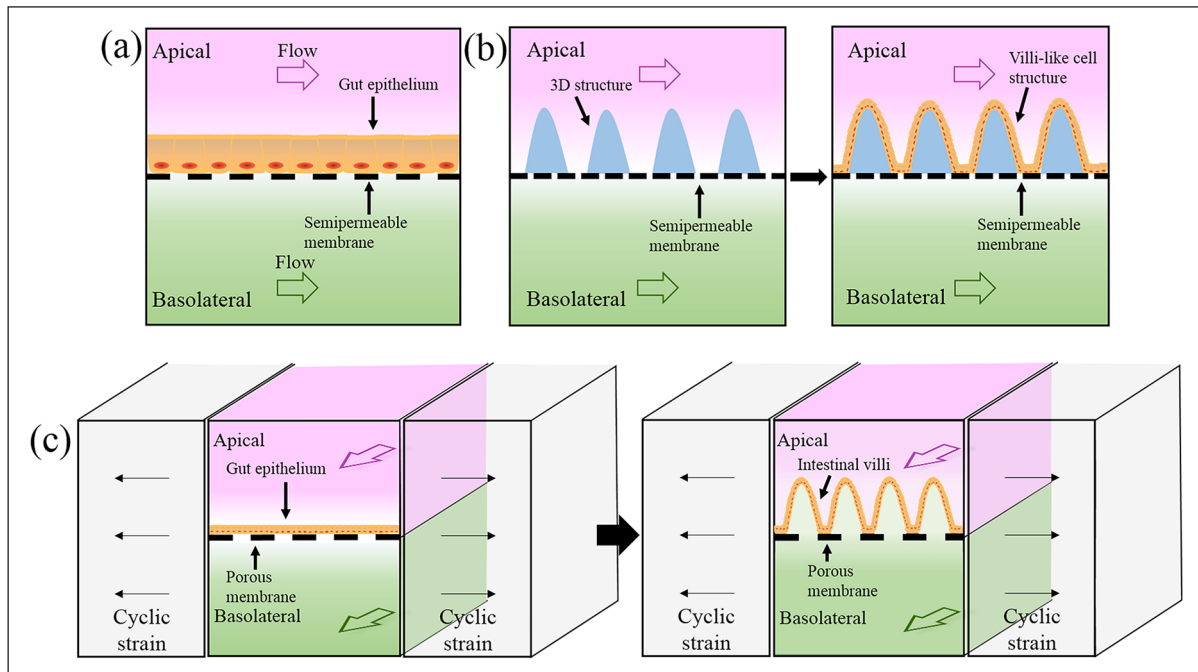


Figure 2. Schematic illustrations of gut-on-chip models. (a) Two-dimensional gut-on-chip model comprising upper and lower channels, with a semipermeable membrane designed to serve as both a microchannel segregator and carrier for growing cells. (b) Three-dimensional villi-like structures on gut-on-chip models formed by culturing intestinal cells on designed microstructures. (c) Villi formation in gut-on-chip by mechanically stretching porous membrane.

have been proposed to reproduce the *in vivo* intestine microenvironment.^{31,52,65,66} For example, Sung et al. fabricated a hydrogel array based on laser ablation and soft lithography technology, and then cultured epithelial cells on the 3D microstructures to simulate the shape and distribution density of human intestinal villi.⁶⁵ Similar studies revealed that these villi-shaped scaffolds could change physiological intestinal functions, such as drug absorption,⁶⁷ mucus production, and resistance to bacterial invasion.^{49,51} When the culture time was extended to 2 weeks, the height of villi microstructures could reach 100 to 150 μm , thus improving intestinal epithelial barrier function and selective permeability.^{50,68} Wang et al. modified a 3D porous substrate to simulate the villi-crypt axis, and cell migration across the crypt axis could be clearly monitored.⁶⁹ Costello et al. improved the scaffold using poly (lactic-*co*-glycolic acid) to support the co-culture of different cell lines, and to evaluate interactions among bacterial adhesion and pathogenic invasion in static culture.^{49,66} Furthermore, they created a bioreactor containing villus scaffolds and fluid flow.⁷⁰ Besides the physical fabrication of a 3D scaffold for villi-like structure formation (Figure 2(b)), other reported protocols employed mechanical stretching against an elastic porous membrane to which intestinal epithelial cells attach.^{52,53} The stretching process can simulate peristaltic motions that the other microstructures above cannot perform. Artificial peristalsis can then induce the morphogenesis of 3D villi lined by the four types of

intestinal epithelial cells (Figure 2(c)). Kim et al. used this strategy to induce human Caco-2 cells to undergo villus differentiation and produce intestinal villi-like structures.⁷¹ Based on this method, they found that optimized experimental conditions to transform monolayer Caco-2 cells to villous morphology were medium flow speed at 30 $\mu\text{L}/\text{h}$ in both upper and lower channels, and a cyclic strain frequency of 0.15 Hz.^{52,53} Moreover, Caco-2 cells not only formed intestinal villi, but also developed basolateral proliferative crypts and ultimately differentiated into multiple intestinal cell types. The resulting vascular endothelial cells could then be further cultured within the lower vascular channel to recreate the intestinal tissue-tissue interface.⁵⁵ This tissue-level gut-on-chip exhibited gut functions such as nutrient digestion, mucus secretion, intestinal barrier formation, and even multilineage differentiation.^{57,72} Thus far, all the reviewed work was performed in an aerobic environment, which is easier to fabricate and manipulate. However, human gut, especially the intestinal cavity, is a strictly anaerobic environment. Therefore, to emulate the anaerobic condition of the intestinal tract, biologists further developed anaerobic intestinal chips.³¹ Shin et al. developed an anoxic-oxic interface-on-a-chip (AOI Chip) to support the growth of anaerobes.⁵⁸ In their work, anoxic culture medium was perfused into the upper channel for bacterial growth, whereas oxic culture medium was added to the lower channel for intestinal cells. Their results revealed that both the epithelial layer

and medium flow are necessary and sufficient to create a steady-state vertical oxygen gradient in the AOI Chip. Two obligate anaerobic commensals were successfully co-cultured without compromising cell viability. Recently, a more complex anaerobic gut-on-chip system was built to maintain dynamic interactions between mucus-producing human intestinal epithelium and aerobic/anaerobic commensal microbes, and the villus structure and hollow vascular lumen were well-lined with endothelial cells.³¹ In this work, six micro-sensors were integrated into the device for in situ oxygen measurements, and the whole device was incubated in a custom anaerobic chamber.

In general, 3D gut-on-chip models provide a better solution to simulate the human gut in vitro, including the epithelial/endothelial layer, villous microstructures, mucus, anaerobic environment, and even peristalsis. These advanced units ensure more realistic functions of the human gut and permit increased applications, such as drug screening, host–microorganism interactions, and nutrition metabolism.

Applications of in vitro gut-on-chip models

Oral delivery is considered to be the most convenient drug administration method. Before reaching the systemic circulation, orally administered drugs are absorbed and metabolized first by the small intestine epithelium.⁶⁴ Thus, understanding oral drug absorption, intestinal transportation, and toxicity is indispensable to the drug development process.⁷³ Many foods can cause allergic reactions in different people. Similar to drug pharmacokinetics, nutrients of food are absorbed first in the small intestine and then delivered to the rest of the body through blood vessels.⁷⁴ Thus, it is important to understand absorption, metabolism, and distribution of digested food to identify treatments for food allergies as well. Traditional in vitro models based on an intestinal epithelial monolayer lack gut physiological characteristics such as intestinal structures, tissue-tissue interactions, and host–microbiome crosstalk, which is strongly related to human health. Although animal models are physiologically relevant to human, there are huge discrepancies between different animal models and humans in terms of absorption, metabolism, microbiome, and gastrointestinal tract. Gut-on-chip allows engineering of an artificial gut containing various human cell types (intestinal epithelial, endothelial, and immune) with a controlled biochemical microenvironment. Importantly, the creation of gut-on-chip models supports drug pharmacokinetic research, the testing of food allergenicity, and prediction of drug efficacy, thus accelerating both the drug development process and analysis of relationships between food and inflammation.^{75,76} Furthermore, the balance between the gut microbiome or gut flora and host is of

great significance to maintaining intestinal homeostasis. Human gut-on-chip can be exploited for co-culture with the microbiome, to recreate intestinal disease models, test targeted therapeutics, and dissect complex disease mechanisms. In this section, we review applications of gut-on-chip systems for evaluating drug pharmacokinetics, host–gut microbiota crosstalk, and nutrition metabolism (Figure 3).

Drug pharmacokinetics and development

Drug absorption mainly occurs in the intestinal tract, but studying intestinal permeability in animal models in vivo is very challenging. Although drugs absorbed by passive transport show a good correlation between animals and humans, there are significant distinctions between animal models and humans. Thus, many alternative in vitro assays have been developed to investigate intestinal absorption mechanisms for diverse types of drugs.⁷⁶ With regard to these investigations, gut-on-chip provides an efficient method because of the aforementioned advantages (Figure 3(a)).^{47,64} For instance, Park et al. employed a gut-on-chip platform with a microhole-trapping array to measure the cellular permeability of ten well-known drugs commonly used as antihypolipidemic agents or diuretics.⁷⁷ Gao et al. integrated a microdevice with mass spectrometry to measure the permeability of curcumin (a polyphenolic antioxidant), that could further improve detection capability.⁴⁵ More importantly, gut-on-chip can serve as one part of a body-on-chip system to evaluate continuous pharmacokinetic processes such as absorption, distribution, metabolism, and excretion (ADME) of various drug administration routes, and predict drug efficacy.^{78–80} An et al. created a laminated microfluidic device to mimic the drug ADME response in vivo. They measured three drugs (propranolol, thiopentone and pentobarbital) pharmacokinetics on chip and found that propranolol was better absorbed than thiopentone and pentobarbital.⁷⁸ Kimura et al. developed a body-on-chip model including an intestinal barrier, liver metabolism, and lung model for in vitro evaluation of three anticancer drugs (epirubicin, irinotecan, and cyclophosphamide).⁸¹ Imura et al. integrated an intestine–liver model to evaluate correlations among intestinal drug absorption, hepatic metabolism, and bioactivity of breast cancer cells.⁸² Li et al. designed an intestine–kidney chip to investigate the absorption of a drug (digoxin) from the small intestine, as well as toxicity to the downstream kidney, thus providing a powerful platform to test drug absorption and nephrotoxicity. Meanwhile, gut-on-chip platforms have also been applied for drug screening and development. Sasan et al. engineered an intestinal epithelium-endothelium model to identify new radioprotective drugs by physiologically mimicking radiation damage.⁵⁹ They exposed the above model to dimethylxaloylglycine, a compound that prevents radiation injury in rats, and

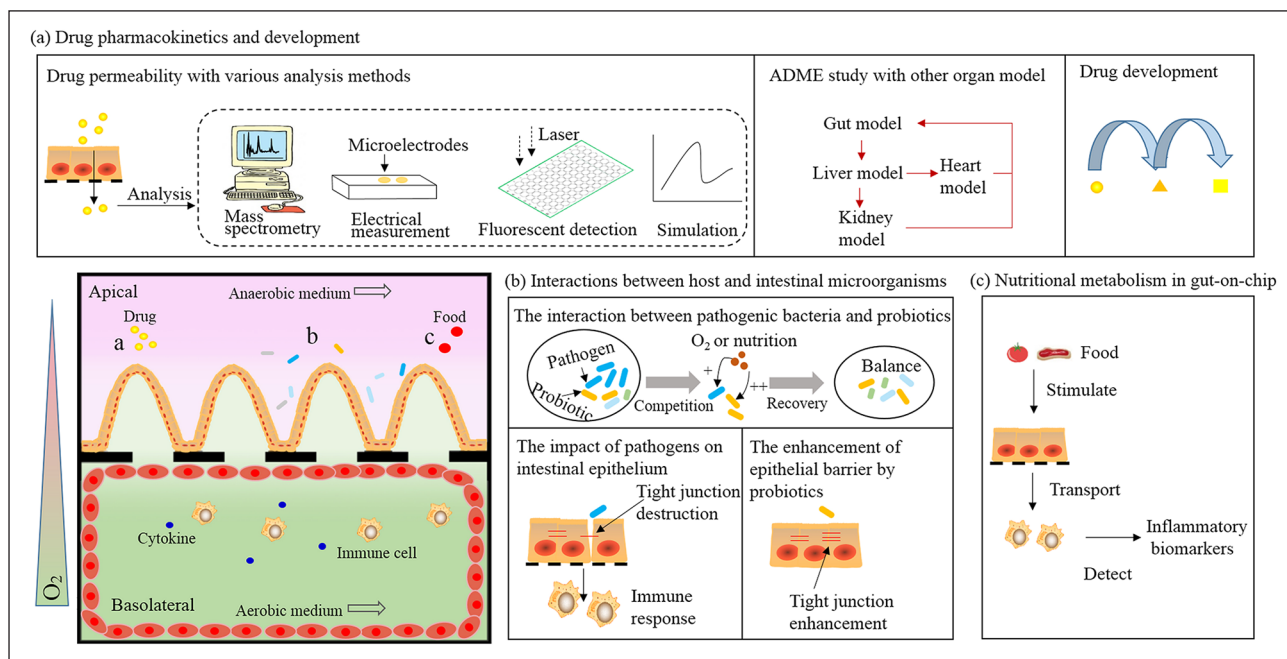


Figure 3. Applications of gut-on-chip system (bottom left). (a) Drug permeability and pharmacokinetics. By combining with multiple analysis tools, gut-on-chip is easy to realize drug absorption and metabolism with the other organ model to accelerate drug development. (b) Gut-on-chip can be used to understand pathogen-induced pathogenesis, analyze host responses, and identify probiotic therapies. (c) Nutritional metabolism in gut-on-chip. Multiple stimuli are applied to the intestinal villi layer, together with digested nutrients, then transported into the basolateral layer. Ultimately, the inflammatory biomarkers are detected to evaluate the ability of nutrient modulation on inflammatory activities.

noticed that intestinal permeability and microvillus injury were efficiently mitigated. Moreover, apoptosis, reactive oxygen species generation, and lipid degradation were significantly reduced in both epithelial and endothelial cells. From another perspective, the gut-on-chip could also be used to create more human-relevant pathological models for screening and development of appropriate countermeasures.⁸³ By utilizing patient-derived stem cells, this platform also provides a novel way to potentially develop personalized therapies in the future.⁸⁴

Interactions between host and intestinal microorganisms

Through long-term co-evolution, the gut microbiome has become inseparable from the host and closely related to human health.^{85,86} In recent years, gut microbes have been implicated in many diseases, such as obesity, inflammatory bowel disease, and neurobehavioral disorders.^{87–89} To decipher the detailed mechanism of such correlations, gut-on-chip technology can provide an appropriate platform by simulating the gut microenvironment, simplifying the microbiome, and permitting monitoring on interactions in vitro (Figure 3(b)).⁶³

In the typical two-channel design separated by a porous membrane, the upper channel can be used to mimic the host intestinal cavity, where the interaction between

epithelium and microbes occurs, while the lower channel can represent blood vessels that provide nutrients and oxygen to epithelial cells, and even be supplemented with immune cells to understand the critical role of the immune system.⁷¹ Kim et al. engineered such a chip and found that the co-culture of non-pathogenic *E. coli* and *Lactobacillus rhamnosus* GG significantly increased epithelial integrity. The bacterial strains and mammalian cells could be labeled with different fluorescence markers, thus allowing interactive dynamics to be detected.⁵² To expand the capabilities of this technology, the authors further recreated a gut inflammation model by combining immune cells, commensal strains, pathogens, probiotics, and antibiotics.⁵⁵ They noticed that the colonization of pathogenic *E. coli* (EIEC) destroyed villi structure and caused gut injury even in the presence of immune cells. They proposed that the pre-colonization with the probiotic VSL#3 could inhibit the growth of EIEC and protect against gut injuries. Moreover, the simulated peristalsis implicated that ileus could be exacerbated by peristaltic interruption and bacterial overgrowth within the small intestine. This work provided a good idea to study the repair effect of probiotics on intestinal inflammation on chip. Shin et al. reconstructed a similar intestinal inflammation model by simulating physiological flow and movement, and found that a complete epithelial barrier was necessary to maintain gut homeostasis in host–intestinal microbiota crosstalk.⁵⁶ Moreover,

pathogen infection model is a significant application area of gut-on-chip. Grassart group, who utilized gut-on-chip to interrogate the impact of intestinal mechanical forces on *Shigella* infection, found that a 3D gut-on-chip with intestinal flow and peristaltic motion enhanced *Shigella* invasion and infectivity compared with a conventional 2D system with no mechanical forces.⁹⁰ Other than bacteria, gut-on-chip has also been explored as an in vitro model for interactions between the host and infective viruses, and to depict mechanisms of enterovirus pathogenesis.⁵⁴ Pathogen infection model as described above can offer a new insight in pathogenesis study and even provide guidance for the clinical treatment of infectious diseases.

The human gut microbiome contains thousands of different bacterial strains, many of which are actually obligate anaerobes.⁹¹ Thus, another pivotal role for gut-on-chip is the investigation of crosstalk between the gut microbiome and host under anaerobic conditions. One recent example is the HuMix model, which was proposed to investigate the interface across host and microbiota.⁴⁶ For HuMix, the authors co-cultured human intestinal epithelial cells with the obligate anaerobe *Bacteroides caccae* and facultative anaerobe *Lactobacillus rhamnosus* GG (LGG), and analyzed interactions by transcriptomics, metabolomics, and immunological assays. They found that the addition of *B. caccae* resulted in significant changes in transcriptional and metabolic responses of intestinal cells compared with intestinal cells co-cultured with only LGG. Shin et al. co-cultured obligate anaerobic symbiotic *Bifidobacterium adolescentis* and *Eubacterium hallii* on a similar gut chip. Epithelial cell viability was unaffected, even after 1 week, and the two bacterial strains gradually colonized. It is noteworthy that the integrity of the epithelial barrier was significantly higher in the presence of *B. adolescentis* in anoxic medium compared with control culture in oxic medium. However, co-culture with *E. hallii* did not cause any significant difference in barrier integrity, possibly because of complicated anaerobic interactions among the microenvironment, bacterial growth, and epithelial regulation.⁵⁸ Using an anaerobic gut-on-chip, Jalili-Firoozinezhad et al. also illustrated that anaerobic conditions could physiologically maintain higher microbial diversity than aerobic conditions.³¹ In this work, the authors co-cultured microbial cells and epithelial cells in anaerobic and aerobic conditions, respectively, by modulating the oxygen concentration. This method provided a new opportunity to simulate the real interface between anaerobe and host as the human intestinal environment. With the gut-on-chip technology developing from initial two-dimensional models to current more comprehensive systems, studies on host-microbial interactions range from a monolayer to the multi-tissue interface, from a single strain to the complex microbiome community, even from aerobic to anaerobic environment that highly restores the intestinal microenvironment and reveals the role of intestinal microorganisms in maintaining human health. Thus,

gut-on-chip as a precisely controllable system for biochemical microenvironment can effectively maintain the cell viability and monitor real-time responses from the cells, providing excellent technical support for the mechanisms between host-microorganisms interactions.

Nutritional metabolism

Food is not only a source of nutrients, but also a modulator of physiological functions that can induce inflammatory responses in the human GI tract. Different types of diets cause various body responses. High-fat meals elicit a systemic rise in pro-inflammatory cytokines that contributes to the development of chronic inflammation. In contrast, several food products, such as orange juice, tomato, and dairy products, have been demonstrated to reduce the post-prandial inflammatory response to high-fat meals.⁹² However, clear interactions between nutrition and organismal inflammation have not been expounded. Thus, understanding the molecular fate of nutrients in ingested food, including ADME, is crucial to human health. During the process of screening food products using in vitro models, digested nutrients are transported through an intestinal cell monolayer, and the ability of transported nutrients to ultimately modulate the inflammatory activity of immune cells is measured.⁹³ Microfluidic-based gut-on-chip models provide a fast and more efficient approach to study the immunomodulatory potential of food and model food allergies compared with traditional Transwell systems.⁹² Most gut-on-chip systems for nutritional analysis contain an intestinal cell culture module on the apical side and an immune cell co-culture module on the basolateral side.^{48,75} Specific food stimulates the gut epithelium in the upper channel to simulate nutrition digestion, and detecting the secretion of pro-inflammatory cytokines by immune cells in the lower channel helps to identify the stimulating food (Figure 3(c)). For example, researchers used lipopolysaccharide (LPS) to stimulate immune cells and then quantified different responses. Their results indicated that LPS induced interleukins 1 and 6 secretion by immune cells.⁷⁵ By combining microfluidic technology-based cell culture and manipulation with time-resolved fluorescent imaging, gut-on-chip platforms have the potential to optimize nutrient-kinetic measurements and alleviate some restrictions of traditional systems in vitro.⁷⁵ By providing a physiologically relevant gut microenvironment and sensitive real-time analysis, gut-on-chip offers a simpler, faster platform for screening products before they enter the increasingly rich and varied food market. Despite the potential of microfluidic devices, such as a controlled fluid environment that more closely mimics human physiology, use of gut-on-chip systems for nutrition analysis has been challenged. Food is a very complex sample, thus, to analyze food at a micro-scale, complex sample processing and analysis methods need to be developed. Some foods stimulate the host by interacting with gut cells and gut bacteria,

leading to an immune response.^{94,95} Therefore, co-culture of different types of cells, even interacting with bacteria in the microfluidic network, is indispensable. We believe gut-on-chip technology and nutraceutical science can be integrated to establish platforms for investigating food allergies on-chip, as well as the influence of food quality on health, thus supporting the identification of treatments for nutrition-related disease.

Challenge and prospect

Despite its advantages and wide applications, the technology itself still faces many challenges. For example, one of the largest concerns is reproducibility and robustness in different laboratories. Therefore, strategies to standardize different modules for gut-on-chip technology are imperative, particularly for commercialization. Secondly, although simplification and modulization speed up development, current gut-on-chip platforms do not fully comprise the real complexity of the human gut, thus, parts of gut functions have been lost. Therefore, how to add more functional modules while maintaining the current merits of this platform requires more wisdom and practice. For instance, the human intestine is surrounded by large numbers of enteric nerves that control peristaltic contractions,³⁹ maintain gut barrier functions, tune endocrine secretions, and regulate blood flow.^{38,96} Indeed, the absence or lack of such enteric nerves has been implicated as the primary cause of several gastrointestinal disorders, such as Hirschsprung's disease.^{97,98} There is no denying that gut-on-chip as an excellent biomimetic technology *in vitro*, it has the potential to be exploited to study more biological and medical problems. One of the potentials of gut-on-chip is to construct different enteropathy models, for instance, IBD, ileus and endotoxemia.^{55,99} Importantly, the system can create combination of different kinds of cell and independently control each parameter, so that it paves a new way to reveal the corresponding pathogenesis. In addition to intestinal diseases, we envision that gut-on-chip will be used in many other fields in the near future. For example, antibiotic misuse has led to the emergence of drug-resistant bacteria. Using gut-on-chip, we can establish a drug resistance model to investigate how resistance evolves *in vivo*, and potentially identify other treatments such as phage therapies.^{100,101} Probiotics play an important role in resisting intestinal dysfunction, which can fight against pathogenic invasion and regulate intestinal immune functions. It is noteworthy that there have already been several related probiotic research on-chip, such as LGG, VSL#3.^{52,55} Thus, gut-on-chip can be then explored to develop new commensal-derived therapeutics. Meanwhile, the multi-organ model established by gut-on-chip and other organ models (liver, kidney) can be effectively evaluate drug toxicity and accelerate the process of drug development. Furthermore, the next possible milestone for gut-on-chip

could be the pioneering of patient-derived gut-on-chip prepared from diseased tissue or human intestinal stem cells from patients *in situ*. Using organoid culture derived from the primary patient intestinal cells, immune cells and local gut microbiome can build a personalized platform.¹⁰² This would allow researchers to conduct personalized diagnostics and provide precision medicine at the same time.

Conclusion

The complete function of human gut, the relevance of gut microbes to human diseases, and the corresponding treatment protocols for intestinal diseases still remain unclear in a large extent. One reason for this is the complexity of human intestine, and the numerous challenges associated with decoupling hundreds of different parameters. Gut-on-chip platforms can be employed to resolve many of these challenges by extracting key parameters to simplify the whole system, combining different modules to uncover interactions, and realizing *in vitro* detection to monitor real-time dynamics. In this article, we reviewed various types of gut-on-chip systems and described the characteristics of different models in structure, function and application. Gut-on-chip can be applied in drug pharmacokinetics, host-microbiome interaction research and nutrition metabolism but no limited within it. As in practical clinics, gut-on-chip can soon be served as a flexible, valuable and powerful tool to predict drug responses. In the process of continuous development, gut-on-chip with human intestine physiology will provide a personalized platform for intestinal diseases research and accelerate the personalized medicine.

Author contributions

Y.X. performed the literature search and wrote the manuscript. H.W. participated in the discussion. H.W., Y. Y., M.L., and S.H. edited and revised the manuscript. Y.X., H.W., X.F., and S.H. conceived this review.

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