



Intestinal Models for Personalized Medicine: from Conventional Models to Microfluidic Primary Intestine-on-a-chip

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Abstract

Intestinal dysfunction is frequently driven by abnormalities of specific genes, microbiota, or microenvironmental factors, which usually differ across individuals, as do intestinal physiology and pathology. Therefore, it's necessary to develop personalized therapeutic strategies, which are currently limited by the lack of a simulated intestine model. The mature human intestinal mucosa is covered by a single layer of columnar epithelial cells that are derived from intestinal stem cells (ISCs). The complexity of the organ dramatically increases the difficulty of faithfully mimicking *in vivo* microenvironments. However, a simulated intestine model will serve as an indispensable foundation for personalized drug screening. In this article, we review the advantages and disadvantages of conventional 2-dimensional models, intestinal organoid models, and current microfluidic intestine-on-a-chip (IOAC) models. The main technological strategies are summarized, and an advanced microfluidic primary IOAC model is proposed for personalized intestinal medicine. In this model, primary ISCs and the microbiome are isolated from individuals and co-cultured in a multi-channel microfluidic chip to establish a microengineered intestine device. The device can faithfully simulate *in vivo* fluidic flow, peristalsis-like motions, host-microbe crosstalk, and multi-cell type interactions. Moreover, the ISCs can be genetically edited before seeding, and monitoring sensors and post-analysis abilities can also be incorporated into the device to achieve high-throughput and rapid pharmaceutical studies. We also discuss the potential future applications and challenges of the microfluidic platform. The development of cell biology, biomaterials, and tissue engineering will drive the advancement of the simulated intestine, making a significant contribution to personalized medicine in the future.

Keywords Intestine-on-a-chip · Organ-on-a-chip · Intestinal model · Intestinal stem cells · Microengineered intestine · Personalized medicine

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Introduction

The intestine is a primary organ for digestion, absorption, and metabolism of nutrients and drugs, as well as a major site for the host-commensal microbiota interaction and mucosal immunity [1]. The intestinal epithelium is one of the most frequently renewed tissues in humans and mammals. The architecture of the small and large intestinal epithelia is different. The small intestinal epithelium is composed of a typical crypt-villus axis, with at least six crypts linking to each villus. In the colon, the villus is specialized into a flat luminal surface associated with multiple inner crypts, accommodating the secretion of feces [2]. The intestinal epithelium is covered by a monolayer of enterocytes, including absorptive cells and secretory types of goblet cells, enteroendocrine cells, tuft cells and Paneth cells [3]. The absorptive cells account for more than 90 %

of the enterocytes in the adult small intestinal epithelium, while goblet cells account for 5 %, enteroendocrine and tuft cells account for 1 % and Paneth cells account for 10–15 cells per crypt [4–6]. The villous absorptive enterocytes secrete hydrolytic enzymes to facilitate digestion of luminal food and then absorb the digested nutrients. The goblet cells are scattered throughout the crypt-villus axis and secrete mucins to form a mucosal physical barrier and to facilitate the passage of stool towards the colon. The Paneth cells are restricted to the small intestinal crypts, produce bactericidal substrates such as lysozyme and cryptidins, and serve as the nurse cells of intestinal stem cells (ISCs) [7]. Currently, two populations of ISCs have been identified in the intestinal epithelium: fast-cycling Lgr5⁺ stem cells and quiescent stem cells [8]. The small populations of enteroendocrine cells are essential for hormone production, allowing these cells to control intestinal motility, regulate appetite and metabolism, and orchestrate mucosal immunity [9, 10]. Additionally, the intestinal epithelium contains at least three other cell types named cup cells [11], tuft cells [12], and microfold (M) cells [13] with limited defined functions, such as mucosal immunity. Furthermore, by using single-cell mRNA sequencing, rare cell types, such as the revival stem cell, have been identified in both healthy and diseased organs [14, 15]. Summarily, the architecture and function of human intestine is fundamentally dependent on the constitution and organization of specialized cell lineages.

The intestine is also the major site for crosstalk between the intestinal epithelium, the commensal microbiome resident in the intestinal mucus layer, and immune cells from the lamina propria and subepithelial regions, as well as other cell types of enteric nerve cells, vascular endothelial cells and myofibroblasts [16, 17]. The crosstalk between the intestinal epithelium, microbiome, mucosal immunity, and endothelial cells is complex, dynamic and context-dependent. Recent advances in microbiome studies indicated that the intestinal microbiome actively impacts multiple gastrointestinal and extra-intestinal functions, including circadian rhythmicity, nutritional responses, metabolism and immunity [18]. Studies showed that gut-vascular barrier impairment can lead to systemic dissemination of bacteria and metastasis of colorectal cancer [19, 20]. Therefore, it's critical to integrate vascular endothelial cells into a simulated intestinal model to improve the predictive accuracy of *in vitro* drug screening [21]. The disruption of the intestine microbiome, impairment of host-microbiota interactions, disruption of gut-vascular barrier, and alterations of the immune system usually lead to susceptibility to pathogenic infection and dysfunction of immunity [20, 22]. Additionally, perturbing homeostasis of the intestinal epithelium can result in a variety of diseases within and beyond the intestine, such as inflammatory bowel disease (IBD) [23], celiac disease [24], metabolic syndrome

[25], rheumatic arthritis [26], neurodegenerative disorder [27], and carcinoma [28].

The commonly used conventional intestine models include *in vitro* intestinal epithelial cell lines [29], *ex vivo* everted sacs [30] and Ussing chambers [31], and *in vivo* animal models [32], as well as Transwell inserts embedded with intestinal epithelial cell lines [33], among which human intestinal epithelial cell lines are most commonly used. Importantly, there are species-specific differences in intestinal architecture, physiology, and pathology, and thus, there has been a great urge to establish new models to accurately recapitulate living human intestine instead of animal models [34]. Although human intestinal cancer-derived cell lines historically have been valuable in intestinal research, they fail to recapitulate the physiological 3-dimensional (3D) architecture or emulate the functional properties of the living human intestine to some extent [35]. The emergence of 3D intestinal organoids derived from either intestinal crypts containing endogenous intestine stem cells or induced pluripotent stem cells (iPSc) has revolutionized the field of intestinal models by maintaining various intestinal lineages and the functions of mucus production and villi formation [36]. However, intestinal organoids also have limitations, for instance, they lack other supporting cells that exist in living intestines, such as immune cells and vascular endothelial cells, which are important for pharmacokinetics analysis of drugs [37]. Happily, these shortcomings have recently been overcome by the development of microfluidic organ chip models of the human intestine. A microfluidic intestine-on-a-chip (IOAC) can be generally defined as a microfabricated cell culture device that mimics the functional units of the intestine *in vitro* [38]. The emergence of microfluidic IOAC models will offer a powerful new approach to promote the development of personalized medicine.

In this article, we summarized the advantages and disadvantages of *in vitro* and *ex vivo* conventional intestine models, intestinal organoid models (from 3D organoids to 2D monolayers), and microfluidic models (from gut-on-a-chip to intestinal organoid-on-a-chip). We then proposed a primary IOAC (pIOAC) model and determined its future prospects in personalized medicine, expecting to help promote the simulation degree of the IOAC model and its use in personalized medicine.

Current Intestine Models

Conventional Intestine Models (*in Vitro* and *ex Vivo*)

In past decades, conventional *in vitro* and *ex vivo* intestine models, including classic cell culture, everted sacs, Transwell inserts, and Ussing chambers, have made a significant contribution to intestinal research and played a vital role in

understanding the physiology and pathology of the human intestine. However, many processes of the human intestine are difficult to recapitulate when using *in vivo* animal models and *ex vivo* human intestinal samples. *In vitro* models are widely employed for the study of complex *in vivo* responses, due to their accessibility for high-throughput testing under well-controlled and repeatable conditions and relatively good human predictive power for the *in vivo* situation [39]. With appropriate approaches, *in vitro* cell models create an opportunity to improve cellular biology studies, while reducing the ethical issues and expense associated with the application of human intestinal samples and animals [40].

Primary cells are isolated from intestinal biopsies and retain the majority of their *in vivo* properties, particularly gene expression patterns. Thus, the retained genome is highly supportive of personalized therapy, but the reproducibility of results may vary from one cell to another [41]. Perreault et al. [42] reported a primary culture of viable differentiated enterocytes generated from the human fetal small intestine. However, they survive only a few days in a conventional culture system. Fully differentiated enterocytes isolated from human intestinal biopsies are rarely used in practice due to a wide range of limitations, such as ethics and regulatory issues, that govern the collection and transport of biopsies and isolation, storage and use of these cells. Alternatively, immortalized cell lines derived from gut adenocarcinoma, such as Caco-2, HT-29, T84, SW480, LS174T cells and so on, are widely used in biological mechanistic studies.

Caco-2 cells were isolated from colorectal adenocarcinoma of a 72-year-old male and are commonly used as a model of the human intestinal epithelium. When cultured in a 2D interface, Caco-2 cells grow into a confluent monolayer and then differentiate, polarize, and connect to each other, forming an apical brush border structure and expressing genes and proteins relevant to human intestine [43]. When grown on collagen-coated porous membranes of Transwell inserts, they should represent a valuable model to evaluate transport, barrier and interaction for the intestinal epithelium [44, 45]. Another immortalized cell line, HT-29, was

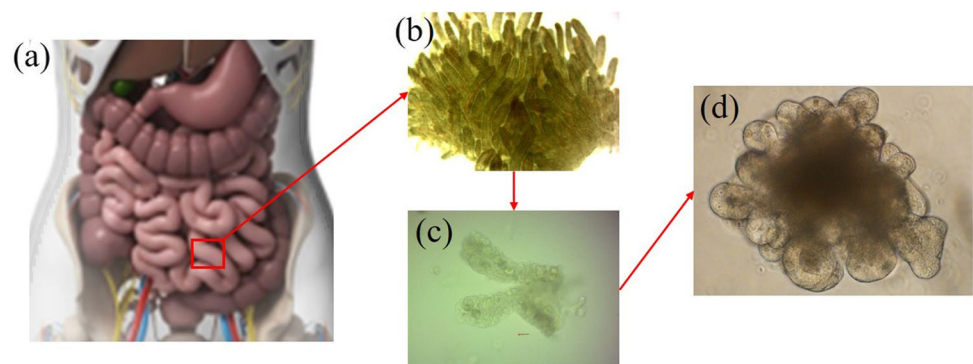
isolated from the colorectal adenocarcinoma of a 44-year-old female in 1964 [46]. Ultrastructural examination showed that these cells have a rich microvillar surface, a moderate amount of intermediate filaments, a few desmosomes, few primary and many secondary lysosomes, and a well-developed intercellular lumina [47]. T84 colon carcinoma cells present considerable heterogeneity in their morphology, an irregular cellular surface, many large vesicles in the peripheral cytoplasm, pleomorphic microvilli, and well-demarcated enlarged nuclei [48]. Usually, results obtained from adenocarcinoma-derived immortalized cell lines cannot be directly applied to make conclusions about the responses of the living human intestine. It is important to establish consistent and reliable models that faithfully mimic *in vivo* conditions.

Intestinal Organoid Models (from 3D Organoids to 2D Monolayers)

Intestinal organoids can be derived from induced pluripotent stem cells [49], embryonic stem cells [50], and ISCs isolated from an intestinal biopsy [51]. Commonly, enteroids and colonoids refer to organoids derived from the small intestine and colon, respectively [52]. Mimicking the *in vivo* niche, ISCs spontaneously develop into a crypt-villus hierarchy containing multiple intestinal epithelial cell types, including enterocytes, goblet cells, Paneth cells, enteroendocrine cells and so on, under conditions with a 3D extracellular matrix (ECM) and special growth factors [53, 54]. Intestinal organoids undergo self-renewal and intestinal histogenesis for prolonged periods when cultured in an appropriate environment (Fig. 1). The culture of intestinal organoids has greatly developed since the first *in vitro* culture of intestinal crypts isolated from rats in 1992 [55]. Currently, intestinal organoid models have been applied to human [56], mouse [57], pig [58], horse [59], bovine [60] and so on. It is worth noting that a tissue-engineered intestine is established based on intestinal organoids, scaffolds, and receptor animals [61].

The development of intestinal organoid models is a milestone in studies on intestinal health. Intestinal organoids can

Fig. 1 Derivation of small intestinal organoids. Small intestinal epithelium (b) is isolated from intestinal biopsies (a), and is applied to crypt (c) isolation. Crypts and crypt derived crypt cells and intestinal stem cells can expand to form organoids (d), under conditions with a 3D extracellular matrix and special growth factors



be derived from an intestinal biopsy of an individual and provide intestinal epithelium-like morphology that contains multiple differentiated intestinal epithelial cell types [62]. In addition, compared with the limited lifespan of several *ex vivo* models, such as the everted sac and the Ussing chamber, the long-term intestinal organoid model is sufficient to expand its application for developing intestinal disease models and mimicking normal intestinal physiology [63]. Presently, intestinal organoids have been introduced to explore intestinal nutrition [64, 65], wound healing [66, 67], host-microbiota interactions [68], inflammatory bowel diseases [69], toxicology [70–72], signaling transduction [73], malignancies [74], and regenerative medicine [75]. Regardless of its advantages, such as spontaneous histogenesis, long-term culture, and gene manipulation possibilities, there still are several limitations of intestinal organoid models in mimicking the *in vivo* intestine.

A major limitation of conventional intestinal organoids is their 3D closed cyst-like configuration in which villi protrude into the inside lumen of the organoids. This is inverted from the *in vivo* intestinal epithelium and makes the apical side inaccessible for peripheral stimuli, such as nutrients, toxins, and pathogens. Apical-out 3D intestinal organoids were developed to reverse organoid polarity, thus enabling the apical surface to face ECM and culture media. However, the 3D intestinal organoids lack a lumen, resulting in the diffusion of intestinal epithelial secretions, such as mucins, into the culture media [76]. One of the common solutions is microinjection. However, this technique is microinjection apparatus- and skillful operator-dependent and inefficient, thus limiting its application [77]. Additionally, organoids growing in 3D ECM are variable in size, shape, morphology, and localization. Thus, the opportunity to access media differs from one organoid to another, and it is difficult to achieve real-time monitoring [78]. Furthermore, nutrient supply, gas exchange, and waste removal are extraordinarily restricted at the interior of the organoids [79]. Then, 3D intestinal organoid-derived monolayers were firstly developed by Moon and colleagues in 2013 [80]. In the monolayer model, essentially, the apical side faces outwards, while the basolateral side is attached to an ECM-coated surface [78]. Importantly, the monolayer model reveals an autonomous WNT and bone morphogenetic proteins (BMP) circuit controlling a homeostasis state with balanced proliferation, differentiation, and apoptosis and can be used for high-throughput microscopy-based experiments [81]. Thus, the monolayer model can potentially overcome the shortcomings described above [82].

Another obstacle in conventional intestinal organoid models is the lack of several essential components of *in vivo* intestine, such as microflora, immune system, vascular system, and nervous system [82]. Hou and colleagues [83] built a co-culture system with intestinal organoids, lamina

propria lymphocytes, and *L. reuteri* and found that lamina propria lymphocytes and *L. reuteri* can improve the anti-inflammatory ability of the intestinal organoids to some extent. However, the microbiome diversity, interaction period, uniformity, and reproducibility of the model are limited. Considering the shortcomings of closed cyst-like configuration, co-culture models based on 3D intestinal organoids are not described here. The application of 2D intestinal organoid models, which actually form crypt-villous axis-like 3D structures like living human intestine, recently has been presented as an alternative to 3D intestinal organoid models [84]. Roodsant *et al.* [85] established a monolayer model of the human intestinal polarized epithelium characterized for epithelial cell lineages, polarization, barrier function, and gene expression. Enterovirus A71 infection and *Listeria monocytogenes* translocation for several hours were evaluated in this model, demonstrating it is a valuable tool to study host-pathogen interactions. Additionally, interactions between intestinal epithelium and mucosal immune cells [86], between the intestinal epithelium and enteric nerve cells [17], and between the intestinal epithelium and subepithelial myofibroblasts and vascular endothelial cells [87] are also explored using 2D intestinal organoid models. The development of these co-culture systems has greatly recapitulated the cellular composition, structure, and functional properties of the human intestine *in vitro*. However, even though they are much more advanced in simulating *in vivo* intestinal epithelial morphology than 3D intestinal organoid models, 2D intestinal organoid models are still far from a faithful model mimicking the human intestine *in vivo* due to several vital drawbacks such as time-limited host-microbe crosstalk, a lack of fluid flow, the absence of peristaltic movement, and deficiencies in intestinal mucus formation and cytochrome P450-based metabolism [85, 88].

Microfluidic Models (from Gut-on-a-chip to Intestinal Organoid-on-a-chip)

The emergence of engineering-derived models is complementary to biology-based techniques and provides new approaches to simulate the complex anatomical, mechanical, and biophysical properties of human intestine *in vitro*. An organ-on-a-chip is a microfluidic cell culture device containing continuously perfused microchannels inhabited by living cells [89]. These devices simulate the activities, mechanics and physiological responses of an entire organ or organs, producing a type of artificial organ functionality not possible with conventional 2D or 3D models [90]. Microfluidic IOAC models are developed to overcome the shortcomings of conventional intestine models and intestinal organoid models in recent years, depending on the advances of biomaterial-based microfabrication techniques [91]. However, the development of IOAC models does not happen overnight.

Pocock et al. [92] used hydrophobic polydimethylsiloxane to fabricate a single-channel IOAC model embedded with a monolayer of differentiated Caco-2 cells and demonstrated its ability to mimic intestinal fluidic conditions and its potential to evaluate the uptake of silica particulate drug carriers. However, it was not possible to evaluate transcellular transportation using this model because of the lack of a permeable surface [93, 94]. Therefore, the application of single-channel IOAC models to simulate intestinal properties *in vivo* is limited greatly. Generally, an IOAC contains two hollow microchannels separated by a porous, flexible, and ECM-coated membrane. Intestinal epithelial cells and other cell types can be co-cultured on opposite sides of the membrane, emulating a transmural lumen-capillary interface in the human intestine [95]. In the past few years, Caco-2 cells have been widely used in establishing intestinal microchips, which have a special name: gut-on-a-chip models [96, 97]. Under peristalsis-like fluid flow and cyclic mechanical motions, Caco-2 cells in a gut-on-a-chip device spontaneously differentiate into a monolayer with 3D villous structure, which partly recapitulates characteristics of human intestine [98]. Several sub-types of these models are fabricated to emulate living human intestine for studies on barrier integrity [99], digestive functions [100], immune modulation [101], drug metabolism [102], and host-microbe crosstalk [103]. Kim et al. [104] established a human gut-on-a-chip model in the presence of vascular and lymphatic endothelium and immune cells, which permits analysis of individual contributors to the pathophysiology of intestinal diseases over a period of weeks and studies on the underlying mechanisms when coupled with peristalsis-like fluidic flow and cyclic mechanical deformations. However, co-culture with the microbiome in the present study is limited to eight strains of beneficial probiotic bacteria, and the interaction time is no more than 3 days.

The human gut harbors a complex community of over 100 trillion microbial cells that play vital roles in maintenance of human health [105]. Imbalances of the microbiome in the gut can contribute to the development of various pathological disorders, particularly within the intestine, including inflammatory bowel disease, colorectal carcinoma, and necrotizing enterocolitis [106–108]. Compared to co-culture with human cells, crosstalk with the microbial community in an *in vitro* model is much more challenging. Several models, including Transwell inserts [109], intestinal organoids [110], and specialized bioreactor models [111], were tested to sustain complex populations of human intestinal microbiota in contact with living human tissues to mimic physiologically and pathologically related human intestine-microbiome crosstalk. In Transwell models, co-culture with bacteria could only be carried out within hours, due to the uncontrolled overgrowth of bacteria [112]. This is also a fundamental issue in 3D organoid models, in addition to their

inwards orientation of the epithelia. A human-microbiota interaction model was developed to co-culture Caco-2 cells with aerobic and anaerobic microbes under an oxygen gradient, which increases the co-culture time to 48 h. However, the microbes need to be separated from the Caco-2 cells by a porous membrane, preventing direct contact between the two sides [113]. Recently, Jalili-Firoozinezhad et al. [114] reported a microfluidic anaerobic IOAC that permits the control and real-time assessment of the transmural hypoxia gradient in the chip, allowing co-culture and maintenance of a complex human intestinal microbiome containing over 200 unique operational taxonomic units from 11 different genera for at least 5 days. Furthermore, this experimental approach was applied to co-culture the fresh gut microbiome with primary human intestinal epithelial cells, resulting in accurately recapitulated bacterial richness. Aguilar-Rojas and colleagues [115] provide a detailed review on the use of various intestinal models to study the interaction between the intestine and microbes, which will not be discussed further in this article.

The intestinal epithelial surface is covered by mucus, which provides a niche for commensal microbiota [116]. Changes in mucus layer homeostasis can influence intestinal barrier function and the crosstalk between bacteria and immune cells [117]. Therefore, a mucus layer with a physiologically relevant structure and constitution is another core challenge for modeling intestine-microbiota interactions *in vitro*. The use of cancer-derived human intestinal epithelial cell lines usually results in secretion of the gastric mucin MUC5AC, but not the predominant intestinal gel-forming mucin MUC2 [118, 119]. Primary human intestinal organoids can extensively retain the mucin secretion characteristics of living human intestine, but the secreted mucins are entrapped in the central lumen of the organoids due to their inwards orientation [120]. Moreover, the thickness of the mucus layer in an *in vitro* intestine model is far behind that of the 600- μm human colonic mucus layer, which is another obstacle to recapitulating living human intestinal mucus [121, 122]. Based on a microfluidic organ-on-a-chip device embedded with primary 2D colonoids, Sontheimer-Phelps et al. [123] established a new model that supports spontaneous goblet cell differentiation and MUC2 secretion, forming a physiologically relevant bilayer structure with a thickness similar to that observed in the human colon. The presence of 2D intestinal organoid monolayer derived from 3D organoid and primary crypt can emulate the features of the living human intestinal epithelium better than 3D organoids and intestinal cell line-derived 2D monolayer, achieving a homeostatic state with balanced proliferation, differentiation, and apoptosis [124]. Kasendra et al. [125] reported a primary human IOAC embedded with a 2D intestinal organoid monolayer in the presence of the human intestine-specific microvascular endothelium, and demonstrated that the

established intestine chip better recapitulates the genome, cell lineages and morphology of the intestinal segment from which it was derived than any of the other *in vitro* intestinal models, including a Caco-2-derived gut-on-a-chip model, Caco-2 Transwell models, and 3D intestinal organoids. The summary of current intestine models is shown in Table 1.

Proposed Primary Intestine-on-a-chip (pioac) Model

Collectively, the constitution of intestinal epithelial cell lineages, mucosal immunity, gut-vascular barrier, prolonged host-commensal microbiota interaction, and mechanical forces (fluid flow and peristalsis-like motions) are key contributors to normal intestinal physiology and disease development and are vital to faithfully mimic living human intestine in an IOAC model. Nowadays, there are several terms referring to intestinal microfluidic chips, such as IOAC [126], gut-on-a-chip [127], and organoid-on-a-chip [38]. We primarily use pIOAC to refer to a proposed microengineered intestine model in this review.

Inevitably, the cellular composition of an intestine model is the crucial factor for its outcomes. As described in the previous sections, a 2D primary intestinal organoid monolayer is valuable as a preferred cell origin to establish a microfluidic chip model, at least in emulating cell lineages, morphology, luminal access and interface, and mucosal immunity of the living human intestine [85]. To improve the physiological relevance of primary intestinal organoid monolayers, there are still other microenvironmental factors, including biological, mechanical, and topological, that must be incorporated into organoid monolayer culture devices to recapitulate the physiological and pathological states of the living intestine. In proper combination, a microfluidic chip device can co-culture and sustain a complex human intestinal microbiome for at least 5 days [114]. Hence, we here propose a pIOAC, based on a 2D primary intestinal organoid monolayer, fluid flow, peristalsis-like motions, and oxygen gradient, as well as other cell types such as gut microbe, mucosal immune cells, enteric nerve cells, endothelial cells and myofibroblasts. To maximize the retention of biological characteristics of living human intestine and meet the requirements of personalized medicine, primary ISCs and gut microbiota, as well as other cell types that need to be incorporated into the chip, should be isolated from an individual. The preparation of these cells is referenced in the literature listed in Table 2. Additionally, a summary of biomaterial-based microfabrication techniques used to enhance the complexity and mechanical cues of the microfluidic organ chip has been published by Rahmani et al. [84].

The design principle to construct a pIOAC device is to emulate intestinal functions of an individual to the maximum

extent based on the simplification of essential elements. The first step is to have a reductionist analysis of the functional units of the living human intestine that has been described in detail in the previous section. In summary, the intestinal epithelial monolayer is covered by one layer of mucus in the small intestine segment and two layers of mucus in the large intestine segment, while the microbiome is attached to the mucus. There are other cell types, such as immune cells, enteric nerve cells, vascular endothelial cells and myofibroblasts, in the lamina propria, subepithelial, and intraepithelial regions (Fig. 2). Next, an analogous model is constructed with polydimethylsiloxane (PDMS) and contains 2 parallel microchannels that are separated by a PDMS porous membrane. An oxygen-sensing organ chip is fabricated as previously described, and an oxygen gradient relevant to living intestine should be established before cell seeding [114]. Primary intestinal epithelial cells/organoid fragments and other needed cell types are embedded in the upper and lower interfaces of the porous membrane, respectively, thus bringing these cell types into physiological proximity. The cells are cultured statically until they form monolayers on both sides of the membrane. Then, relevant culture media are perfused at a desired flow rate through the upper and lower channels, cyclic suction is applied to hollow side chambers, and peristalsis-like stretching motions are exerted on the porous membrane (Fig. 3). Grown for about 2 weeks under these conditions, 2D organoid monolayers will transform into 3D villi-crypt structures, similar to the *in vivo* situation, and then can be applied to morphological analyses, mucus detection, and paracellular permeability measurement. Finally, antibiotic is excluded in the perfusing medium, and an appropriate amount of microbiota isolated from individuals or any other target microbe should be added to the upper channel embedded with differentiated intestinal epithelia.

For details, in addition to primary ISCs and organoid fragments, biopsy-derived crypt cells that contain large amounts of ISCs can also be applied to form intestinal organoid monolayers. Most importantly, the crypt cells are able to undergo gene editing, and the application of these cells can greatly decrease the expense of experimental time and cost [58]. Furthermore, as one of the distinctive properties of microfluidic organ chip devices, monitoring sensors and post-analysis abilities can also be incorporated into the device to achieve high-throughput and rapid pharmaceutical studies. For example, a microfluidic chip with an integrated electrical cell-impedance sensor can efficiently capture single cells on microelectrode arrays for sequential impedance measurement and cell migration [138]. Many sensors, such as sensors of temperature [139], pH and cations [140], therapeutic drugs and metabolites [141, 142], and cellular parameters [143], and sensor combinations [144] are incorporated in a microfluidic chip and confer the ability to perform continuous monitoring.

Table 1 Summary of current intestine models

Intestinal models	Advantage	Limitation	Application	References
Conventional intestine models intestinal biopsies	closely resembles the in vivo properties	ethics and regulatory issues; a small population of samples; unable to be cultured for long periods	differentiation; permeability	[30]
primary intestinal cells	the retained genome is highly supportive of personalized therapy	ethics and regulatory issues; limited population of cells; unable to be cultured for long periods	proliferation; migration; cell interaction; host-microbe interaction	[41, 42]
immortalized cell lines (such as Caco-2, HT-29, T84, SW480, LS174T cells and so on)	accessibility for high-throughput testing under well-controlled and repeatable conditions	limited predictive power for the in vivo situation	proliferation; migration; cell interaction; host-microbe interaction	[43, 47, 48]
Transwell inserts	represent a valuable model to evaluate transport, barrier and interaction for the intestinal epithelium	co-culture with bacteria could only be carried out within hours	permeability; cell interaction; host-microbe interaction	[44, 45, 112]
Intestinal organoid models 3D organoids	spontaneous histogenesis; long-term culture; gene manipulation possibilities	closed cyst-like configuration which is inverted from the in vivo intestinal epithelium	proliferation; differentiation; permeability; cell interaction; host-microbe interaction; tissue regeneration	[58, 72]
apical-out 3D organoids	enabling the apical surface to face extracellular matrix and culture media	lack of a lumen	proliferation; differentiation; permeability; cell interaction; host-microbe interaction; tissue regeneration	[76]
2D organoids	actual crypt-villous axis-like 3D structures like living human intestine	time-limited host-microbe crosstalk; a lack of fluid flow; the absence of peristaltic movement; deficiencies in intestinal mucus formation and cytochrome P450-based metabolism	differentiation; permeability; cell interaction; host-microbe interaction; tissue regeneration	[85, 124]
Microfluidic models Caco-2-derived gut-on-a-chip	spontaneously differentiate into a monolayer with 3D villous structure which partly recapitulates characteristics of human intestine	lack of multiple cell lineages in living intestine; secretion of the gastric mucin MUC5AC, but not the predominant intestinal gel-forming mucin MUC2	permeability; cell interaction; host-microbe interaction; tissue regeneration	[96, 104, 118, 119]
3D/2D organoid-on-a-chip	better recapitulates the genome, cell lineages and morphology of the intestinal segment	limited degree of integration of fluidic flow, peristalsis-like motions, host-microbe crosstalk, and multi-cell type interactions, as well as monitoring sensors and post-analysis abilities	differentiation; permeability; cell interaction; host-microbe interaction; tissue regeneration; physiology; the pathology of certain disease processes	[114, 125]

Table 2 The preparation of cell types related to primary intestine-on-a-chip

Cell types	Origin	References
Lactobacilli and enterococci	Infant gut	[128]
Bacteria	Fresh human feces	[129, 130]
T cells	Human colon biopsies	[131]
Immune subsets	Lamina propria of human intestinal biopsies	[132]
Macrophages	Human blood	[133]
Inflammatory cells and colon Epithelial cells	Human colon biopsies	[123]
Enteric nerve cells	Human postnatal gut	[134]
Intestinal microvascular endothelial cells	Human intestinal biopsies	[135]
Human umbilical vein endothelial cells	Human umbilical cord	[136]
Myofibroblasts	Colon	[137]

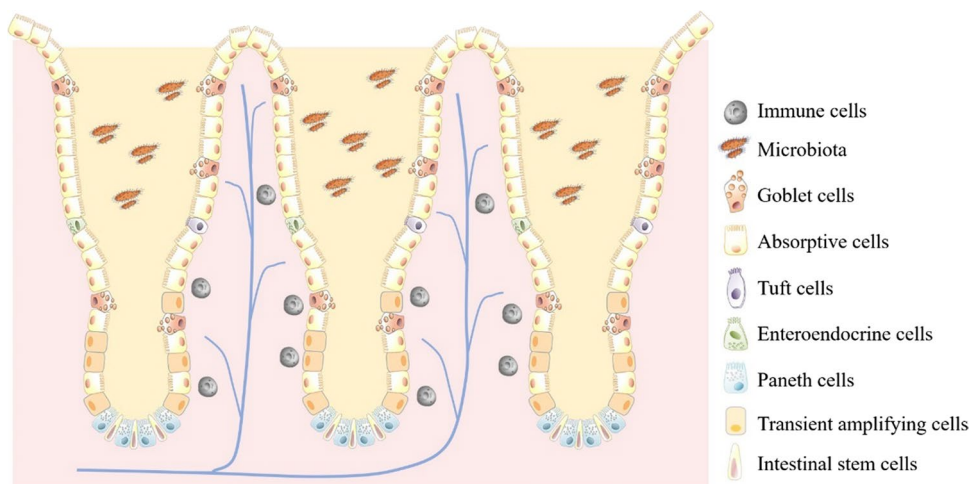


Fig. 2 Schematic representation of native 3D architecture of the small intestinal epithelial tissue. The mature human intestinal mucosa is covered by a single layer of columnar epithelial cells that are classified as intestinal stem cells, absorptive cells, goblet cells, enteroendocrine cells, Paneth cells, M cells, tuft cells and so on. These cells

are strictly organized and interact with each other, laying the foundation for building the intestinal mucosa. In addition, the interactions between the intestinal mucosa and submucosal vascular endothelia, immune cells, neurons, and fibroblasts, as well as luminal microorganisms and digesta, form a functional intestine

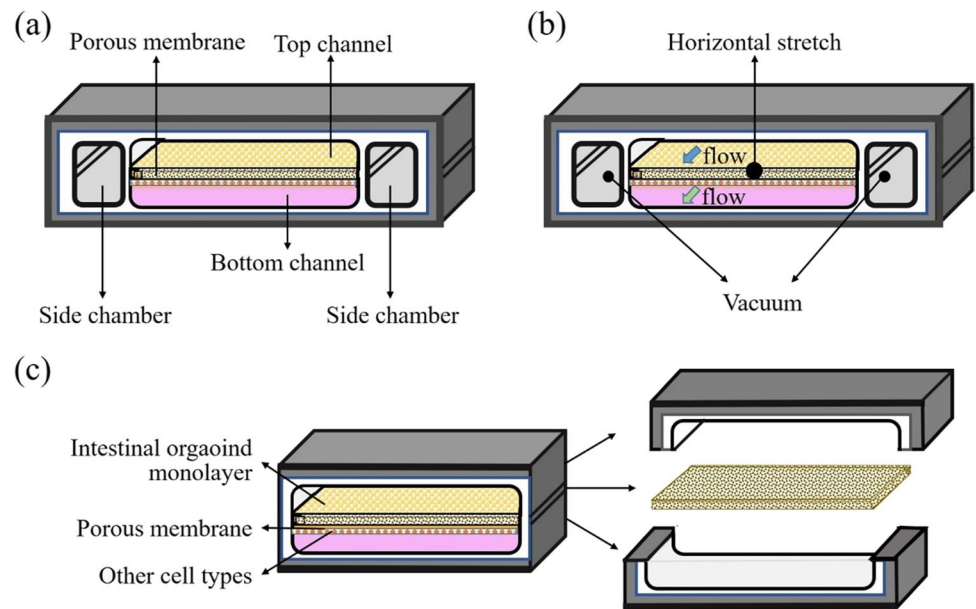
Future Perspectives in Personalized Medicine

It is reported that the results from animal experiments often fail to predict the safety and effectiveness of potential drug candidates, due to the distortion of an animal model and the biological differences between species [145]. Numerous models, such as Caco-2 monolayer culture on a Transwell insert and 3D intestinal organoids, have been applied to mimic human intestinal diseases and evaluate the pharmacokinetics and pharmacodynamics of novel drugs in humans. It is well known that the emergence of organ-on-a-chip models allows the recapitulation of biological characteristics found in native human tissues and enables studies of physiology and the pathology of

certain disease processes. Thus, the application of these models can potentially provide new insights into disease mechanisms and the possibility for advanced drug screening and personalized medicine [146].

To date, a few studies on disease models using microfluidic intestine chip devices have been published, most of which were carried out with gut-on-a-chip models. For instance, a human gut-on-a-chip microfluidic device embedded with Caco-2 cells and vascular endothelial cells was introduced to model the radiation injury response of the human intestine when exposed to γ -radiation [147]. Based on the same framework of this gut-on-a-chip device, a more complex model was fabricated to study the independent contributions of the gut microbiome, inflammatory cells, and peristalsis-like mechanical deformations to intestinal bacterial overgrowth and inflammation, and the results

Fig. 3 Schematic of the primary intestine-on-a-chip device. **(a)** Schematic representation of an analogous model with 2 parallel microchannels and 2 side chambers. **(b)** An analogous model is constructed from three layers to bring primary intestinal epithelial cells, microbiota (added to the top channel), and other cell types into physiological proximity



demonstrated that this gut-on-a-chip device can be used to establish human intestinal disease models and gain new insights into gut pathophysiology [104]. Furthermore, this kind of device is potential to be improved in a patient-specific manner to advance personalized medicine in the future, since the cellular components of the model, such as primary intestinal epithelial cells, connective tissues, immune cells, and commensal gut microbial communities, can be varied independently [104]. Thus, this gut-on-a-chip device was approved in association with IBD [148]. In addition to recapitulating disease phenotypes, the gut-on-a-chip models have also been applied to substrate transport and drug discovery. For instance, a gut-on-a-chip model combined with gas chromatography-high resolution mass spectrometry was developed to assess the transport of individual dioxin congeners [149]. Beaurivage et al. [150] established a robust high throughput gut-on-a-chip model allowing the recapitulation of key aspects of IBD pathogenesis. Moreover, they demonstrated, for the first time, the use of on-chip adenoviral shRNA transduction to knockdown of key inflammatory regulators RELA and MYD88, allowing for larger scale disease modeling, target validation and drug discovery purposes. Similarly, a gut-on-a-chip model was applied to determine the permeability coefficients of lipophilic prodrugs of 7-ethyl-10-hydroxycamptothecin, and results showed that this device is more biologically relevant than Caco-2 Transwell models, and the magnitude of permeability coefficients is higher than that of standard Ussing chamber set up with rat tissues [126].

Because of the many limitations of cancer-derived cell lines in personalized medicine, an intestine chip microfluidic model using biopsy-derived organoids was developed and applied to evaluate nutrient digestion, mucus secretion,

and intestinal barrier function [125]. Gazzaniga et al. [151] have developed a primary mouse IOAC model that support co-culture with living gut microbe and is able to evaluate living gut related characteristics such as barrier function, mucus production, and cytokine release for the analysis of pathogenic bacterial infections. The results of 16 S rRNA sequencing confirmed that *Enterococcus faecium* contributes to the promotion of host tolerance to *Salmonella typhimurium* infection, confirming findings obtained in previous experiments in mice. In addition, a pIOAC incorporating intestinal epithelial cells derived from human intestinal organoids and monocyte-derived macrophages has been successfully fabricated to simulate key aspects of the intestine of individuals with IBD. Intriguingly, the inflammatory trigger-induced gene expression profile in this model was similar to those in patients with IBD, opening the doors to personalized medicine [152]. Recently, studies with human intestinal organoids have shown that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) preferentially infects mature enterocytes over enteroendocrine and goblet cells [153]. However, there are several limitations in the use of organoid technology. Importantly, these organoids are less complex than their counterparts and do not interact with the local microenvironment of the body [154]. Simultaneously, Guo and colleagues [155] created a gut-on-a-chip model that allows the recapitulation of living human intestinal pathophysiology induced by SARSCoV-2, allowing to accelerate coronavirus disease 2019 research and develop novel therapies. Encouragingly, the proposed pIOAC model shows the ability to overcome the deficiencies of 3D organoids and gut-on-a-chip models based on cancer-derived cell lines and represents a crucial tool in the fight against current and future pandemics. It is worth looking forward to the widespread

application of an improved pIOAC model and its help in advancing personalized medicine.

Conclusions

In this article, we reviewed the progress of current intestine models, including conventional intestine models, intestinal organoid models, and microfluidic chip models, and proposed a pIOAC model to emulate the structure, function, physiology, and pathology of the living human intestine. There are four vital components of this model: an individual intestine-derived 2D organoid monolayer, mechanical forces, an oxygen gradient relevant to the living intestine, and a prolonged host-commensal microbiota interaction. The ability of a primary intestinal organoid monolayer to emulate the morphology and functions of the native intestine and to maintain an individual host's genetic profile provides an unparalleled opportunity for modeling human intestinal diseases. Mechanical forces and spatial oxygen gradient collectively create a hypoxic microenvironment and mechanical deformation similar to a living intestine, which provide the possibility of long-term host-microbiota interaction under a controllable situation. The microfluidic pIOAC may provide unique perspectives for the advancement of personalized medicine. Although the proposed pIOAC models can faithfully mimic many physiological and pathological responses of the human intestine, there are still many limitations that might play a significant role in some disorders that must be overcome. For example, in this model, the crosstalk between polarized intestinal monolayer, mucosal immune cells, enteric nerve cells, vascular endothelial cells, and myofibroblasts is restricted to a porous membrane interface, rather than the lamina propria, subepithelial and intraepithelial regions as in the living intestine. However, microfluidic intestine chip models have more advantages over conventional culture systems, and the progress in biology and microengineering will overcome these deficiencies in the future. Overall, the evolution of complex IOAC devices has greatly accelerated *in vitro* models of the living human intestine, which will improve our knowledge of intestinal development, biology, physiology, and pathophysiology. Looking forward, pIOAC devices will provide efficient and clinically relevant alternatives for personalized medicine.

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