Review

Gut-on-a-chip for disease models

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Abstract

The intestinal tract is a vital organ responsible for digestion and absorption in the human body and plays an essential role in pathogen invasion. Compared with other traditional models, gut-on-a-chip has many unique advantages, and thereby, it can be considered as a novel model for studying intestinal functions and diseases. Based on the chip design, we can replicate the in vivo microenvironment of the intestine and study the effects of individual variables on the experiment. In recent years, it has been used to study several diseases. To better mimic the intestinal microenvironment, the structure and function of gut-on-a-chip are constantly optimised and improved. Owing to the complexity of the disease mechanism, gut-on-a-chip can be used in conjunction with other organ chips. In this review, we summarise the human intestinal structure and function as well as the development and improvement of gut-on-a-chip. Finally, we present and discuss gut-on-a-chip applications in inflammatory bowel disease (IBD), viral infections and phenylketonuria. Further improvement of the simulation and high throughput of gut-on-a-chip and realisation of personalised treatments are the problems that should be solved for gut-on-a-chip as a disease model.

Keywords

Gut-on-a-chip, disease model, inflammatory bowel disease, SARS-CoV-2, phenylketonuria

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Introduction

The digestive, absorption and barrier functions of the small intestine are closely related to its unique structural features, such as villi and microvilli structures, mucus layers and periodic peristalsis.[1–3] The large surface area of the villi enhances absorption in the small intestine.[4] The mucus layer is the first significant barrier between the small intestine and external world.[5] Periodic peristalsis can aid digestion and absorption and promote the transfer of waste. Once the intestinal cells become abnormal, intestinal stem cells can repair the intestine via rapid proliferation and differentiation.[6] Additionally, intestinal microorganisms play an important role in intestine functions.[7–12] These processes are essential for maintaining intestinal homoeostasis.[13–16]

Animal models, such as those of mice and pigs, are famous for studying intestinal diseases.[17] Due to differences in species, some animals cannot be used to study human diseases. Moreover, the use of animals in research is controversial. Compared to animal models, culturing cells in vitro to study conditions exhibits the advantages of convenience, low cost and no ethical issues.[18] However, traditional culturing cells in vitro models usually lack in vivo characteristics such as fluid flow, periodic peristalsis, crosstalk between host and microorganism and crosstalk between tissues.[19,20] Therefore, it is critical to reconstruct this complexity by using an in vitro model.

With the development of micromanufacturing and 3D printing technologies, gut-on-a-chip provides a new method for studying intestinal diseases in vitro.[3,21,22] Based on intestinal functions, gut-on-a-chip introduces modules with different parts, such as an injection pump for fluid flow and a pressure system for mechanical deformation.[1,23–25] Many modules, including the trans-epithelial

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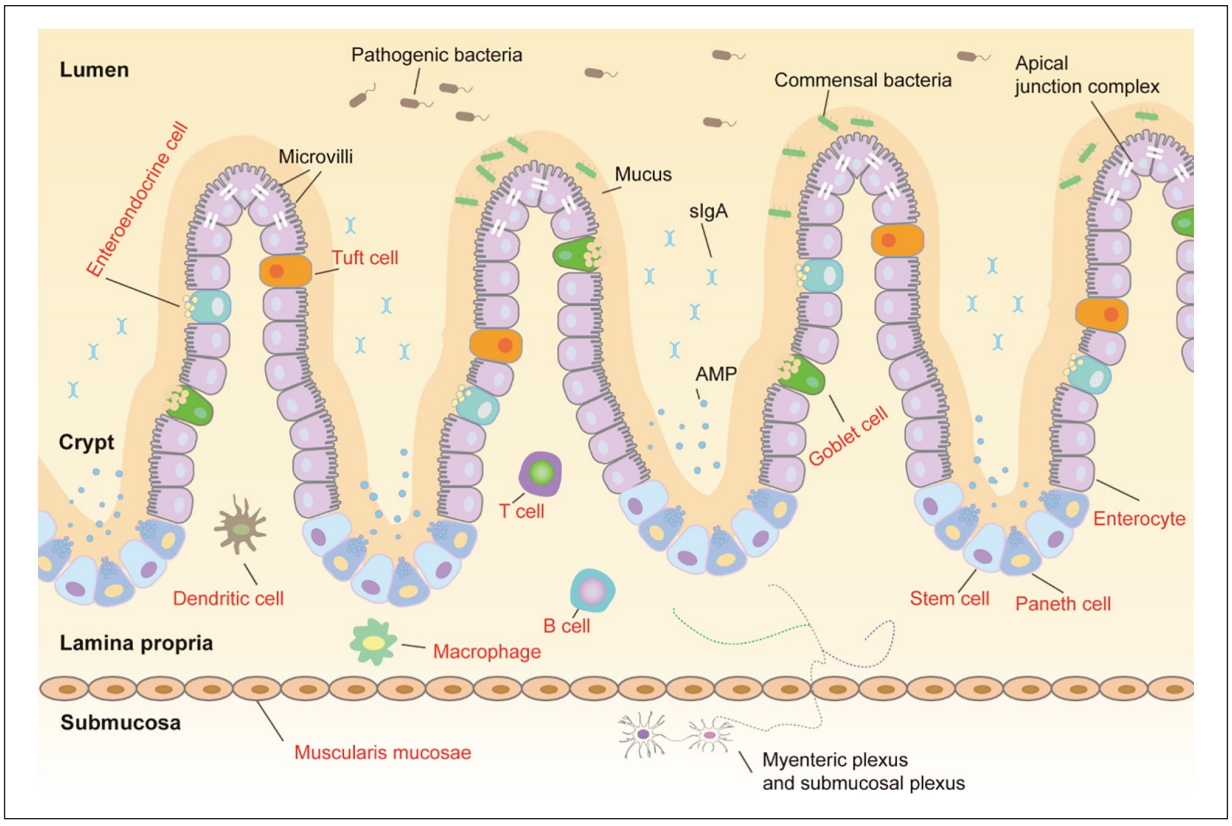

Figure 1. Schematic diagram of the intestinal mucosa. The intestinal structure can be divided into mucus, villi, crypt, lamina propria, muscularis mucosae and submucosa from the outside to the inside. Symbiotic bacteria in mucus play an essential role in intestinal barrier function. The epithelium includes many types of cells, including enterocytes, goblet cells, enteroendocrine cells, tuft cells, Paneth cells and intestinal stem cells. Similar to many vital parts, the lamina propria also contains many immune cells. Tight junctions, adherent junctions and desmosomes are the main components of the apical junction complex. The ENS resides in the submucosa, consisting of two major plexuses: the myenteric plexus and submucosal plexus.

electrical resistance (TEER) module, pH module and metabolite analysis module, have been introduced to detect cell growth on a chip in real-time.[26] Therefore, the concept of 'multi-organs-on-a-chip' can aid in providing new insights into diseases and has also been proposed to examine certain conditions involving multiple organs.[27–31]

In this review, the vital structures and functions of the human intestine in gut-on-a-chip simulations is summarised. Moreover, the development of a gut-on-a-chip is presented by summarising studies on gut-on-a-chip in the literature. We also discuss some of the current applications of gut-on-a-chip and the future direction of development. We believe that the simulation ability and high throughput of gut-on-a-chip are key to gut-on-a-chip as a disease model.

Human intestinal structure and function

Human intestinal structure. The human intestine includes the small and large segments. The small intestine is an integral part of the digestive system that can break down and absorb most nutrients. It is a massive organ with an average length of 3–5 m and can be divided into the duodenum, jejunum and ileum. The large intestine includes the caecum, appendix, colon, rectum and anal canal. Unlike the small intestine, it has a shorter length but much larger lumen.[32,33]

The intestinal mucosa, the innermost layer of the intestine, includes a layer of polarised columnar epithelial cells and subepithelial region that contains the lamina propria, enteric nervous system (ENS), connective tissue and muscular layers.[34] The epithelium incorporates enterocytes, goblet cells, enteroendocrine cells, tuft cells, Paneth cells and intestinal stem cells (Figure 1).[14,35] Enterocytes are primarily responsible for nutrient absorption. Enteroendocrine cells secrete various gastrointestinal hormones. It has been determined that tuft cells, as receptors, play an essential role in anti-parasite infection.[36] Goblet cells can synthesise and release mucin, while Paneth cells can synthesise antimicrobial peptides (AMP). Given the ability of intestinal stem cells, the renewal rate of intestinal epithelial cells is rapid, and the cells last for only 3–5 days.[37,38] The apical junction complex consists of tight junctions, adherent junctions and desmosomes (Figure 1). These structures confer

mechanical strength on the intestinal epithelial barrier and regulate paracellular permeability.[39] The mucus layer above the epithelial barrier separates the luminal contents from the intestinal epithelial cells. Mucus consists of water and glycosylated proteins, termed as mucins, which are O-linked glycan-attached glycoproteins anchored to the intestinal epithelial layer.[40] The lamina propria contains many immune cells (Figure 1), including macrophages and dendritic cells.[34]

The ENS is the most significant part of the peripheral nervous system (PNS), which differs in size, morphology, composition and complexity from the rest of the PNS.[41] Moreover, the ENS originates from neural crest cells that colonise the gut during intrauterine life. The ENS is an intertwined network of neurones and glial cells (Figure 1) consisting of two significant plexuses: the myenteric plexus and submucosal plexus.[34] Many neurones are present in the ENS, approximately 200–600 million in humans. Approximately 20 types of intestinal neurones can be defined with slightly different numbers in different regions.[42]

Microbiota of the intestine. The intestine is a complex ecosystem that contains a wide variety of microorganisms under anaerobic conditions. The human intestine contains approximately 10^{14} microbial cells, including bacteria (the vast majority), viruses (5.8%), archaea (0.8%) and eukaryotes (0.5%).[43] The composition of the intestinal microbiota is influenced by host genetics, diet and environmental factors.[44] Hence, the diversity of the gut microbiota is highly dynamic and differs for each human individual and changes during lifetime.

The intestinal microbiota interacts directly with the host by producing a diverse reservoir of metabolites obtained from exogenous or endogenous substances.[40] Many intestinal diseases are associated with a decreased diversity of the intestinal microbiota. However, aberrant intestinal microbiota are not only associated with intestinal diseases, such as Crohn's disease and ulcerative colitis, but also with non-intestinal diseases, such as obesity, type 2 diabetes mellitus, rheumatoid arthritis and neurological and psychiatric disorders.[45–47] However, it remains unclear whether the imbalance of intestinal microbiota causes or is a consequence of the disease.[48]

The commensal intestinal microbial species can fight evading pathogens by producing antimicrobials, such as bacteriocins and certain metabolites, competing for luminal nutrients and attachment sites and producing signalling molecules that can modulate the gene expression of other bacteria. In return, the human host provides a substrate for microbiota.[43] Commensal microbes benefit from the nutrient-rich intestinal environment. The microbiota produces hundreds of proteins and metabolites, including phenolic metabolites and short-chain fatty acids (SCFAs). These metabolites modulate crucial host functions, including nutrient processing, maintenance of energy homoeostasis and immune system development.[49–52]

Digestive and absorption function of the intestine. The stomach receives and stores food for several hours and secretes acids and enzymes to facilitate digestion. During this time, the smooth muscles of the stomach contract and relax to mix and break down food into smaller particles that are then processed further in the duodenum.[53,54]

The duodenum is the initial portion of the small intestine where absorption begins. Pancreatic enzymes (a complex mixture of proteases, amylases and lipases) interact with other digestive enzymes produced by the inner wall of the small intestine to break down food components. Before reaching the jejunum, bicarbonate is secreted into the duodenum to neutralise stomach acid, maintaining a pH of approximately 6–7 in the small intestine, which is suitable for digestion of proteins, carbohydrates and fats.[55] The primary function of the jejunum is to absorb sugar, amino acids and fatty acids. The ileum absorbs any remaining nutrients that are not absorbed by the duodenum or jejunum, particularly vitamin B12, as well as bile acids that continue to be recycled.[32,33] Moreover, the colon, the last part of the digestive system, reabsorbs the remaining water from the indigestible contents and prepares the luminal contents for elimination.[56] Fermentation is a vital function of the large bowel or colon and is considered as the process by which anaerobic bacteria break down carbohydrates into short-chain fatty acids, gases (hydrogen, methane and carbon dioxide) and other metabolites.[57] In general, the intestinal microbiota plays an essential role in the digestive and absorption functions of the intestine.[47]

Barrier function of the intestine. The intestine constructs three types of barriers: physical, chemical and immunological barriers. The physical and chemical barriers spatially segregate gut microbiota in the intestinal lumen and immune cells in the lamina propria. These two barriers can prevent conflicts between the intestinal microbiota and host immune cells, resulting in intestinal inflammation.[52] Furthermore, immunological barriers can protect the intestine via the powerful immune functions of immune cells.

Mucus prevents microbiota and large molecules from contacting the epithelial cells, but simultaneously allows the passage of small molecules.[56] Intestinal mucus is an organised glycoprotein network with a host-specific glycan structure. However, the maturation and function of the mucus layer are strongly influenced by intestine microbiota.[58–60] The mucus layer in the colon is composed of inner and outer layers, and the intestinal microbiota is confined to the outer layer. Conversely, the mucous layer diffuses in the small intestine and does not form a double layer.[43,61] Additionally, the apical junction complex can physically hamper microbial invasion via the paracellular pathway.[62]

Microbe-associated signals can induce the expression of defensins in enterocytes and antimicrobial factors in Paneth cells, leading to the production of antimicrobial peptides. Furthermore, these signals can also stimulate the maturation of B and T cells. In this case, B cells produce more IgA, and serum amyloid A-dependent T helper 17 cells improve their differentiation ability, which leads to innate immune defence mechanisms for fighting infections.[63,64]

Development and improvement of gut-on-a-chip

Researchers developed many models, such as the intestinal ring, intestinal segment, everted intestinal sac, Boyden Chamber and Transwell, to examined the complex structure and function of the intestinal tract. In a previous study, the advantages and disadvantages of traditional intestinal and gut-on-a-chip models have been extensively discussed.[14] Compared with traditional intestinal models, gut-on-a-chip, based on a microfluidic chip, exhibits certain advantages, such as providing similar fluid velocity and peristalsis, over traditional models. These advantages have led to the rapid development of gut-on-a-chip in recent years, which has become a powerful tool for examining the intestinal tract. However, several limitations of the gut-on-a-chip model, such as complex fabrication processes, strict operating procedures and small chip capacity, must be improved. This in turn can lead to an increase in simulation of in vivo conditions.

Development of gut-on-a-chip. In 2012, Kim et al. used microfluidic system engineering to develop a mechanically active 'human lung-on-a-chip' model that can exhibit cyclic breathing motions. Given the advantages of this model, they explored whether a similar in vitro model of the human intestine, which can replicate the key features in the intestine, can be developed. Thus, they first proposed the concept 'gut-on-a-chip' and used this model to examine the co-culture of Caco-2 cells with living intestinal microbes.[65]

The 'gut-on-a-chip' contains upper and lower microchannels that are separated by a porous Polydimethylsiloxane (PDMS) membrane. On the left and right sides of the channel, vacuum chambers are used to exert cyclic mechanical strain to mimic peristaltic motion. The process of making an intestinal chip has been described previously,[66] and Figure 2(a) depicts a schematic diagram of the general process. They determined that the application of physiological fluid flow and shear stress can promote accelerated intestinal epithelial cell differentiation, formation of 3D villi-like structures and increased intestinal barrier function. The addition of cyclic mechanical strain further enhances these responses. Moreover, they cultured *Lactobacillus rhamnosus* GG (LGG) on the apical surface of Caco-2 cells monolayer for up to 100 h and determined that 'gut-on-a-chip' supports the growth of microbial flora without compromising human cell viability. The authors also showed reprogramming of human intestinal epithelial cell lines to undergo spontaneous villus morphogenesis and small intestinal differentiation.[67] Therefore, the proposed model is highly successful because it can effectively recapitulate many complex functions of the human intestine.

In a follow-up study, Kim et al. analysed as to how probiotic and pathogenic bacteria, lipopolysaccharides, immune cells, inflammatory cytokines, vascular endothelial cells and mechanical forces individually and in combination contribute to intestinal inflammation, villus injury and compromise epithelial barrier function. The results showed that the microfluidic gut-on-a-chip device can be used to create human intestinal disease models to further examine intestinal pathophysiology.[66,68,69] Based on the aforementioned studies, it can be observed that gut-on-a-chip is a complex system composed of many types of structural and functional units in a modular way.[70] In other words, the complexity of the system depends on the number and functionality of modules that should be explored. The aforementioned articles provide an essential idea to use gut-on-a-chip to study intestinal and non-intestinal diseases in the future.

Improvement of gut-on-a-chip. Since the concept of gut-on-a-chip was proposed, many scholars have performed significant studies based on gut-on-a-chip and introduced many practical modules (Table 1). Gut-on-a-chip can be classified into three types according to their structure (Figure 2(b)). The first type of gut-on-a-chip consists of an upper microchannel, a lower microchannel and a porous membrane in the middle. The second type of gut-on-a-chip consists of an upper microchannel, a lower microchannel and a middle channel (usually filled with an extracellular matrix). The third type of gut-on-a-chip consists of a left microchannel, right microchannel and middle channel filled with collagen gel. To further improve the ability of gut-on-a-chip to simulate the in vivo microenvironment, cells with different functions, concentration gradient of oxygen and scaffolds were added to the gut-on-a-chip. HT-29 cells, displaying the properties of goblet cells, were co-cultured with Caco-2 cells with low expression of mucus proteins.[71–75] In previous studies, human primitive intestinal epithelial cells obtained from biopsies can overcome the limitations of using Caco-2 cells.[76–78] Human pulmonary microvascular endothelial cells, human umbilical vein endothelial cells (HUVECs), intestinal subepithelial myofibroblasts and peripheral blood mononuclear cells (PBMCs) were also added to the system to provide cellular crosstalk.[71,79–82] Shim et al.[83] incorporated a collagen scaffold mimicking human intestinal villi into a microfluidic device, and thereby, providing cells with a 3D tissue structure and fluidic shear.

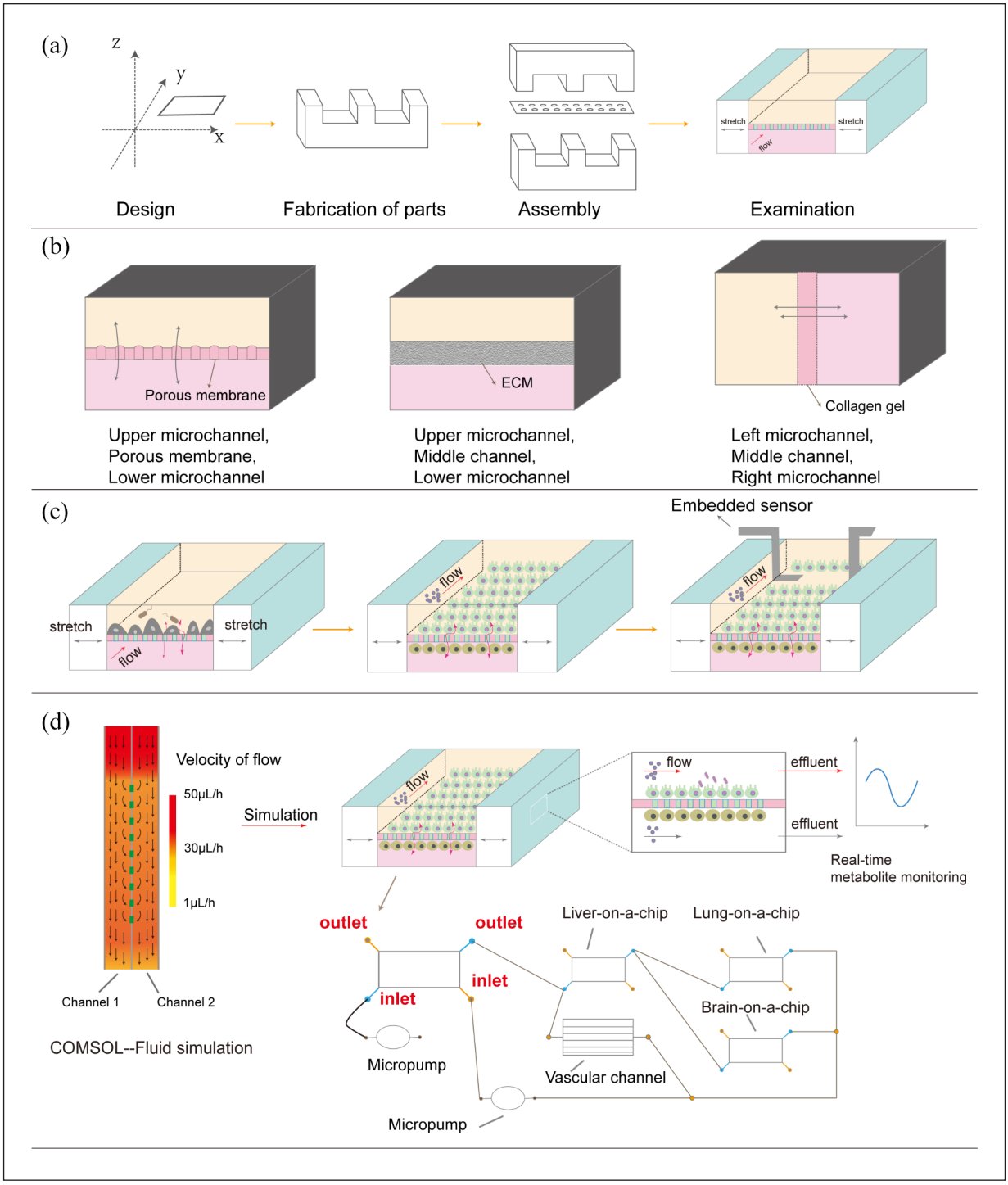

Figure 2. Schematic diagram showing the current development of gut-on-a-chip. (a) The process of making an organ-on-a-chip generally includes: Design, fabrication of parts, assembly and examination. (b) Gut-on-a-chip can be architecturally classified into three types, the first type one contains porous membrane, and the last two use other materials (extracellular matrix and collagen gel) as opposed to porous membrane. (c) The development of a single gut-on-a-chip. Initially, gut-on-a-chip was only able to simply culture monolayer cells. Gradually, gut-on-a-chip was able to co-culture a variety of cells and bacteria. Currently, there are a lot of sensors embedded in gut-on-a-chip, which can be analysed in real time. (d) Schematic diagram of the application of gut-on-a-chip at present. The gut-on-a-chip model is designed according to the anatomical knowledge and then applied to evaluate whether the flow velocity and shear force are in line with the physiological conditions in the human body. To make the experimental process more in line with human physiological conditions, gut-on-a-chip has been used in conjunction with other organ chips (including lung-on-a-chip, liver-on-a-chip and brain-on-a-chip). Simultaneously, each organ chip can use embedded sensors to analyse various metabolites in real time.

Table 1. Characteristics of gut-on-a-chip in different kinds of literature.

Structure	Flow rate	Cell type	Microorganism type	Mechanical strain	Characteristics
Upper microchannel, Porous membrane, Lower microchannel	30 μL/h, (40 μL/h)	Caco-2	*Lactobacillus rhamnosus* GG	10% strain, 0.15 Hz	Mimicking the intestinal peristalsis Co-culture with bacteria[65]
	30 μL/h	Caco-2	N/A	10% strain, 0.15 Hz	Mimicking the intestinal peristalsis[67] Using direct current to measure the TEER values in the chip[84]
	50 μL/h	Caco-2	*Bifidobacterium adolescentis* (DSM 20083), *Eubacterium hallii* (DSM 17630)	10% strain, 0.15 Hz	Oxygen Gradient Co-culture with the obligate anaerobic gut microbiome Computational simulation in COMSOL[85]
	25 μL/h, 30 μL/h, 40 μL/h, 50 μL/h, 400 μL/h, 6000 μL/h	Caco-2	N/A	N/A	Containing four cell culture chambers and NC porous membrane[86] Glass-based chip[87] The embedded electrodes for measuring the TEER[88] Using clinical IBD patient cells[89] Introduction of a collagen scaffold and using gravity flow device[83]
	50 μL/h	Caco-2	Faecal microbiome	5% strain, 0.15 Hz	Convoluted design of microchannels Using organoid-derived epithelial cells Computational simulation in COMSOL[90]
	60 μL/h	Caco-2, HT-29 MTX	Synthetic biotic strain (SYN5183)	10% strain, 0.15 Hz	Analysis of effluent from compartments[73]
	30 μL/h, (60 μL/h)	Caco-2	Coxsackievirus B1	10% strain, 0.15 Hz	Studying viral infections[91]
	50 μL/h, (200 μL/h)	Caco-2, HT-29, PBMCs, HUVECs	SARS-COV-2 strain 107	N/A	Introduction a variety of cells Building a human intestinal SARS-CoV-2 infection model[80]
Upper microchannel, Middle channel, Lower microchannel	Unknown	Caco-2	N/A	N/A	Allowing membrane-free co-culture Real-time imaging High throughput Measuring the TEER values Using interval rocker[92]
Left microchannel, Middle channel, Right microchannel	21 μL/h	Caco-2 HUVECs	*Lactiplantibacillus plantarum* HY7715 probiotic, *Bifidobacterium animalis spp. lactis* HY8002 probiotic	N/A	Osmosis-driven fluidic flow Allowing membrane-free co-culture The embedded electrodes for measuring the TEER[79]

COMSOL: A fluid simulation software; HUVECs: human umbilical vein endothelial cells; IBD: inflammatory bowel disease; PBMCs: peripheral blood mononuclear cells; TEER: trans-epithelial electrical resistance.
The flow rates, cell types, microorganism types and mechanical strains shown in the table can be adjusted independently according to the objective of the study.

Owing to the structure of the intestine, anaerobic gut bacteria play a vital role in human health and disease. It is technically challenging to examine the interactions of oxygen-sensitive bacteria with oxygen-requiring intestinal epithelial cells in vitro.[48] Walsh et al.[93] developed a microfabricated device to generate stable and repeatable defined oxygen gradients from 0% to 4% partial pressure O_2 to emulate the steep oxygen gradient at the colon wall. Shin et al. developed an anoxic–oxic interface-on-a-chip by leveraging a modified human gut-on-a-chip. Furthermore, the results demonstrated a controlled oxygen gradient in the lumen-capillary transepithelial

interface by flowing anoxic and oxic culture media in various physiological environments.[85]

Many studies focused on improving the throughput of gut-on-a-chip. Guo et al.[86] developed a biomimetic human gut-on-a-chip with four culture chambers to model drug metabolism in the intestine. Beaurivage et al.[92] developed a robust high-throughput 3D gut-on-a-chip model containing 40 individual microfluidic chips to investigate the features of inflammatory bowel disease (IBD).

To improve and faster monitor cell growth in gut-on-a-chip, many scholars adopted trans-epithelial electrical resistance, a widely used parameter to characterise the quality of the barrier function of epithelial and endothelial cell monolayers. The internal structure of a single gut-on-a-chip begins to change from simple to complex (Figure 2(c)). Odijk et al.[84] presented a mathematical model to enhance the fidelity of TEER measurements in microfluidic organs-on-chips, and their study illustrated the differences measured in TEER between microfluidic chips and Transwell systems. Van der Helm et al.[88] embedded electrodes in gut-on-a-chip and proposed novel methods for combining impedance spectroscopy with electrical stimulation to measure the cell layer barrier function and detect changes in villus differentiation. Additionally, microchannel modelling can be performed with COMSOL Multiphysics (COMSOL Inc., USA) software to conduct fluidic flow modelling for designing a model that is more consistent with human in vivo condition.[79,90,93] However, PDMS, the preferred material for developing gut-on-a-chip, is unsuitable for certain applications because of its gas permeability and capacity to absorb small hydrophobic molecules.[72,87] Therefore, many materials are also being used to replace PDMS, including Polyethylene terephthalate, Polycarbonate and Polyester.[78,94] Owing to the growing demand for gut-on-a-chip, an increasing number of sensors have been introduced, including pH sensors, SCFA biosensors and cytokine biosensors.[95] The combination of gut-on-a-chip and other organ chips, such as liver-on-a-chip, brain-on-a-chip and lung-on-a-chip, is another way of examining enteric diseases further.[26,76,96–98] Different organ chips can be connected in same or different layers via pipes. By appropriately adding a micropump to the pipeline, the flow rate of the fluid can satisfy the requirements of each organ chip. Vascular channels that simulate blood flow can be added between organs, which stabilises metabolism between organs in the device.[76] Hence, a novel multi-organ-on-a-chip model, combined with gut-on-a-chip, liver-on-a-chip, brain-on-a-chip and lung-on-a-chip models (Figure 2(d)), is proposed in this study. However, experimental data must be compared with anatomical knowledge to ensure the accuracy of multi-organ chips.

When designing microphysiological systems (MPSs), there are usually three basic elements to consider: anatomy, physiology and cell sources.[99,100] Through a unique understanding of these three basic elements, we can design an in vitro model that is highly similar to the human body. First, for anatomy, we should understand the basic structure of the intestinal tract (such as the type and number of intestinal cells and morphology of intestinal villi). Second, for physiology, we should understand the biological and abiotic factors that exist in the intestinal tract. These factors include pathogenic and symbiotic bacteria, extracellular matrix (ECM) that supports cell growth, intestinal pressure, shear forces due to intestinal fluid flow and external physical stimulation of the intestinal tract. Communication and interactions between the intestinal tract and other organs are also important abiotic factors. Under low shear stress and cyclic strain, the columnar epithelium polarises rapidly and spontaneously grows into folds that recapitulate the structure of the intestinal villi. Simultaneously, cells can form a high integrity barrier, which cannot be realised via traditional cell culture.[65,101] However, the shear stress and cyclic strain can have different values owing to different diseases; therefore, these parameters should be constantly verified with anatomical knowledge in the experiment. Finally, for cell sources, we can select cells from different sources for accurate replication of diseases according to different diseases for providing a better scheme for personalised treatment. Therefore, through a comprehensive consideration of these three basic elements, gut-on-a-chip will be more accurate than the traditional model, and the experimental results obtained using the gut-on-a-chip can be easily repeated.[102] The gut-on-a-chip can realise real-time control of the experimental variables using microfluidic devices, and the visual observation and analysis of the experimental process can be realised using a variety of embedded sensors. Based on the many advantages mentioned above, research and application of gut-on-a-chip can be rapidly developed in the future.

Disease models using human gut-on-a-chip

Gut-on-a-chip for studying IBD. Inflammatory bowel disease, including ulcerative colitis and Crohn's disease, is a devastating chronic inflammation of the human intestine (Figure 3(a)). However, its exact aetiology remains unknown. Reduced production of mucus and antimicrobial peptides has been observed in some IBD patients.[52] Generally, it is considered to be due to interactions between the environment, heredity, infection, immunity and mental factors. However, it is difficult to confirm the interactions and contributing factors that play a crucial role in the development of IBD. Therefore, it is essential to develop an in vitro IBD model that can recapitulate the contributing factors to the maximum possible extent and reconstruct the structure and microenvironment of the intestine. Moreover, gut-on-a-chip can satisfy these requirements via microfluidic control and modules with

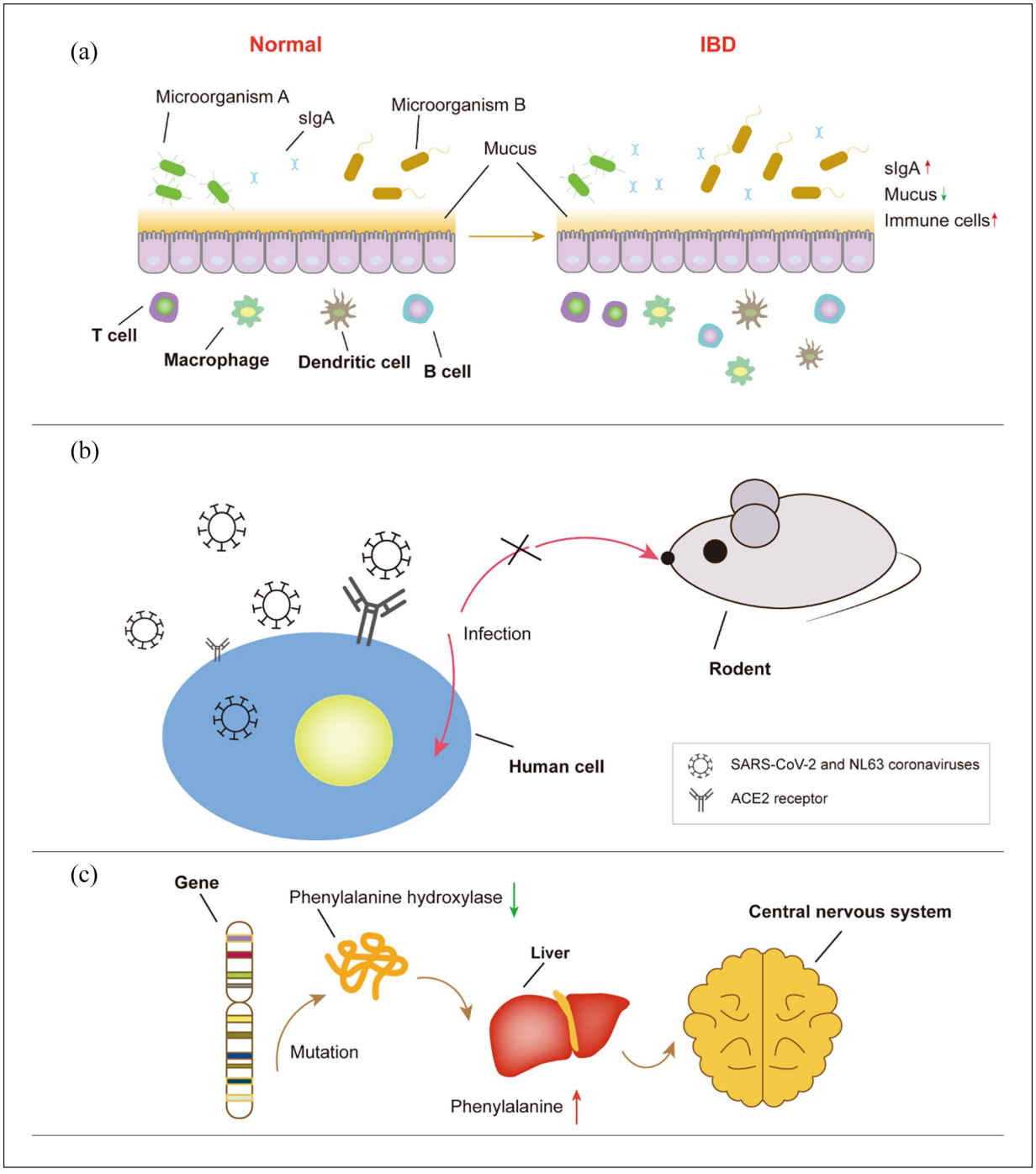

Figure 3. Schematic diagram of the mechanism of three diseases related to the gut. (a) Changes in intestinal structure and composition in IBD patients. The type and quantity of intestinal microorganisms in patients change. Compared with earlier, mucus secretion decreases; on the contrary, the number of immune cells increases. (b) A schematic diagram of invasion of two types of epidemic coronaviruses of the human body. These coronaviruses recognise angiotensin-converting enzyme 2 (ACE2) receptor to infect human cells. Given that rodents do not possess this receptor, they are not suitable for applications involving the infection of these viruses. (c) A schematic diagram of the primary mechanism of Phenylketonuria (PKU). Gene mutation reduces the activity of phenylalanine hydroxylase, resulting in a large accumulation of phenylalanine in the liver. Eventually, these physiological processes will have toxic effects on the nervous system.

different functions.[103] Furthermore, it is easy to obtain the effluent of gut-on-a-chip for analysing metabolic activity, which can aid in understanding the mechanism of IBD.

Beaurivage et al. demonstrated the application of a robust high-throughput gut-on-a-chip model to investigate the hallmarks of IBD. They applied an optimised

immune-relevant cytokine trigger that mimicked the effect of *Escherichia coli*-activated dendritic cells (DCs) on intestinal epithelial cells (IECs) to mimic inflammatory characteristics in this model. Furthermore, they determined that TPCA-1, an anti-inflammatory compound, can prevent inflammation in gut-on-a-chip, which demonstrated the validity of this model for drug discovery purposes.[92]

However, when stimulated, Caco-2 cells do not express some of the major inflammatory cytokines involved in IBD. Furthermore, patients with IBD often lack the necessary regulatory mechanisms and exhibit abnormal activation of certain types of immune cells, leading to a persistent inflammatory state. Beaurivage et al. integrated IECs derived from human intestinal organoids with monocyte-derived macrophages on a gut-on-a-chip. They used lipopolysaccharide and interferon-gamma to induce IBD hallmarks, leading to the activation and increased cytokine production in human intestinal organoids and macrophages. Under microfluidic conditions, they determined that the transcriptome of gut-on-a-chip resembled that of a normal adult human colon in vivo. In this study, TPCA-1 played a similar role in preventing inflammation.[104]

Recently, Yoon et al. used gut-on-a-chip to culture IBD patient cells with and without peptide-hydrogel treatment to validate the synergistic actions of peptides and hydrogels used to treat IBD. The data showed that peptide-hydrogel treatment for 96 h induced significant structural recovery of IBD patient cells in gut-on-a-chip, supported by improved villi formation and ZO-1 expression.[89]

It is also a new method to examine IBD based on the interaction between the microbiota and IBD. Significant variations in intestinal microbiota have been associated with IBD. The intestines of IBD patients show relatively lower bacterial diversity, particularly the loss of anaerobic bacteria.[105] Some studies have suggested that an altered intestinal microbiome can be considered as the core of IBD. However, it remains unclear whether dysbiosis precedes disease development or is a by-product of the disease.[81,106] Some clinical trials have shown that faecal microbiota transplantation (FMTs) can contribute to a positive outcome in IBD. Based on these data, we can take advantage of gut-on-a-chip and FMTs to find a promising treatment for IBD.

Gut-on-a-chip for studying infection of virus. Conventional methods for studying infections include the use of transformed cell lines, primary tissue-derived human cells, stem cells and animal models. Although animal models are one of the most popular models in studies involving infections, some animal models are unsuitable for examining viruses associated with humans (Figure 3(b)). Rodents are evolutionarily distant from humans. Additionally, there are ethical issues associated with experiments involving rodents.[91] Furthermore, conventional cell cultures exhibit other problems such as differences in gene profiles, epigenetics and functions with natural tissues. However, the limited source of primary cells and lack of a microenvironment similar to that of the human body are also problems that cannot be ignored in traditional cell culture.[96]

Villenave et al. used coxsackievirus B1 as a prototype enterovirus strain to analyse human enterovirus infection and replication using a human gut-on-a-chip. They determined high coxsackievirus B1 replication efficiencies in gut-on-a-chip, which almost completely destroyed the villi within 24 h after infection.[91]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and human coronavirus NL63 (HCoV-NL63) recognise angiotensin-converting enzyme 2 receptors in cells for attacking the human body. According to statistics, a large number of patients infected with these coronaviruses exhibit gastrointestinal symptoms. Some viral marker proteins are found in the human gastrointestinal (GI) tract. Therefore, the epithelial lining of the GI tract has been suggested as a potential transmission route and target of SARS-CoV-2 infection.[107] Furthermore, it was discovered that the gut microbiota influences the occurrence and development of lung diseases and are, in turn, perturbed by the respiratory virus infection.[108]

Bein et al. used the gut-on-a-chip to examine host cellular and inflammatory responses to infection with NL63 coronavirus. They determined that cells cultured in gut-on-a-chip significantly increased angiotensin-converting enzyme 2 (ACE2) protein levels when compared to those cultured statically. Furthermore, under the infection of NL63, gut-on-a-chip showed certain characteristics of inflammation, such as loss of barrier function, increased cytokine production and recruitment of circulating peripheral blood mononuclear cells. Moreover, gut-on-a-chip infected with NL63 was used to test the antiviral effects of nafamostat and remdesivir, indicating that gut-on-a-chip can also aid as a human preclinical model for studying coronavirus.[109]

Guo et al. used SARS-COV-2 strain 107 and a gut-on-a-chip to model the mechanism of the virus in infecting the intestine. Caco-2 and HT-29 cells were co-cultured in the upper channel, and HUVECs were cultured with PBMCs in the lower channel. Destruction of intestinal villi, decreased integrity of the intestinal barrier, abnormal mucus secretion and activation of the immune response were observed on the chip infected with SARS-CoV-2. Guo et al. determined that intestinal mucin secretion changes from a concentrated distribution to a dispersed distribution after viral infection. Although transcriptomic analysis demonstrated significant alterations in the intestinal epithelium and endothelium in RNA and protein metabolism pathways, cell cycle regulation and oxidative phosphorylation, similar to the clinical manifestations of COVID-19, intestinal epithelial cells were more susceptible to SARS-CoV-2 infection than endothelial cells.[80]

Although using a gut-on-a-chip to study SARS-CoV-2 infection can be a practical approach, organs in the human

body do not exist in isolation, and infectious diseases often have systemic pathological symptoms.[96] Zhang et al.[110] developed a human alveolar infection model of SARS-CoV-2 using an organ chip. Therefore, combining gut-on-a-chip with lung-on-a-chip to develop a 'multi-organs-on-a-chip' can contribute to a better understanding of SARS-CoV-2.

Gut-on-a-chip for studying phenylketonuria. Phenylketonuria (PKU) is a genetic disease characterised by a metabolic disorder of phenylalanine, which has toxic effects on the central nervous system (Figure 3(c)). PKU is generally treated with reasonable doses of phenylalanine for normal growth and other nutrients to prevent nutritional deficiency.

Microbes can respond to environmental signals within the human body to metabolise many compounds, including potentially toxic compounds. Some studies suggested that bacterially delivered phenylalanine (Phe) ammonia lyase is a potential therapy for PKU. Moreover, *Escherichia coli* Nissle does not colonise humans and is not present in the faeces of humans a week after ingestion. Isabella et al. constructed SYNB1618, a Phe-degrading derivative of *Escherichia coli* Nissle, to create a biotherapeutic agent that is expected to be suitable for treating PKU. Two pathways for Phe degradation were engineered in *Escherichia coli* Nissle. The results showed that SYNB1618 might have an excellent therapeutic effect on this disease as it can consume Phe in the human gastrointestinal tract, which defines a strategy for the translation of live bacterial therapeutics to treat metabolic disorders.[111] Similarly, Nelson et al.[73] used SYN5183, a synthetic live biotherapeutic, to study the treatment of PKU. They determined that SYN5183 resulted in dose-dependent increases in the biomarker trans-cinnamic acid and a corresponding 26.9% decrease in systemic Phe.

Discussion

In general, gut-on-a-chip technology is rapidly developing. Scholars examined and improved the primary conditions of gut-on-a-chip, such as oxygen concentration gradients, cell types, microorganisms and production materials, in a relatively short time. In recent years, research on gut-on-a-chip has focused on improving the flux and efficiency of gut-on-a-chip, monitoring the chip in real time and realising the combination of different organ chips. However, it is still difficult to fully reconstruct intestinal structure and function in vitro. To better design gut-on-a-chip, the use of fluid simulation software for simulating the channel of the chip is also a popular method. Nevertheless, there is no need to add every element to gut-on-a-chip, which should depend on the subject. Furthermore gut-on-a-chip should exhibit a high degree of simulation, but should not be overly complex. A basic module of gut-on-a-chip, which has the characteristics of a particular flow rate and regular peristalsis, can be developed. Subsequently, the module can be modified and adjusted based on the research such as bacteria and cytokines. More importantly, the development of modularisation is beneficial for the development of chips and reduces their cost.

Given the microfluidic system of gut-on-a-chip, the related symptoms of IBD can be easily induced by adding certain triggers (such as lipopolysaccharide and interferon-gamma). Similarly, microfluidic systems can also be used to change many variables that affect IBD to gain an in-depth understanding of the role of each variable and relationship between each variable. Furthermore, given that the materials for preparing gut-on-a-chip are highly transparent, the cell morphology can be observed and recorded directly using a microscope. However, gut-on-a-chip has a disadvantage in that it cannot directly study the impact of psychological factors on the condition of patients with IBD, which also has a particular impact on IBD. Gut-on-a-chip can efficiently study the two predominant problems of IBD (genetic susceptibility and immune abnormality). Therefore, it is expected that gut-on-a-chip can be used to realise personalised treatment of patients with IBD.

Given the particularity of viral infection, gut-on-a-chip provides a new method for studying specific viruses. Gut-on-a-chip can be used to cultivate human-related cells to study the infection of some viruses, enhance the credibility of the experiment and avoid ethical problems. More importantly, gut-on-a-chip can be easily combined with other organ chips, and thereby, the infection of certain viruses in different organs or parts can be more easily understood. In the face of the recent COVID-19 epidemic, it may be a breakthrough to study SARS-CoV-2 using the gut-on-a-chip.

Engineered bacteria represent a new type of therapy that uses synthetic biological tools. Gut-on-a-chip can realise co-culture between cells and microorganisms for a relatively long time. Based on microfluidic control, it is relatively easy to collect metabolites produced by the gut-on-a-chip system, which can be used to study drug metabolism. Therefore, gut-on-a-chip provides a powerful platform for studying the use of biotherapeutic agents to treat PKU. With respect to the treatment of PKU, improving the high throughput of gut-on-a-chip and using the chip for personalised therapy can be the future direction.

Conclusion

To address the complexity of intestinal diseases, the structure and function of gut-on-a-chip are constantly optimised via computer simulations and cell experiments. To date, several practical modules and functions have been introduced. Therefore, the gut-on-a-chip has served as a powerful platform for studying the treatment of IBD, viral infection and phenylketonuria. Owing to the flexibility of the chip, factors that affect a disease or the interaction

between them can be examined. Different modules can be adopted for diseases with different mechanisms to build gut-on-a-chip. Furthermore, models can be developed for some diseases with individual differences by adjusting parameters in some modules, such as taking primary cells from patients and adjusting the flow rate. However, intestinal diseases also affect not only the intestine but also other organs. Hence, 'multi-organs-on-a-chip' presents another method for examining intestinal diseases in the future. Chips with high simulation, high throughput, multiorgan nature, real-time detection and other characteristics can potentially become powerful disease models.

Author contributions

Changxiu Xian performed the literature review and wrote the original draft. Jiaxin Zhang sorted out the table and figures of this article. Suqing Zhao provided support for review and editing. Xiang-Guang Li was responsible for the study concept, performed critical revision and editing of the manuscript for important intellectual content and obtained funding. All authors approved the final manuscript.

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