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Review

# Advances in reconstructing intestinal functionalities *in vitro*: From two/three dimensional-cell culture platforms to human intestine-on-a-chip

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ABSTRACT

Standard two/three dimensional (2D/3D)-cell culture platforms have facilitated the understanding of the communications between various cell types and their microenvironments. However, they are still limited in recapitulating the complex functionalities in vivo, such as tissue formation, tissue-tissue interface, and mechanical/biochemical microenvironments of tissues and organs. Intestine-on-a-chip platforms offer a new way to mimic intestinal behaviors and functionalities by constructing *in vitro* intestinal models in microfluidic devices. This review summarizes the advances and limitations of the state-of-the-art 2D/3D-cell culture platforms, animal models, intestine chips, and the combined multi-organ chips related with intestines. Their applications to studying intestinal functions, drug testing, and disease modeling are introduced. Different intestinal cell sources are compared in terms of gene expression abilities and the recapitulated intestinal morphologies. Among these cells, cells isolated form human intestinal tissues and derived from pluripotent stem cells appear to be more suitable for *in vitro* reconstruction of intestinal organs. Key challenges of current intestine-on-a-chip platforms and future directions are also discussed.

## 1. Introduction

Intestine plays an important role in the human digestive system, absorption system, barrier function and immune system [1,2]. A series of human diseases are related to the dysfunction of the intestine, including obesity, inflammatory bowel disease. Intestinal diseases are increasingly threatening human life and health [3,4]. For example, 1.8 million people died due to colorectal cancer in 2018 according to the WHO report, which is the third leading cause of cancer death [5,6]. Long development time and increased cost in new drug discoveries directly affect the treatment expense of cancer patients and a five-year survival rate. For example, Fruquintinib is used to treat metastatic colon cancer, in which research and development (R&D) takes 13 years and costs billions of dollars [7].

In terms of the traditional drug screening methods like SD mouse everted Sac and intestinal ring [94], intestinal tissues or organs were directly harvested from animals. As a consequence, 95% of drugs that passed the animal testing showed inadequate therapeutic effects and even toxicity to humans due to the species differences between animals and humans. Novel intestinal cell culture platforms can culture human cells for drug screening. *In vitro* culture platforms have developed from two-dimensional (2D) culture dish [8,9] to the Boyden room and the Transwell chamber [10,11], to the multi-functional three-dimensional (3D) culture platform [12–14]. Standard 2D/3D culture platforms were generally used in drug screening [15]. However, the simplicity of those 2D/3D culture plates impeded reconstructing multi-modal and complex intestine tissues *in vitro* in terms of morphologies and functional properties [18–21]. For example, Trans-epithelial electric resistance (TEER) was commonly used to evaluate tight junctions in intestinal villus structure and permeability in intestinal function. TEER values showed a significant difference between the monolayer cells cultured in static Transwell chambers and that cultured in the microfluidic intestinal,

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which is integrated with stimulation function to mimic mechanical microenvironments *in vitro* (1000 Ω $cm^2$ vs. 3000 Ω $cm^2$ after culturing for 12 days) [22].

Starting from the concept of the lung-on-a-chip proposed by Donald Ingber [23], multiple organs (e.g. heart, intestine, liver) have been modeled on different types of microfluidic chips [24,25]. Organ-on-chips have demonstrated many advantages [26]. They could establish human organoids *in vitro* by mimicking the living physiological environments [27]. Since the cells used in organ-on-chips were harvested from human body or derived from human cells, drug screening

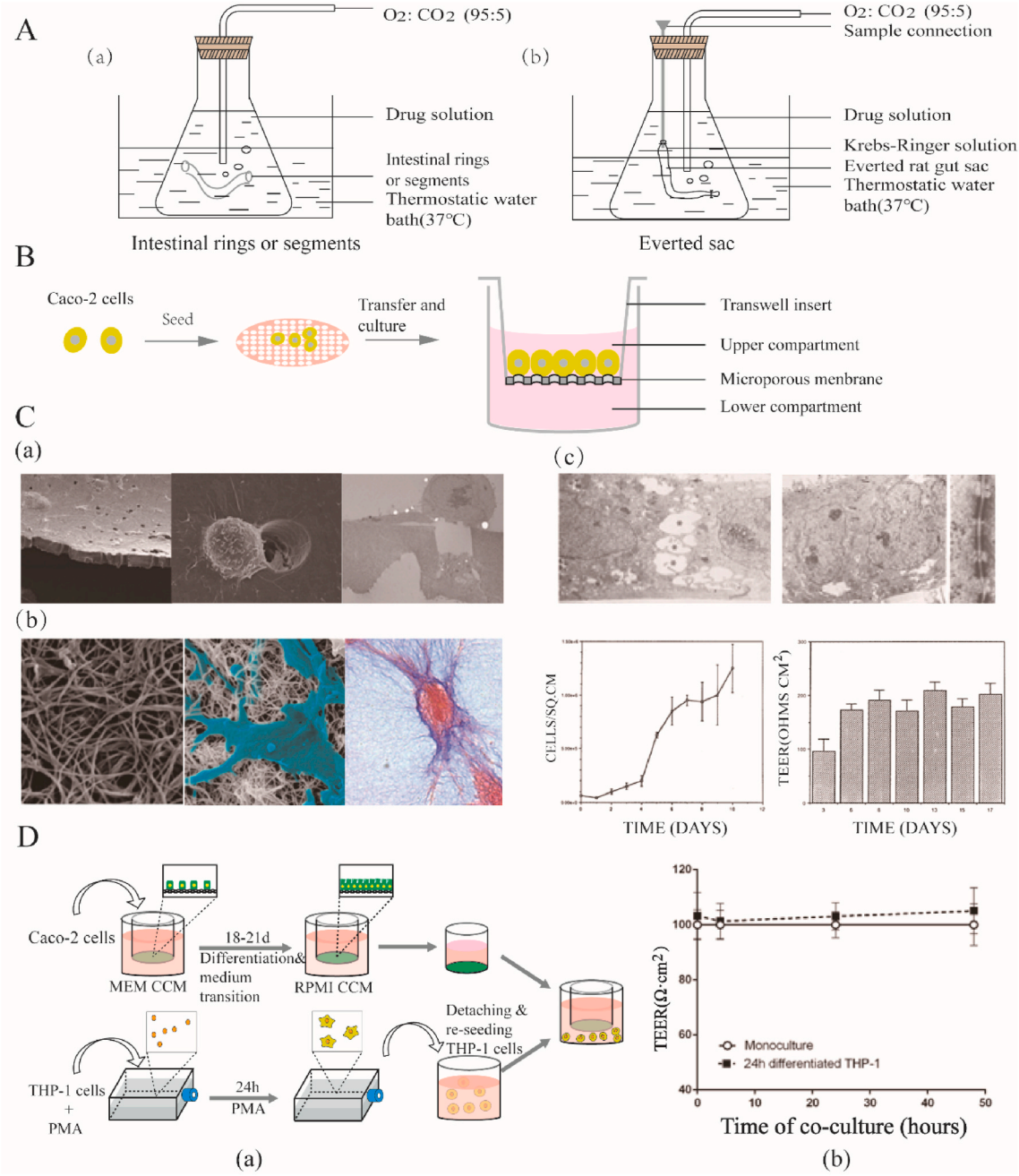



**Fig. 1. Schematic representation of different traditional intestinal models.** A) Platform schematics demonstrating the analysis of nutrient absorption by using (a) intestinal rings/segments and (b) everted gut sac. B) Schematic diagram of a Transwell invasion chamber cultured with Caco-2 cells. C) (a) Freeze fracture SEM image of an 8 μm pore-sized polycarbonate membrane on a Transwell chamber. SEM of Squamous carcinoma cells (SCCs) in the process of migrating from a serum-free medium toward medium with 10% FCS in the lower chamber. TEM image showing the migration process of a SCC cell through an 8 mm pore. Reprinted with permission [10]. Copyright 2011 Springer. (b) Scanning electron microscopy (SEM) illustrates the structure of collagen I fibers and a mesenchymal cell (pseudo-colored in blue) invading through a collagen I (white fibers) matrix. The third subplot shows a Cason's trichrome staining of a section of formalin-fixed paraffin-embedded collagen I gel. An embryonic intestinal fibroblast embedded in collagen I gel attaches to, restructures and remodels the ECM fibers on its movement through. Collagen fibers are stained in blue whereas the cell body is reddish-brown. Reprinted with permission [47]. (c) Ultrastructural features of Caco-2 cell monolayers grown on collagen-coated microporous membranes. Growth of Caco-2 cells on in Transwell. Cell densities and transepithelial electrical resistances of Caco-2 monolayers at different stages of confluence and differentiation. Reprinted with permission [51]. D) (a) Schematic description of the stable co-culture set-up. (b) Barrier integrity as TEER of Caco-2 cells over 48 h co-culture with 24 h PMA-differentiated THP-1 cells. The co-culture of Caco-2 cells with 24 h-differentiated THP-1 cells did not induce significant changes in the TEER over a period of 48 h. Reprinted with permission [52]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

failures caused by species differences can be avoided. In addition, different organs can be minimized and integrated to a single chip system for disease modeling and drug testing by using diseased cells extracted from patients during a biopsy.

Studies have revealed that intestinal functions can be partly kept in *ex vivo* (e.g. migration and absorption) using animal intestine segments [28–30] and everted gut sac [31–33]. The technology of organ-on-a-chip combined with human intestinal cells (Caco-2 cells) [34], *ex vivo* human intestinal tissues [35], human intestinal stem cells [36]) would offer new platforms for drug discovery and studying disease mechanism. For intestine-on-a-chip, micro-engineering technology [37] were applied to rearrange intestinal cells (e.g. enterocyte) into three-dimensional cavity structures, to investigate cellular responses to the mechanical cues, such as mechanical stress, flow shear stress and to study intestinal behaviors such as peristalsis and intestinal contraction [16,17,38]. Recently, other *in vitro* tissue models, such as liver and blood vessels, could be connected with intestine models by porous membrane or micro-channel to establish multi-organs-on-a-chip systems [39–41].

In this paper, we discussed current advances in the reconstruction of intestinal functions *in vitro*. We summarized the contribution of *in vitro* animal intestinal models and different human intestinal cell sources (e.g. Caco-2 cells, human pluripotent stem cells, and the cells isolated from living intestinal tissues) on the development of the gut on the chip. The transition from traditional 2D/3D cell culture platforms to intestinal organ chips has been proved to promote the reliability and efficiency of platform applications in drug screening. Furthermore, the combination of intestinal organ chips with other organ chips (e.g., liver) were also discussed for studying different organ functions such as tissue metabolism and nutrition absorption and drug transfer. Finally, we highlighted the current needs for the development of intestine chips and provided theoretical and practical information for the follow-up works in future.

## 2. Traditional intestinal models and their research progress

### 2.1. Traditional intestinal models in vitro

#### 2.1.1. Intestinal ring and intestinal segment

The intestinal ring was proposed by Agar et al., in 1954, which was constructed by a part of the intestine [42], A fresh intestine was washed thoroughly and cultured in the high oxygenated buffer. Both models were applied for recording compounds transport process from enterocytes to capillary and counting accumulation of compounds absorbed by enterocytes themselves [43]. Compared with the intestinal ring model, the intestinal segment model removed the outer muscle layers. As intestinal cells lack the normal physiological environment (e.g. pH, temperature), the tissues in both models survive hardly more than 2 h (Fig. 1A. (a)). A concern was that the compound in the buffer may interfere with the interesting compound, thus the radio-labeled interesting compounds would be preferable.

#### 2.1.2. Everted intestine sac

For obtaining the transport process of glucose and amino acid glucose in rat and hamster intestine, Wilson and Wiseman proposed the everted intestine sac system in 1954 [32]. To construct the everted sac model, one end of the everted intestine segment was tied and the other

**Table 1**
Single intestinal cells and different types of cells or cells in combination with microorganisms and other organ cells to reproduce organ function.

| Cell composition | Function and characteristics | Advantages | Disadvantages | Whether there is a function of screening drugs | References |
|---|---|---|---|---|---|
| Ring and segment of rat's intestine<br>Everted sac of tat | Studying on the absorption of l- and D-histidine by isolated intestinal and intestinal rings in rats<br>Evaluating the role of P-gp in the intestinal absorption of drug | Wide variety of sources<br>Easy to operate | Short survival time<br>More attached microorganisms | NO | [42–45] |
| Caco-2 cells | Having a certain epithelial barrier function<br>Three-dimensional small intestine villus structure<br>Enzymatic activity of brush border enzyme and mucus production<br>Containing a variety of mutated genes, has certain limitations in the study of human intestinal cancer, drug development, etc. | Easy to cultivate<br>Wide variety of sources | Cancer cells themselves | YES | [27,48,49,137,138,141,149,153-155] |
| Intestinal pluripotent stem cells (iPSCs) | Three-dimensional small intestine villus structure<br>Nearly complete intestinal epithelial barrier function | Complete human intestinal cell characteristics | Less source<br>The number of differentiated cells is relatively low | YES | [157,159–161] |
| primary human intestinal epithelial cells | Three-dimensional small intestine villus structure<br>Nearly complete intestinal epithelial barrier function<br>Enzymatic activity of brush border enzyme and mucus production<br>Multi-lineage differentiation, production of a variety of intestinal epithelial cell types, including absorptive intestinal cells, enteroendocrine cells, goblet cells and Paneth cells | Complete human intestinal cell characteristics | Less source | YES | [121,142,156] |
| Caco-2 + microorganism | Benign symbiosis with non-pathogenic *E. coli*<br>Culturing with pathogenic *E. coli* causes loss of intestinal barrier function and spatial structure damage<br>Probiotics can significantly improve intestinal barrier function<br>Co-incubation with strains increases the transport rate of biotinylated ovalbumin and initiates degranulation of RBL-2H3 cells | Easy to cultivate | Microorganisms tend to overgrow | / | [13,134,135] |
| Caco-2 + other organ cells | Replication of joint effects between different organs<br>Caco-2 and HepG2 cells have good effects on absorption, transport and metabolism of genistein and dacarbazine<br>Digoxin (DIG) is effective in combination with Verapamil (VER) and Colestyramine (COL) for detection of intestinal cell transport and nephrotoxicity | Easy to cultivate | Cancer cells themselves | YES | [193,208,210] |

was connected to tubing (Fig. 1A.(b)). Buffer was flowed into the intestine for forming a sac. This model was commonly employed for exploring mechanisms of drug metabolism and drug absorption, and investigating the role of intestinal enzymes and transporters during drug transport through the intestine [44,45]. However, the viability of the cells in this model also only keeps up to 2 h under the ideal environmental condition (Table .1). Thus, the disadvantages were also apparent. For example, the absorption process in the *ex vivo* intestine model consumes much more time than that in the living intestine in vivo, since the compound has to cross the muscle layers and the fluid is stagnant.

### 2.1.3. Boyden Chamber and Transwell

Boyden Chamber and Transwell belongs to the static culture platform, which cannot reflect the dynamic properties of intestinal cells (Table .2). The Boyden chamber consists of a two-filled chamber separated by a microporous membrane for analyzing leukocyte chemotaxis. Later researchers used Boyden Chamber for cell culture, migration observation and invasion testing [46,47]. Since cells in the Boyden chamber can migrate from the upper chamber to the lower chamber through the porous membrane, it is usually called a Transwell chamber in the research of intestinal cells. For example, Caco-2 cells can be penetrated through the polycarbonate membrane with a diameter of 8 μm pores, which reflects the process of cell migration and invasion (Fig. 1C.(a),(b)) [10]. Caco-2 cells were cultured on Transwell polycarbonate membranes with smaller pores (diameter = 2 μm) (Fig. 1B), and cell growth and reproduction were observed over time using a hemocytometer (Fig. 1C.(c)). Results showed that polarity and tight

**Table 2**
Typical three-intestinal cell culture model *in vitro* simulation capability device.

| Device | Definition | Function and characteristics | Disease model | Advantages | Disadvantages | Cultured cell | References |
|---|---|---|---|---|---|---|---|
| Transwell | Cup-shaped device with permeability | Short-term co-culture of intestinal cells and microorganisms<br>Static culture of single cells<br>Determination of cell invasion and migration<br>Different pore diameter porous membranes can be selected according to cell type<br>Bubbles generated during the experiment need to be cleared in time<br>Simple structure, convenient operation, short experimental steps, multiple repeatability experiments<br>Measuring the tight junctions of TEER monitoring cells | Malignant tumor cells invade the matrix gel and migrate through the porous membrane<br>CD155/PVR protein inhibits invasion and migration of tumor cells<br>Detection and enumeration of invasiveness of circulating tumor cells (CTCs) | Easy to operate<br>Repeatable experiment<br>Lower cost | Short cultured time<br>No continuous infusion function | Animal cells (e.g.mouse)<br>Caco-2 cells | [18,21,42,46,47,51] |
| Gut-on-a-chip | Microfluidic device made by soft lithography technology and having continuous infusion function | Self-generating into a villus structure similar to human intestinal organs under continous fluid flow and shear stress<br>Continuous culture input and metabolite collection form a stable intestinal physiology system<br>Using cyclic mechanical stress to cause the cell population to continuously expand and contract<br>Co-cultivation with microorganisms<br>Replicating intestinal barrier function<br>Measuring the tight junctions of TEER monitoring cells | Benign symbiosis of probiotics (such as Lactobacillus rhamnosus GG(LGG)) with long cells<br>Loss of intestinal barrier function (pathogenic *E. coli* invades intestinal cells)<br>Intestinal inflammation (peripheral blood mononuclear cells (PBMCs) synergize with green fluorescent protein-labeled *E. coli* (GFP-EC) or lipopolysaccharide (Lps))<br>Coxsackievirus B1 (CVB1) invades intestinal cells, resulting in loss of barrier function and structure | Long-term culture<br>Continuous infusion<br>Producing mechanical stress | Two-dimensional:<br>No mass contraction<br>No axial contraction<br>No peristaltic movement<br>Three-dimensional:<br>Difficult to cultivate<br>No mechanical factors | iPSCs<br>Primary human intestinal epithelial cells | [13,24,27,134,135,141–143] |
| Multi-organ chip | Device for integrating multiple organ chips | Double channel implantation of different organ cells<br>Drug absorption and metabolism in multiple organs<br>Establish a complete multi-organ physiological system | Effects of combination therapy with genistein and dacarbazine on cell viability, hepatotoxicity and cell cycle arrest<br>Relationship between generated ROS levels and apoptosis<br>Using epirubicin (EPI), irinotecan (CPT-11) and cyclophosphamide (CPA) as substrates to simulate the effects of oral or bioactive anticancer drugs on an on-chip organ model | Continuous infusion<br>Integration of multiple organ cells | The connection between the various organ chips is simple | Caco-2 cells<br>HepG2 cells<br>A549 cells | [193,208,210] |

junctions of the cells resembled those of human intestinal epithelial cells [48–50]. Another application of Transwell, when co-culture of THP-1 cells with caco-2 cells were compared to only Caco-2 cells by measuring TEER, the results indicate that THP-1 cells have no negative effects on the barrier formation of caco-2 cells (Table .1) (Fig. 1D) [51,52].

Because the structure of the Transwell chamber is suitable for batch production, convenient operation and consistent results can be obtained in different experiments. To a certain extent, Transwell has promoted research on intestinal cells. However, the limitations of the Transwell chamber are also obvious. Setups were not integrated for inputting culture medium and evaluating the excretion of cellular metabolites. All experiments were carried out in a closed container, and the culture medium cannot keep constant concentration with the time change. Especially, it is difficult to determine the effective concentration of a drug due to different drugs with different digesting time. In addition, Transwell cannot stimulate cells using external disturbance (e.g. electrical or mechanical stimulation) during the cultivation of physiological activities.

### 2.2. Reconstructions of the intestinal functions based on traditional intestinal models

#### 2.2.1. Reconstruction of digestion and absorption

Digestion and absorption are important physiological activities of the intestinal organs in humans and animals [53,54]. After undergoing the mechanical step and chemical step of the activities, nutrients (such as peptides, amino acids) can be converted to the energy used in the body [55]. The mechanical step includes mouth chewing, gastrointestinal peristalsis and extrusion; the chemical step utilizes digestive enzymes for converting mashed food into nutrients [56]. Traditional intestinal models focus on the study of chemical steps.

In the 1950s, researchers have studied the basic process of human digestion and absorption using the normal small intestine. Ages of volunteers ranged from 16 to 40 years old. Polyvinyl alcohol (inner diameter 2.1 mm) tube intubation technique [55], was used to sample intestinal contents in the gastrointestinal tract. This technique provides data on the absorption position and extent of absorption of various substances contained in the test meal. Results revealed that the pH value of the intestinal contents was about six during duodenal digestion and would slowly increase to eight along the distal direction. The pH in the duodenum and jejunum is constant during digestion. After that, the research focus was transplanted to explore the role of certain cells or enzymes during digestion. For example, Spencer et al. used mice to verify whether the cells of the jejunum and ileum can absorb gluconate [57]. And mice was tested whether anthocyanins can be absorbed by the cells of small intestine villi [58].

There are a large number of microorganisms in the intestinal system. They play important roles in intestinal absorption and digestion and in maintaining the stability of the intestinal system [59–61]. For example, Lactobacillus can secrete a large amount of lactic acid to promote intestinal digestion and can also synthesize vitamins and amino acids in the intestine. In the intestine, changes in the number and type of colonies are related to human health [62]. For example, previous studies have shown that the proportion of Bacteroidetes and Firmicutes in the intestine is closely related to obesity. Compared with non-obese people, the proportion of Bacteroidetes in obese people decreased and the proportion of firmicutes increased [63].

#### 2.2.2. Research on intestinal barrier function

The intestinal epithelium can prevent harmful substances (e.g. bacteria and toxins) from entering the intestinal mucosa and entering other tissues, organs and blood circulation in the human body [64,65]. Which is called the intestinal barrier function [66,67]. Abnormalities in intestinal barrier function can lead to pathogenic toxins or antigens to invade into the tissues/organs with blood flowing [2,68]. For example, Ulcerative colitis occurs along with the increase of intestinal permeability, leading to the damage in the intestinal epithelial barrier, resulting increase of antigen uptake in the lumen [69].

The intestine is important for maintaining the physiological balance of the human body. Many studies on intestinal function [70] and pathology [71–73] have been attracted researchers' attention. However, there are still many unknown pathologies. For example, it is well accepted that enteropathogenic *Escherichia coli* (EPEC) is the main cause of diarrhea in infants and children in many developing countries [74]. However, its pathogenesis is still unclear due to the complex predisposing factors. Previous studies have shown that EPEC's type III secretion system delivers EspF to intestinal cells. EspF is required for EPEC-induced loss of transepithelial electrical resistance and an increase in paracellular permeability in intestinal epithelial monolayers. This provides an important conclusion for the study of the pathogenesis of diarrhea.

Intestinal mucosal epithelial cell structure [75], tight junctions between cells [76], and parasitic membranes on the intestinal surface can effectively prevent harmful bacteria and Pathogenic toxin into the blood system [77]. The tight junctions of intestinal epithelial cells consist of cell membrane protein and cytoplasmic protein. Cell membrane protein locates on the cell membranes, including transmembrane structural proteins such as claudin protein [78], occludin protein [79], junctional adhesion molecule (JAM) [80]. Cytoplasmic protein locates inside the cell membrane. It establishes the connection between membrane proteins and the cytoskeleton and transmits informational molecules [81]. Kim et al. studies hypertensive patient's relationship between dysfunction of intestinal barrier and accumulation of intestinal bacteria [82]. Zonulin is used to regulate the epithelial tight junction proteins. The secretion of zonulin increased in the intestines of hypertensive patients. However, the bacteria number of producing butyrate was reduced [83]. It has been experimentally proven that in the intestines of hypertensive rats, the increase in intestinal sympathetic communication activity and the decrease in intestinal tight junction protein occur prior to the occurrence of hypertension [84]. The result revealed that the increased permeability of the intestinal epithelial barrier was one of the factors for inducing hypertension [85]. Future detection of intestinal bacteria and intestinal barrier function probably provides a new way to the diagnosis and treatment of hypertension.

#### 2.2.3. Research on intestinal immune function

Mucosal immunity and microbiota co-regulate the intestinal system [86–88]. For example, the invasion of pathogenic antigens could be prevented by intestinal mucosal lymphoid tissue and secretory antibodies from intestinal plasma cells (SIgA) through the immune system [89]. Inflammatory bowel disease (IBD) is a kind of chronic intestinal inflammatory disease, including ulcerative colitis [69] and Crohn's disease (CD) [90,91]. The pathogenesis of this kind of disease has not yet been clarified. Recent studies have shown that the disease could partly explain from the dysfunctions of the intestinal epithelial barrier and intestinal immune [92]. Pei et al. explored the role of colonic microbiota in the pathogenesis of ulcerative colitis (UC) [93] through a mouse model. To establish a pathological model of ulcerative colitis, a mouse was induced by injecting sodium dextran sulfate. Samples from intraluminal metabolites, inner mucus layer and the outer mucus layer of the intestine were analyzed. Results showed that changes in composition and quantity of the UC mouse flora induced by sodium dextran sulfate mainly occurred in the outer layer of mucus, which resembled Human UC patients. Whereas the change in the intestine lumen micro-population was not obvious, which differed with the human UC patients. The difference verified that the current artificial UC model cannot completely to explore the disease mechanism. But, results indicate that it is meaningful to study the change in microorganisms on the outer layer of colonic mucus in the UC model in the future.

### 2.3. Limitations in the traditional intestinal models

Over the years, animal models were commonly used in the study of the intestine [94,95]. Mice are the predominant choice because 95% of their homologous genes are the same as the human. Furthermore, mice have advantages of small size, fast reproduction, convenient operation, and inexpensiveness [96]. At present, most new drugs for treating intestinal diseases were commonly tested by mice intestinal cells *in vitro* or mice disease model in vivo and then following with clinical trials [97–99]. Therefore, animal experimental models can avoid the risks brought by experiments on humans. In experiments, in order to study human diseases, animals can be used as substitutes for human beings and repeatedly observed and studied under artificially designed experimental conditions. However, many limitations exist in mice models. (1) Species difference between animal and human [100], the National Institutes of Health (NIH) has noted that 95% of all drugs that are shown to be safe and effective in animal tests fail in human trials because they don't work or are dangerous. For example, TGN1412, a drug developed by TeGenero, was believed to treat diseases with lacking T cell activations (such as B-cell lymphoma) or for treating autoimmune diseases such as rheumatoid arthritis. Experiments have shown that TGN1412 is safe and effective in drug testing in animal (such as mouse) models. However, when TGN1412 was tested in volunteer patients, it was immediately withdrawn from the first phase of clinical trials [101] due to the worsening the condition of volunteer patients (multiple organ failure). (2) With the increase in animal welfare issues, one of the important issues in scientific research is to consider ethics in animal experimentation.

More importantly, the models *in vitro* lack intestinal inherent properties (especially for 2D/3D culture platforms and animal isolated intestinal tissues), such as functions of peristalsis [102,103] and radial contraction [104,105], microenvironment of shear stress [106,107], and other intrinsic multimodal motion [108,109] and micromechanical environment [110]. Therefore, it cannot effectively form morphological features and functions compared to the living intestine in vivo, such as the difference in villi of the small intestine, and the ability to digest, absorb. In addition, existing intestinal organ-level models *in vitro* cannot be cultured for a long time (up to 2 h) [51]. If the intestinal cells from the human body are cultured in a similar living microenvironment, whether the cells reproduce the morphology and function of human intestinal organs, and whether they are more suitable for drug testing and pathological research. For solving the above problems, the intestine chip platform has the potential to replace the traditional intestinal models.

## 3. Intestine-on-a-chip used for reconstruction of intestine *in vitro*

### 3.1. The current intestine-on-a-chip developed from lung-on-a-chip

Organ-on-a-chip was developed from microfluidic devices, which was commonly fabricated by soft-lithography on substrates of silicon or glass [111,112]. The chip involves microfluidic technology [113], micro/nano processing technology [114] and cell biology [115,116]. Key features of an organ were included in an organ-on-a-chip, such as organic differentiation, tissue-tissue interface, fluid biology and mechanical stress [117]. It could mimic the main structural and functional characteristics of human organs *in vitro*. On this basis, it can be used for investigating disease mechanisms and detecting drug absorption [118,119]. Donald Ingber first proposed a model for the construction of lung-on-chips [23], and which initial purpose is to reproduce lung function. After that, many organ-on-chips, were developed, such as the intestinal chip [120,121], heart chip [122,123], kidney chip [124,125], liver chip [126–128], vascular chip [129,130], human body chips [131,132] and other organ chips. Among these organs-on-chips, the intestine-on-chips were proposed based on lung-on-chips. Based on the lungs and intestines have similar periodic mechanical activities (the expansion and atrophy of lung cells during breathing *vs.* the expansion and contraction of the intestine during digesting food). Kim et al. directly applied the architecture of the lung-on-a-chip for constructing an intestine-on-a-chip. However, the difference in mechanical movements exists between lung and intestines [22]. For example, intestines have twisting motion and peristaltic movement, whereas lungs without similar actions.

The basic schematic of the intestinal chip is shown in Fig. 2.: the chip was made by polydimethylsiloxane (PDMS), which is a translucent, biocompatible elastomer [133]. The intestine-on-a-chip is divided into upper and lower channels, and the middle is inserted by a PDMS membrane. The culture medium is continuously replenished in the upper chamber, the lower chamber is used to collect metabolites and efflux them out. Chambers on both sides are vacuum generators that could apply cyclic mechanical stress to the porous membrane. This can control the lateral contraction and elongation of the porous membrane. Intestinal epithelial cells are planted onto the porous membrane. The designed chip could simulate the intestinal microenvironment includes controlling nutrient supplementation, metabolite emissions, mechanical stress, fluid concentration gradients, and other external conditions.

### 3.2. Current progress of intestine chip

Kim et al. established a model for the co-culture of Caco-2 cells and microorganisms on chip (Table .2) (Fig. 3A) [27,134,135]. The long-term symbiosis between microbes and intestinal organs, a pathological model of intestinal inflammation and bacterial overgrowth were all investigated. Results have shown that Caco-2 cells grown on the chip can spontaneously differentiate into intestines with villous structure, the tight junction between cells mucus secretion (Fig. 3D.E). These phenomena cannot be achieved by traditional culture models. In exploring intestinal inflammation, PBMCs were used to interact with lipopolysaccharide (LPS) or pathogenic *E. coli*. This can produce a series of pro-inflammatory cytokines (IL-8, IL-6, IL-1β and TNF-α), and damage intestinal villus structure, finally disorder the intestinal barrier function. The presence of PBMCs accelerates and exacerbates intestinal epithelial cell damage caused by pathogenic *E. coli*. Compared with the study of clinical patients with enteritis disease, this type of chip cannot completely reflect the factors that cause inflammation. This is due to many complicated factors in inflammatory diseases that failed to be considered, such as the participation of multiple immune factors, regulation of intestinal nerve cells, immune-regulation of the microbiota.

Gut microbiota is an important role in the gut system [136], which aids its host to accomplish physiological functions [137–140]. For example, non-pathogenic *E. coli* were co-cultured with intestinal cells in the intestine-on-a-chip, a good symbiotic state of non-pathogenic *E. coli* and intestinal epithelial cells was observed by a differential interference microscopy (Fig. 3F). More complex models could be done in the future, for example, different kinds of microorganisms are co-cultured with the intestines, including different probiotics and pathogenic colonies. This may provide a way to treat certain intestinal diseases.

There are other studies that have contributed to the development of intestinal organ models. For example, Kyu-Young Shim et al. incorporated a collagen scaffold to reproduce the two major characteristics of the gut tissue (Fig.3.H,I) [141], the 3D villi structure, and the fluidic shear in a microfluidic chip. They compared the tissue morphology and essential functions of Caco-2 cells in 2D monolayer culture on Transwells, 2D monolayer culture on microfluidic chips, and 3D culture on microfluidic chips. This study shows that the presence of mechanical stimulus and morphological and biochemical stimulus both enhances the metabolic function of Caco-2 cells. And Caco-2 cells were able to proliferate well and form intestinal barriers in both perfusion and 3D conditions. In addition, exposing cells to perfusion culture in 3D culture condition improved their metabolic activity considerably. Another study, Trietsch, Sebastiaan J et al., developed a model of intestinal tract

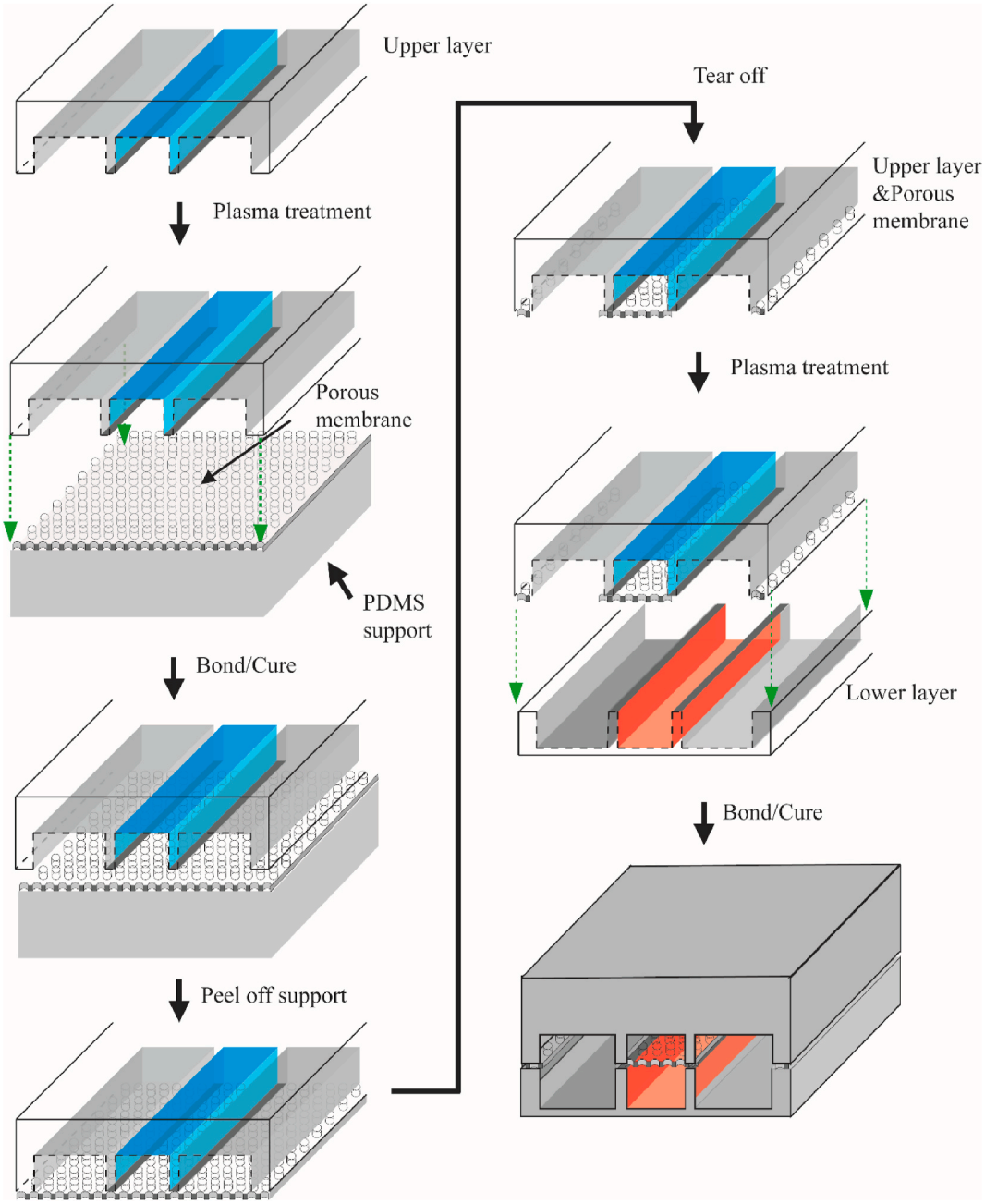
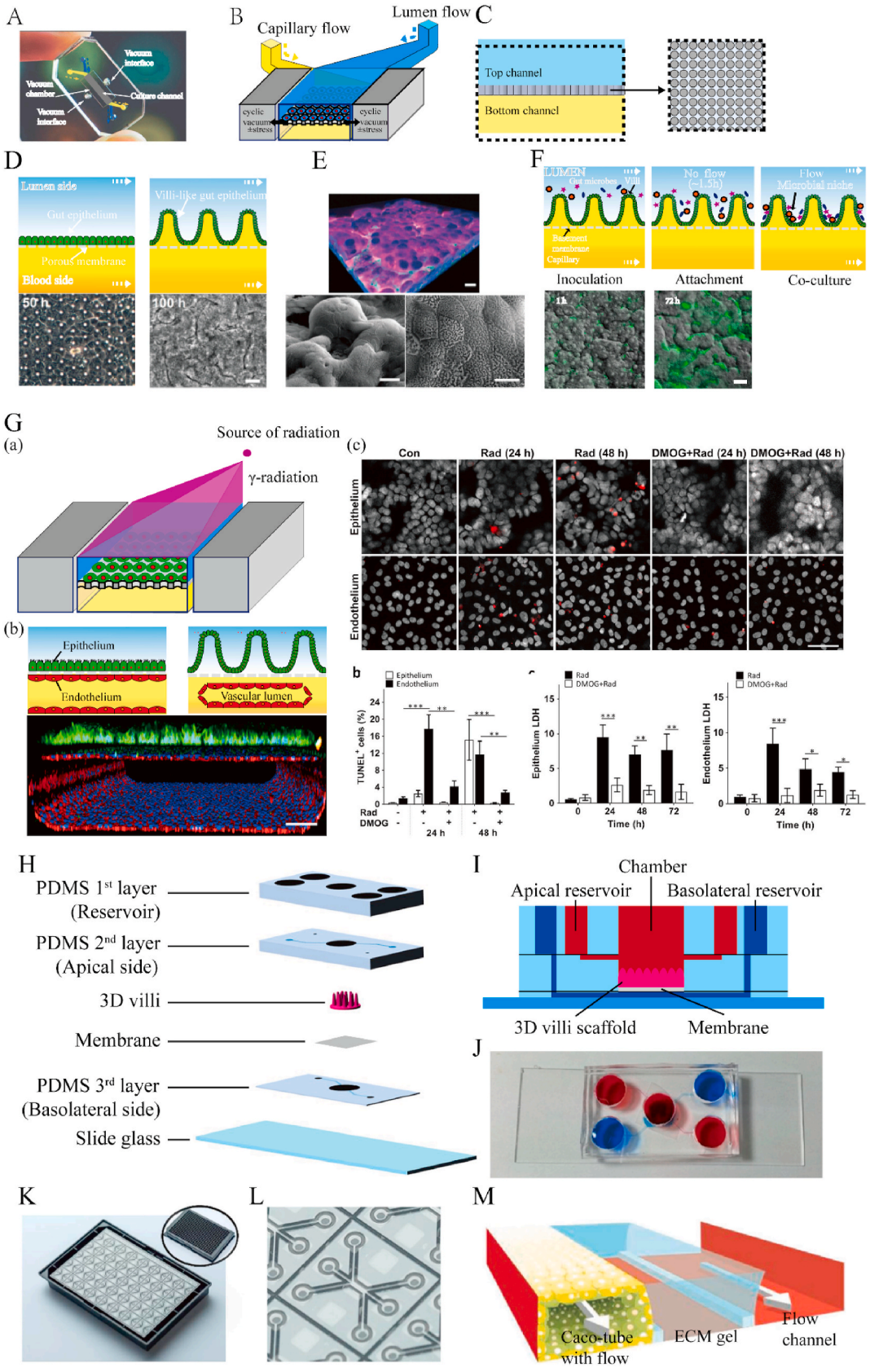



**Fig. 2.** Microfabrication process. The gut-on-a-chip microdevice fabricated from three PDMS layers (an upper layer, a porous membrane, and a lower layer), which were sequentially bonded and modified to create the central cell culture channel with upper (blue) and lower (red) channels, and two lateral vacuum chambers. The regions of the porous PDMS membrane that spanned the vacuum chambers (grey) were physically torn off during the process to create full height chambers [22]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

epithelium that forty gut models were grown in a tubular shape in the OrganoPlate platform(Fig. 3J,K,L) [142]. This platform uses culture perfused, extracellular matrix supported epithelia and interrogate their barrier function in a membrane-free manner.

Sasan Jalili-Firoozinezhad used a gut-chip to establish a radiation-induced cell death model (gastrointestinal syndrome) (Fig. 3G) [143]. The model well reflects the abnormalities of cells when the cell was exposed to γ-radiation damage. The protective effect of dimethyloxalylglycine (DMOG) on intestinal cells was verified during γ-radiation in the intestine. Intestinal endothelial cells were also found that they would participate in damaging the initial targets of radiation, which leads to producing intestinal diseases. This is consistent with existing research findings [144]. This model also confirmed that intestine chip can be used for discovering new medical countermeasures (MCMs) [145,146]. In this model, the Caco-2 cell line was used, which can replicate the process of intestinal differentiation, produce villus structures, and secrete mucus [147–149]. However, two factors need to be considered when it comes to evaluating the effects of radiation on the intestines. (1) the Caco-2 cell line was originally isolated from colon cancer, studies have shown that it includes most characteristics of human ileum, but it does not fully represent the function of the human intestines, such as the secreted antibodies from Caco-2 was less than that from normal villi. Another example, the intestinal cancer-derived Caco-2 is more sensitive to radiation than normal human intestinal epithelial/endothelial cells. Further work should compare the changes in Caco-2 cells with human intestinal cell after radiation. During this process, primary human




(caption on next page)

**Fig. 3. A.** A photographic image of an on-chip intestinal organ device made of PDMS elastomer. **B.** A schematic of the gut-on-a-chip device showing the flexible porous ECM-coated membrane lined by gut epithelial cells cross horizontally through the middle of the central microchannel, and full height vacuum chambers on both sides. **C.** A cross-sectional view of the top and bottom channels of the gut-on-a-chip. **D.** Formation of intestinal villi by Caco-2 cells within in Gut-on-a-Chip cultures. **E.** A 3D reconstruction of Z-stacked images of Caco-2 villi stained for nuclei (blue), F-actin (green), and mucin 2 (magenta) is shown. **F.** The human Gut-on-a-Chip microphysiological system for host-gut microbiome co-culture [27,134,135]. **G. (a)** Schematic diagram of intestinal radiation simulation. **(b)** Schematic showing the positions of the human intestinal epithelium and endothelium when initially plated on opposite sides of the matrix-coated porous membrane within the two-channel microfluidic device (top), and how this progresses to form a villus epithelium in the top channel interfaced with a planar endothelium that forms a lumen in the bottom channel (middle). **(c)** Radiation-induced apoptosis and cytotoxicity in intestinal epithelium and vascular endothelium, and radio-protective effects of DMOG [137]. **H.** Schematic of different components of an intestine-on-a-chip with villi structure. **I.** Side-view of a gut chip. **J.** Picture of a gut chip showing two sets of reservoirs for the apical (red) and basolateral sides (blue) [141]. **K.** Photograph of the bottom of an OrganoPlate showing 40 microfluidic channel networks with inlay showing the top view of the 384-well plate format device; **L.** Zoom-in on a single microfluidic channel network comprising three channels that join in the center. **M.** Schematic diagram of 3D chip, an extracellular matrix gel and a perfusion lane; two phase guides (white bars) define the three distinct lanes in the central channel [142]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

intestinal epithelial cells would be integrated into the chip (such as the intestine excision part of the patient). (2) Although current research is capable of replicating multiple clinical responses to radiation using intestinal epithelial cells and endothelial cells, the importance of immune cells and commensal flora should be explored in radiation-induced model. Therefore, the intestine-on-a-chip integrated types of commensal cells is urgent for investigating co-response for radiation.

For evaluating an intestinal disease model *in vitro*, the fundamental rule is whether the employed cells could completely replicate functions of the human intestine and whether the physiological state of the cells in the disease model is similar to the abnormal cell state of the patient. Thus, the development of new cell types is also key to improving the application of intestine chip.

To date, researchers have considered many conditions in the development of intestinal organ chips, such as flow shear stress, mechanical stimuli and anaerobic environment. These conditions cannot be achieved by traditional intestinal organ models (Intestinal ring, Intestinal segment, Everted Sac and Transwell). For flow shear stress, Qasem Ramadan 1 et al. developed a dynamic microfluidic human intestinal barrier model by co-culture of Caco-2 cells and U937 to investigate the immune responsive of cells [150]. The controlled shear stress can be achieved by changing the fluid flow rate. In addition, perfusion-based media supply allows delivery and removal of soluble molecules from the extracellular microenvironment. Compared to static culture, cells maintained a higher survival rate, and a larger value of TEER under perfusion-based condition. This was because the cells in the perfusion system could respond to the fluid shear stress through mechano-transduction. Flow shear stress simulates the flow of the succus entericus and the chyme in the real intestinal environment. It can improve the differentiation of intestinal epithelial cells, and improve the generation of small intestinal villi and other physiological features. Mechanical stretching is another important type of simulation in intestine-on-a-chip. Alexandre Grassart et al. determined whether cyclic stretching (mimicking peristalsis of intestine) could impact the infectivity of the human pathogen Shigella within a 3D colonic epithelium [151]. Compared to non-mechanically stimulated conditions, the application of stretching forces (peristalsis) significantly increased the efficiency of infection by approximately 50%, and bacterial invasion from the apical side of the epithelium was strongly boosted by peristaltic motion. The microbiota in the gut have many crucial functions in human health and affect the host via different host-microbiota interaction pathways. Microbiota could induce transcriptome changes in Caco-2 cells, that more closely resemble the intestinal tract in the body. Because most gut bacteria are facultative anaerobes, the intestinal cell-microbe co-culture system in the anaerobic environment is helpful to understand the role of gut microbiota in health and disease. Mehdi Sadaghian Sadabad et al. developed a coculture system for oxygen-requiring human gut epithelial (Caco-2) cells and an anaerobic gut bacterium [152]. This study, for the first time, showed that mutualism relationship was observed between oxygen-requiring intestinal epithelial (Caco-2) cells and anaerobic F. prausnitzii bacteria. In general, flow shear stress, mechanical stimuli and anaerobic environment are important components in intestinal organ chips.

### 3.3. Cell sources used in intestine chip

Studies have shown that the use of Caco-2 cells can reproduce the basic structure and function of the intestinal organs, such as the production of villous morphology, drug transport, mucus production, and a certain barrier function [153,154]. While experimenting with Caco-2 cells, the researchers also attempted to compare the characteristics of human pluripotent stem cells and living tissue primary epithelial cells with caco-2 cells [121,155,156] and provided ideas for subsequent intestinal chip research (Table .1).

Workman M J used an induced pluripotent stem cells (iPSCs) instead of Caco-2 cells to create a gut-on-a-chip (Fig. 4B) [157]. The chip combines iPSCs-derived intestinal tissue hod for preparing human small intestine chips [121]. Tissue containing intestinal epithelial cells isolated from the healthy area of the intestinal biopsy, the primary epithelial cells are expanded as 3D organoids [158], dissociated, and cultured on a porous membrane (Fig. 4F). Gene transcriptome analysis in the study showed that the performance of this on-chip intestinal cell is closer to that of the human duodenum compared these results to those previously obtained micro-engineering techniques. During differentiation of iPSCs into a part of intestinal tissue, mesenchymal cells would also be produced which would affect (Fig. 4. A) [159–161]. In order to eliminate the unexpected interference caused by mesenchymal cells, the differentiated intestinal tissue would be dissociated into individual cells in suspension and screened by fluorescence-activated cell sorting, and then seeded on the chip to form a cell monolayer [162]. Through comparison of the induced intestinal cells and Caco-2 cells, the induced cells exhibited much better in physiological functions (e.g. intestinal barrier function) than the Caco-2 cells (Fig. 4C). Microbes in each body's intestine play different roles in regulating the intestinal environment and physiological activities [163–165]. Intestine-on-chips can simulate the pathological micro-environment of the patient's intestine, which was achieved by flowing bacteria and viruses with cell culture medium [166]. Thus, the chip would contribute to developing personalized medicine by utilizing more similar to in vivo behavior compared to Transwell or other static culture systems [167,168].

Kasandra M developed a method for fabricating a primary human small intestine-on-a-chip containing human epithelial cells isolated from healthy regions of intestinal biopsies [121]. The primary epithelial cells were expanded as 3D organoids, dissociated, and cultured on a porous membrane within a microfluidic device with human intestinal microvascular endothelium cultured in a parallel microchannel under flow and cyclic deformation (Table .1). Firstly, intestinal epithelial cells were firstly isolated from a healthy area of the intestinal biopsy, then primary epithelial cells were expanded as 3D organoids (Fig. 5). After that, the organoids were dissociated and cultured on a porous membrane. In this study, gene transcriptome analysis showed that the performance of this on-chip intestinal cell is closer to that of the human duodenum compared to these results from previously obtained with the Caco-2 human Gut Chip and Caco-2 Transwell cultures. However, the sources

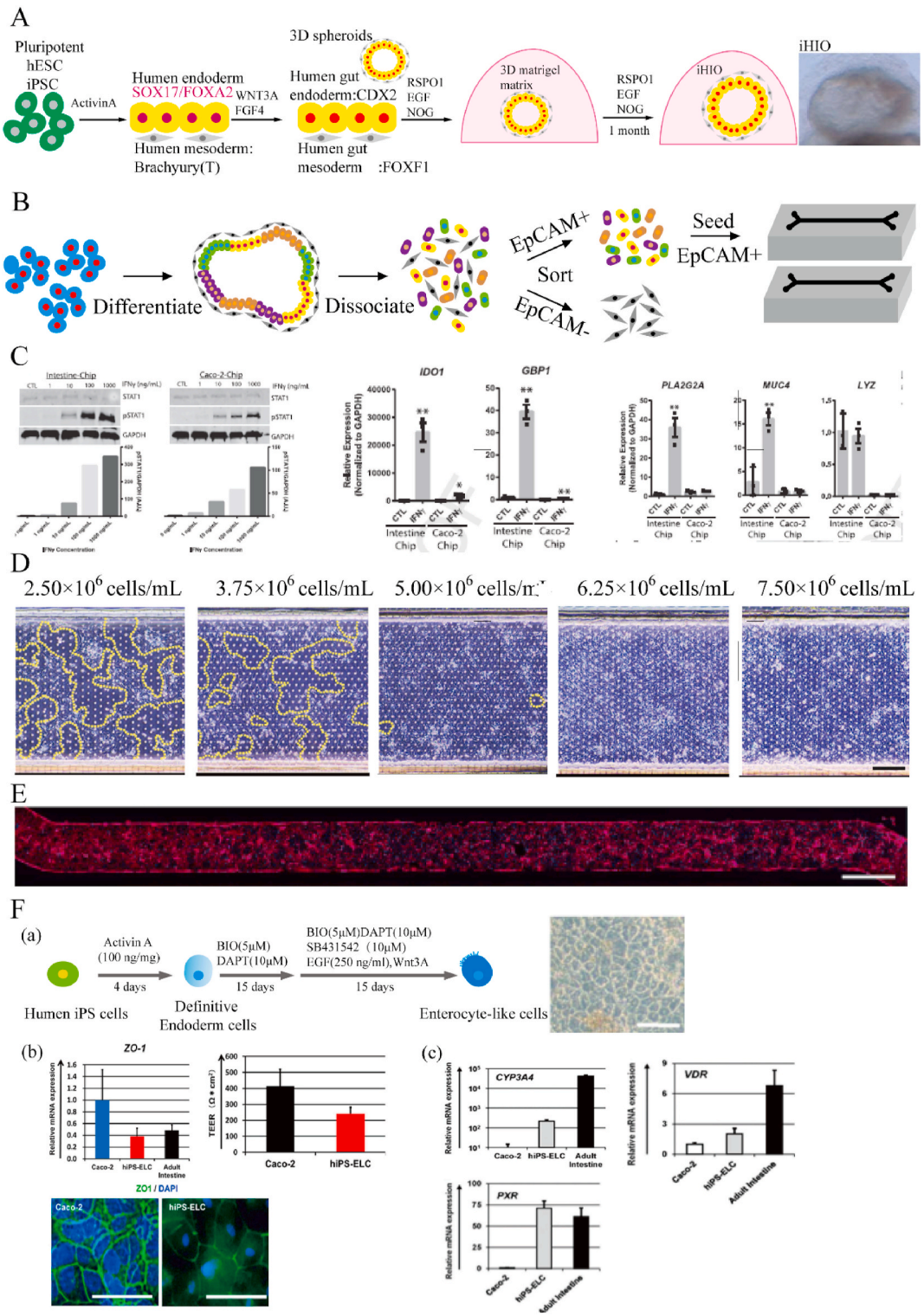



**Fig. 4.** **A.** Schematic of human pluripotent stem cell-derived intestinal organoids [155]. **B.** Schematic of workflow for the incorporation of iPSC-derived HIOs into the Intestine-Chip. **C.** Western blot analyses and quantification of phosphorylation of STAT1, Quantitative reverse-transcription polymerase chain reaction analyses and IFN-g drug response. **D.** Phase-contrast images of HIO- after culturing 24 h. **E.** A fully confluent monolayer confocal images of intestinal epithelial cells along the entire channel of the Chip [157]. **F.(a)** Modified enterocyte differentiation protocol is illustrated. **(b)** Analysis of barrier formation capacity in the human iPS-derived enterocyte-like cell monolayers. **(c)** CYP3A4 expression level and induction potency in the human iPS-derived enterocyte-like cells [162].

from the human body were limited. Previous studies have shown that Caco-2 cells in intestine-on-a-chip behaves like human ileum. To a certain extent, it is feasible to establish an *in vitro* intestinal model with caco-2 cells, when the integrity of differentiation and gene-specific expression don not strictly followed in intestinal cells. Therefore, the Caco-2 cell line would be still the mainstream in these intestine-chips in the future.

Other researchers have also established different 3D organoid intestinal models. Claudia Beaurivage et al. integrated intestinal epithelial cells (IEC) derived from human intestinal organoids with monocyte-

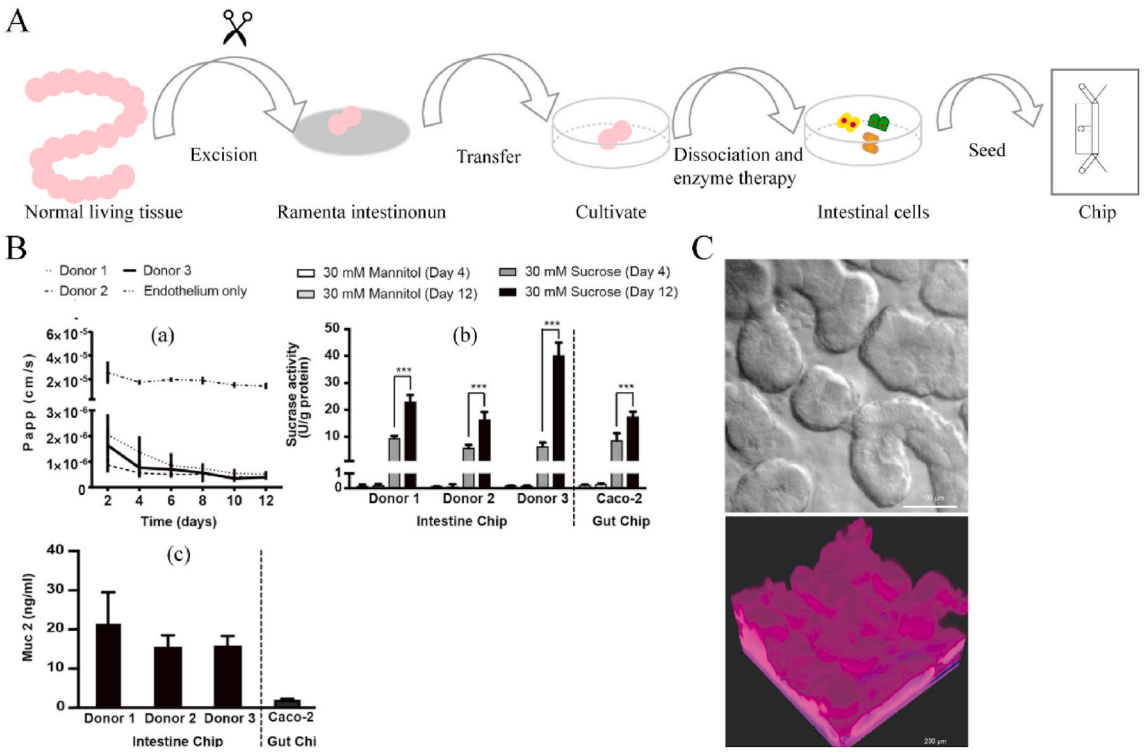



**Fig. 5. A.** Cells derived from normal intestinal tissue excision were cultured in the chip. **B.**(a) Comparison of barrier function measured in the intact Intestine Chips lined and primary HIMECs versus chips lined by endothelium alone. (b) Comparison of intestinal epithelial cell sucrase-isomaltase activity in the Intestine Chip and Caco-2 Gut Chip. (c) Comparison of mucin 2 (Muc2) levels in the apical secretions collected in the effluent from the epithelial channel of the Intestine Chip generated form organoids. **C.** Microscopic views showing the finger-like protrusions of the primary intestinal epithelium cultured on-chip for 12 days under continuous flow (60 μl hr−1) [121].

derived macrophages, in a gut-on-a-chip platform to model the human intestine and key aspects of IBD [169]. The gut-on-a-chip model differs from previous models not only by integrating primary human macrophages together with IEC, but also by inducing an inflammatory state of the epithelium similar to the one observed in IBD patients. The study used human primitive intestinal epithelial cells from biopsy to construct an intestinal organ model, which can solve the limitations of using Caco-2 cells. In addition, culturing the IEC in microfluidic conditions corrected the inside-out configuration of human intestinal organoids (HIO) and increased the expression of intestinal transporters. For example, up-regulation of Solute carrier (SLC) superfamily members can enhance the transport of drugs through the epithelium. Based on the 3D intestinal organ model, Huanhuan Joyce Chen et al. developed a modular gastrointestinal (GI) tract - liver system by co-culture of primary human intestinal epithelial cells (hIECs) and HepG2/C3A cells (3D liver mimic) [170]. In this co-culture GI-Liver system, metabolic rates (urea or albumin production) of the cells were comparable to those of HepG2/C3A cells in single-organ fluidic culture system, however, the induced cytochrome P450 (CYP) activities significantly increased.

### 3.4. Indicators used in evaluating intestine chip

#### 3.4.1. Differentiation and tight junction of intestinal cells

Differentiation of intestinal epithelial cells and tight junctions among cells are important markers for evaluating intestinal physiology [171,172]. They both are also important markers for the evaluation of the cells in the intestinal models. Thus, the quantitative measurement of markers could provide the necessary context to judge cellular physiology. TEER measurement is a non-invasive technique, which provides ion resistance across a single layer of cells. This technique has been used to detect the barrier properties of the cell layer [173–175], which was related to tight junctions. Impedance spectrum and electrical stimulation were combined [176] to measure the resistance of the cultured cell layer [174,177], which results can indicate the changes in villus differentiation and intercellular tight junctions in human intestinal epithelial cells cultured on a chip. By integrating multiple pairs of electrodes, the corresponding small areas could be simultaneously monitored. During the culture with fluid culture medium, TEER value was measured as an indicator for evaluating differentiation of the intestinal villi and tight junction between cells [178–180]. This method realizes continuous monitoring of the formation process of the cell layer [181]. Importantly, it was a label-free method that could not cause any damage to the cells on the intestinal chip.

#### 3.4.2. Living micro-environment of the intestinal cells

The cell living micro-environment plays an important role in reconstructing cellular features (morphology and function). When the micro-environment of the intestinal cells is similar to the living intestine, the intestinal features *in vitro* would be similar to the intestine in vivo. For human intestine, the micro-environment including many parameters, including osmotic pressure [182,183], pH [184,185], temperature [186] and others.

Changes in intestinal osmotic pressure can lead to abnormal function of intestinal cells [56]. For example, the intestine in a mouse would improve vitamin absorption under isotonic conditions; however, hypotonic and hypertonic solutions would alter or damage the vitamin absorption in the mucosal tissue of intestine [187]. The intestine is an important site of absorption and digestion, its pH is maintained at a weak acidity (about 6.8).

pH changes in the intestine could cause intestinal microbial abnormalities and decrease sugar absorption in the body [188], leading to intestinal diseases. The temperature of the human intestine usually maintained at approximately 37.7°, changes in human intestinal environment temperature can affect intestinal cell activity [189]. During the

cultivation of intestinal cells, some variables can also indirectly reflect the information of the cells. For example, the oxygen consumption in the device can represent the physiological activity of the cells [190–192], and the production of reactive oxygen species can represent the degree of cell apoptosis [193–195].

## 4. Intestine related multiple organs-on-a-chip

Pharmacokinetics is dedicated to the study that how a drug affects the actions of absorption, distribution, metabolism and excretion in organisms [196,197]. It is often adopted to evaluate drug toxicity in humans [198,199]. The development of new drugs [200,201], biocompatibility [202,203], and drug availability [204,205].

Drug absorption is a complex process in the body from a series of biochemical reactions in multiple organs, transfer, primary metabolism, secondary metabolism, to the final reaction of the effective metabolic drug product of the acting on the disease focus [196,197].

Organs-derived cells have been used for drug absorption studies. However, this approach often fails in drug screening as it cannot simulate the process of drug absorption. Multi-organ combined chips cannot be realized by cell culture techniques alone, different kinds of cells in different organs need different culturing environment. For example, the pH for the intestine is approximately 6.80 while the pH of the liver is approximately between 7.35 and 7.45. Multiple organs-on-a-chip integrates different parts for culturing different organs. Different parts are connected by microchannels. These microchannels are used to transfer nutrients and achieve cellular communications of chemicals and signals among different organs on the chip.

To study drug metabolism and absorption in intestine and liver, Jie M cultured caco-2 cells and HepG2 cells to construct an intestine-liver-on-a-chip (Table .2) (Fig. 6A) [193]. Different concentrations of genistein and dacarbazine were injected into the hollow fiber (HF) cavity. Mass spectrometry was used to analyze the drugs and the metabolic products, which investigates the intestinal absorption and liver metabolism of the two drugs. The results indicated that a high concentration of the drug-combination inhibited the growth of HepG2 cells [206], while moderate concentrations of the drug-combination maintained the normal metabolic function of hepatocytes. In addition, Caco-2 cells cultured in hollow fiber bioreactors promoted the expression of intestinal function, compared to that cultured in the traditional two-dimensional Transwell culture system [207]. This also revealed that Caco-2 cell line takes an important role in drug absorption and transport in the intestine. In multiple organs combined studies, this intestine-liver model provided a reference design for the studies of drug transport and metabolism for drug therapy. In this device, a channel made by hollow fiber and an elliptical chamber were used to form an up-down structure to co-culture intestinal cells and hepatocytes. For this structure, there were two problems to solve: firstly, the intestinal cells were seeded in the hollow fiber channel, which was difficult to cover the entire channel completely; secondly, there was no experiment on drug availability in this model while drug availability is a key judging criteria whether a device is suitable for drug screening.

In pharmacodynamics study, Hiroshi Kimura established an intestine-liver-lung organs-on-a-chip using a microfluidic device (Fig. 6B.C). This model was successfully used for exploring the dynamics of doxorubicin (EPI), irinotecan (CPT-11), and cyclophosphamide (CPA) [208]. The number of cells for each organ in the device was determined according to the volume ratio of the human organs. Caco-2 cells, HepG2 cells, and A549 cells [209] can be co-cultured for more than 3 days in this device. By detecting EPI, CPT-11, and CPA, functionalities of the small intestine and liver organs were determined during the absorption and transportation of anticancer drugs. This is the first time to use a microfluidic device that integrates both simulated physiological parameters and continuous perfusions in a single chip. The physiological parameters of blood flow between organs are critical in the simulation, which plays a stabilizing role in the metabolism between organs in the device. In the future, the organ volume parameters of the human body, external environmental conditions of the organs, and the primary organ cells or pluripotent stem cells in the human body would be used in the next generation of the chip. This could make the *in vitro* organ simulation system closer to the human organ system. In addition, only the data at the end of the experiment was demonstrated in this device, the intuitive information during the drug effect on cellular activity was not detected.

Ilka Maschmeyer et al. built a co-culture microfluidic device including four organs - skin, intestine, liver and kidney [210]. The four organs were separated by using different chambers (Fig. 6F). The number of different organ cells is determined with reference to the volume ratio of these organs in humans. This device could maintain four organ cells with normal physiological activity for 28 days. Metabolic and excretory circuits in this device kept a balance of glucose and other nutrients. The device also simulated the process of drug transfer and metabolism in the gut, the secondary metabolism in the liver, and the final absorption in the kidneys. The concentration of glucose and protein between the culture medium pools of the device is different due to the differences in the organ living environment. By detecting the indicators of each organ (such as TEER of intestinal barrier function), it can determine whether the physiological activity between various organ systems within the device is stable. However, the complexity of this research brought in too many challenges. For example, the environments of various organs were too difficult to maintain when the number of organs began to increase. Moreover, the intensity of cellular expression under *in vitro* culture conditions is also uncontrollable. This could be solved by optimizing the device design.

## 5. The limitation and optimization direction for industrial and clinical setting

In terms of drug development, clinical trials and immune testing, the transformation from animal models to intestinal organ chips is a promising trend. Animal models are ethically controversial to use and fail to pass human toxicity tests while intestine-on-a-chip uses induced pluripotent stem cells (iPSCs) and primary epithelial cells as cultured cells. Moreover, the intestinal organ chip exhibits intestinal features such as the formation of three-dimensional villi structures and the intestinal microenvironment such as fluid shear stress, and mechanical stimulation. etc.) that can give cells to survive in the chip. Nevertheless, the intestinal organ chip still needs to invest resources to design and develop for industrial applications, and it still has many issues to consider.

To enhance their practical applications, the materials used in the chip may affect the performance of the intestinal organ model. Regarding a question about chip preparation materials, since most intestinal organ chips utilize PDMS which can absorb small molecules, and its hydrophobicity can cause lipophilic compounds to adsorb to the chip wall [211]. Therefore, whether PDMS can find a way to reduce the absorption of small hydrophobic molecules and better materials to replace PDMS is also worthy of attention. In intestinal organ chips, electrospinning of polycaprolactone/gelatin polymer blends was used to model the membrane extracellular matrix [212]. Although electrospinning was able to simulate different micro-architectures relevant to normal and abnormal lung tissue, changes in substrate stiffness and architecture can influence cell development. Hydrogels can provide a mechanical and biochemical environment that is similar to in vivo cell-ECM interactions, promoting the formation of tissue-like structures [213]. Nonetheless, in large scale 3D-tissue culture, the cumbersome steps necessary for dispensing viscous hydrogels and changing growth media over time, the difficulty in imaging 3D-cultured cells, and limited diffusion of nutrients within a 3D construct need to be improved [214].

Companies have begun to commercialize intestinal organ chips. For example, Mimetas' OrganoPlate platform has shown a 3D cell co-culture intestine model. This platform includes the selection of various

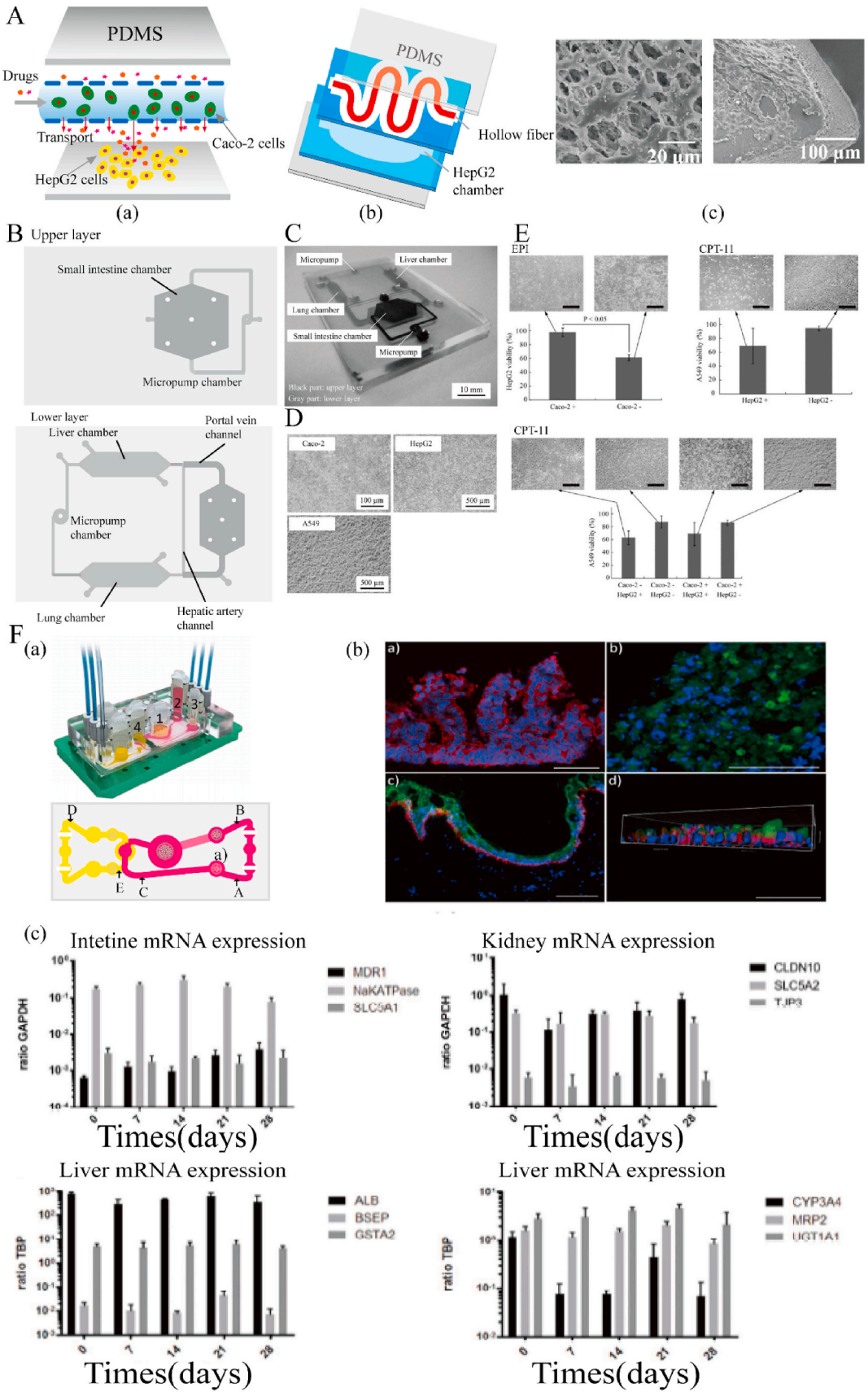



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**Fig. 6.** A Schematic illustration of the microfluidic intestine-liver model. **(a)** schematic of drug transport and absorption in HF. **(b)** schematic illustration of the double-layer microchip. **(c)** SEM images of HF wall and Caco-2 cells cultured in the HF lumen respectively [193]. **B.** Schematic representation of the microfluidic device. **C.** Three chambers and micro-pumps represent the small intestine, liver, lungs, and heart. **D.** Photographs of cells co-cultured in the microfluidic device for 72 h. **E.** Viability and morphology of target cells treated with 10 μM epirubicin (EPI), 50 μM irinotecan (CPT-11), and 1 mM cyclophosphamide (CPA) dissolved in culture medium [208]. **F(a)** 3D view of the device comprising two polycarbonate cover-plates. Numbers represent the four tissue culture compartments for intestine, liver, skin, and kidney tissue. Top view of the four-organ-chip layout illustrating the positions of three measuring spots (A, B and C) in the surrogate blood circuit and two spots (D, E) in the excretory circuit. **(b)** Performance of human tissues in the 4OC after 28 days of co-culture. **(c)** Gene expression in co-cultures of the four-organ-chip over 28 days [210].

conditions such as cell source type, extracellular matrix (ECM), culture fluid flow rate, and growth medium. The goal of Mimetas is to continuously improve the intestinal organ model for clinical applications and select treatment methods for the intestinal diseased cells of individual patients.

For clinical settings, intestinal disease models need to be established based on intestinal organ chips, and then therapeutic drugs and treatment plans can be screened. Currently, these models have not truly reproduced in vivo intestinal diseases, such as the lack of immune cells and enteric nerve cells. Ying C et al. grew human intestinal enteroid-derived epithelial cells *in vitro* in a biocompatible 3D tubular silk scaffold system [216]. In this system, the 3D non-transformed intestinal epithelium tissue model closely mimics human infection, and the tissue model demonstrates significant antibacterial responses to *E. coli* infection. These results indicated potential applications of the 3D stem-cell derived epithelium for the *in vitro* study of host-microbe-pathogen interplay and IBD pathogenesis. Workman M J et al. demonstrated an intestine chip that contained all the intestinal epithelial subtypes, and was biologically responsive to exogenous stimuli [157]. To lay the foundation for personalized treatment of patients with intestinal diseases, further efforts are required to develop intestinal organ disease models on intestinal organ chips is closer to better recapitulate in vivo pathological characteristics.

## 6. Conclusion and outlook

In this review, the reconstruction of intestinal organ functions *in vitro* was summarized in aspects of *in vitro* animal models at organ/tissue level, 2D/3D culturing platforms, intestine chip, and multi-organ chips. Although the development of intestine chips has been less than ten years, they have shown advantages over the traditional culturing systems. The intestinal organ chip could create a culture condition closer to the human microenvironment than that of the traditional two/three dimensional-cell culture models, especially in the nutrient output and metabolite excretion circulatory system, concentration gradient, cell tight junction expansion, implantation of different microbial species and multi-organ joint effect. Much progress has been made in the development of intestinal cell/tissue culture platforms and in the investigation of intestinal-related pathology and intestinal drug screening, In the meanwhile, the problem of species differences in animal models and the limitation of current devices in reproducing human intestinal microenvironments and tissue functions *in vitro* need to be solved. Therefore, research institutes and drug industry are anticipated to focus on optimizing the structure of intestinal chip, industrializing device applications in drug testing to reduce cost and improve effectiveness.

There are several challenges to be tackled. Intestine chip should be optimized such as the stretching frequency and amplitude of mechanical stimulation. The hollow fiber structure device showed better performance (simulation of intestinal absorption and transport functions) in the porous membranes and could be adopted in the intestine chip. There is a need to quantitatively evaluate how the culture medium affects the physiological activities of the host cells, including the composition and concentration gradient of culture medium and the shear stress generated by the velocity of the fluid in the channel. Thousands of intestinal microorganisms in the body do not grow in isolation but live together. Intestinal chips should realize co-culture of more bacteria. Since the density and distribution of the gut microbiota vary at different locations in the gut (e.g., at sites of inflammation) and change over time, in situ measurements of both the microbiota and the host responses in intestine-on-a-chip with a sufficiently high temporal and spatial resolution are essential but are lacking. Finally, intestinal cells and microbiota on the intestinal organs of dialysis patients can be integrated on the device, which is more suitable for personalized treatment of patients.

## Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

## Informed consent

Informed consent was obtained from all individual participants included in the study.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

[1] A. Ouwehand, E. Isolauri, S. Salminen, The role of the intestinal microflora for the development of the immune system in early childhood, Eur. J. Nutr. 41 (2003), https://doi.org/10.1007/s00394-002-1105-4, 1–1.
[2] A. Fasano, T. Shea-Donohue, Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases, Nat. Clin. Pract. Gastroenterol. Hepatol. 2 (2005) 416–422, https://doi.org/10.1038/ncpgasthep0259.
[3] N. Khan, F. Afaq, H. Mukhtar, Lifestyle as risk factor for cancer: evidence from human studies, Canc. Lett. 293 (2010) 133–143, https://doi.org/10.1016/j.canlet.2009.12.013.
[4] P. Munkholm, E. Langholz, M. Davidsen, V. Binder, Intestinal cancer risk and mortality in patients with Crohn's disease, Gastroenterology 105 (1993) 1716–1723, https://doi.org/10.1016/0016-5085(93)91068-S.
[5] Z. Winawer, et al., Prevention of colorectal cancer by colonscopy polipectomy, N. Engl. J. Med. 329 (27) (1994) 1977–1981, https://doi.org/10.1056/NEJM199312303292701.
[6] M.J. Gu, Q.C. Huang, C.Z. Bao, Y.J. Li, X.Q. Li, D. Ye, Z.H. Ye, K. Chen, J.B. Wang, Attributable causes of colorectal cancer in China, BMC Canc. 18 (2018) 38, https://doi.org/10.1186/s12885-017-3968-z.
[7] J. Li, S. Qin, R.H. Xu, L. Shen, J. Xu, Y. Bai, L. Yang, Y. Deng, Z.D. Chen, H. Zhong, H. Pan, W. Guo, Y. Shu, Y. Yuan, J. Zhou, N. Xu, T. Liu, D. Ma, C. Wu, Y. Cheng, D. Chen, W. Li, S. Sun, Z. Yu, P. Cao, H. Chen, J. Wang, S. Wang, H. Wang, S. Fan, Y. Hua, W. Su, Effect of fruquintinib vs placebo on overall survival in patients with previously treated metastatic colorectal cancer the FRESCO randomized clinical trial, JAMA, J. Am. Med. Assoc. 319 (2018) 2486–2496, https://doi.org/10.1001/jama.2018.7855.
[8] M.A. Nandkumar, M. Yamato, A. Kushida, C. Konno, M. Hirose, A. Kikuchi, T. Okano, Two-dimensional cell sheet manipulation of heterotypically co-cultured lung cells utilizing temperature-responsive culture dishes results in long-

term maintenance of differentiated epithelial cell functions, Biomaterials 23 (2002) 1121–1130, https://doi.org/10.1016/S0142-9612(01)00225-3.
[9] Y. Ohta, T. Sato, Intestinal tumor in a dish, Front. Med. 1 (2014) 1–4, https://doi.org/10.3389/fmed.2014.00014.
[10] J. Marshall, Cell migration - chapter 8: transwell invasion assays, Stress 769 (2011) 137–147, https://doi.org/10.1007/978-1-61779-207-6.
[11] K.E. Sloan, B.K. Eustace, J.K. Stewart, C. Zehetmeier, C. Torella, M. Simeone, J. E. Roy, C. Unger, D.N. Louis, L.L. Ilag, D.G. Jay, CD155/PVR plays a key role in cell motility during tumor cell invasion and migration, BMC Canc. 4 (2004) 1–14, https://doi.org/10.1186/1471-2407-4-73.
[12] H.F. Lu, K.N. Chua, P.C. Zhang, W.S. Lim, S. Ramakrishna, K.W. Leong, H.Q. Mao, Three-dimensional co-culture of rat hepatocyte spheroids and NIH/3T3 fibroblasts enhances hepatocyte functional maintenance, Acta Biomater. 1 (2005) 399–410, https://doi.org/10.1016/j.actbio.2005.04.003.
[13] F. Leonard, E.M. Collnot, C.M. Lehr, A three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro, Mol. Pharm. 7 (2010) 2103–2119, https://doi.org/10.1021/mp1000795.
[14] J.L. Murphy, M.J. Arrowood, Cell Culture Infectivity to Assess Chlorine Disinfection, 2020, pp. 283–302, https://doi.org/10.1007/978-1-4939-9748-0 (chapter 2), 2052.
[15] P.C. Collins, W.M. Miller, E.T. Papoutsakis, Stirred culture of peripheral and cord blood hematopoietic cells offers advantages over traditional static systems for clinically relevant applications, Biotechnol. Bioeng. 59 (1998) 534–543, https://doi.org/10.1002/(SICI)1097-0290(19980905)59:5<534::AID-BIT2>3.0.CO;2-B.
[16] Z. Jianmin, W. Hongfang, F. Meifu, Static three-dimensional cul- ture of human hepatocarci- noma cell with microcarriers, (n.d.) 3–7.
[17] L.A. Schwerdtfeger, S.A. Tobet, Designing Multicellular Intestinal Systems, Elsevier Inc., 2020, https://doi.org/10.1016/b978-0-12-819178-1.00010-1.
[18] R.H. Dosh, A. Essa, N. Jordan-Mahy, C. Sammon, C.L. Le Maitre, Use of hydrogel scaffolds to develop an in vitro 3D culture model of human intestinal epithelium, Acta Biomater. 62 (2017) 128–143, https://doi.org/10.1016/j.actbio.2017.08.035.
[19] C.O. Yilmaz, Z.S. Xu, D.H. Gracias, Curved and Folded Micropatterns in 3D Cell Culture and Tissue Engineering, first ed., Elsevier Inc., 2014 https://doi.org/10.1016/B978-0-12-800281-0.00009-9.
[20] C.M. Costello, J. Hongpeng, S. Shaffiey, J. Yu, N.K. Jain, D. Hackam, J.C. March, Synthetic small intestinal scaffolds for improved studies of intestinal differentiation, Biotechnol. Bioeng. 111 (2014) 1222–1232, https://doi.org/10.1002/bit.25180.
[21] S. Sato, K. Hisaie, S. Kurokawa, A. Suzuki, N. Sakon, Y. Uchida, Y. Yuki, H. Kiyono, Human norovirus propagation in human induced pluripotent stem cell–derived intestinal epithelial cells, Cmgh 7 (2019) 686–688, https://doi.org/10.1016/j.jcmgh.2018.11.001, e5.
[22] H.J. Kim, D. Huh, G. Hamilton, D.E. Ingber, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, Lab Chip 12 (2012) 2165–2174, https://doi.org/10.1039/c2lc40074j.
[23] D. Huh, B.D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Yuan Hsin, D. E. Ingber, Reconstituting organ-level lung functions on a chip, Science 328 (2010) 1662–1668, https://doi.org/10.1126/science.1188302.
[24] R. Villenave, S.Q. Wales, T. Hamkins-Indik, E. Papafragkou, J.C. Weaver, T. C. Ferrante, A. Bahinski, C.A. Elkins, M. Kulka, D.E. Ingber, Human gut-on-a-chip supports polarized infection of coxsackie B1 virus in vitro, PloS One 12 (2017) 1–17, https://doi.org/10.1371/journal.pone.0169412.
[25] M.W. van der Helm, A.D. van der Meer, J.C.T. Eijkel, A. van den Berg, L. I. Segerink, Microfluidic organ-on-chip technology for blood-brain barrier research, Tissue Barriers 4 (2016), https://doi.org/10.1080/21688370.2016.1142493.
[26] V. De Gregorio, B. Corrado, S. Sbrescia, S. Sibilio, F. Urciuolo, P.A. Netti, G. Imparato, Intestine-on-chip device increases ECM remodeling inducing faster epithelial cell differentiation, Biotechnol. Bioeng. 117 (2020) 556–566, https://doi.org/10.1002/bit.27186.
[27] H.J. Kim, D.E. Ingber, Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation, Integr. Biol. (United Kingdom). 5 (2013) 1130–1140, https://doi.org/10.1039/c3ib40126j.
[28] C.J. Savory, M.A. Mitchell, Absorption of hexose and pentose sugars in vivo in perfused intestinal segments in the fowl, Comp. Biochem. Physiol. – Part A Physiol. 100 (1991) 969–974, https://doi.org/10.1016/0300-9629(91)90324-6.
[29] L. Biasutto, E. Marotta, A. Mattarei, S. Beltramello, P. Caliceti, S. Salmaso, A. Bernkop-schnürch, S. Garbisa, M. Zoratti, C. Paradisi, Cellular physiology biochemistry and biochemistr y absorption and metabolism of resveratrol carboxyesters and methanesulfonate by explanted rat intestinal segments, Cell. Physiol. Biochem. (2009) 557–566.
[30] D. Heller, T h i s, 151 (1965) 1–4.
[31] M.A. Alam, F.I. Al-Jenoobi, A.M. Al-Mohizea, Everted gut sac model as a tool in pharmaceutical research: limitations and applications, J. Pharm. Pharmacol. 64 (2012) 326–336, https://doi.org/10.1111/j.2042-7158.2011.01391.x.
[32] B. Carreno-Gómez, J.F. Woodley, A.T. Florence, Studies on the uptake of tomato lectin nanoparticles in everted gut sacs, Int. J. Pharm. 183 (1999) 7–11, https://doi.org/10.1016/S0378-5173(99)00050-2.
[33] X. Sha, X. Fang, Transport characteristics of 9-nitrocamptothecin in the human intestinal cell line Caco-2 and everted gut sacs, Int. J. Pharm. 272 (2004) 161–171, https://doi.org/10.1016/j.ijpharm.2003.12.023.
[34] A.C. Thierry, E. Bernasconi, A. Mercenier, B. Corthésy, Conditioned polarized Caco-2 cell monolayers allow to discriminate for the ability of gut-derived microorganisms to modulate permeability and antigen-induced basophil degranulation, Clin. Exp. Allergy 39 (2009) 527–536, https://doi.org/10.1111/j.1365-2222.2008.03185.x.
[35] P. Annaert, J. Brouwers, A. Bijnens, F. Lammert, J. Tack, P. Augustijns, Ex vivo permeability experiments in excised rat intestinal tissue and in vitro solubility measurements in aspirated human intestinal fluids support age-dependent oral drug absorption, Eur. J. Pharmaceut. Sci. 39 (2010) 15–22, https://doi.org/10.1016/j.ejps.2009.10.005.
[36] F.E. Holmberg, J.B. Seidelin, X. Yin, B.E. Mead, Z. Tong, Y. Li, J.M. Karp, O. H. Nielsen, Culturing human intestinal stem cells for regenerative applications in the treatment of inflammatory bowel disease, EMBO Mol. Med. 9 (2017) 558–570, https://doi.org/10.15252/emmm.201607260.
[37] R. Gauvin, R. Parenteau-Bareil, M.R. Dokmeci, W.D. Merryman, A. Khademhosseini, Hydrogels and microtechnologies for engineering the cellular microenvironment, Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology. 4 (2012) 235–246, https://doi.org/10.1002/wnan.171.
[38] J.D. Hardcastle, C. V Mann, Study of large bowel peristalsis, Gut 9 (1968) 512–520, https://doi.org/10.1136/gut.9.5.512.
[39] A.R. Perestrelo, A.C.P. Águas, A. Rainer, G. Forte, Microfluidic organ/body-on-a-chip devices at the convergence of biology and microengineering, Sensors 15 (2015) 31142–31170, https://doi.org/10.3390/s151229848.
[40] Y. Kato, Y. Hirai, K. Kamei, T. Tsuchiya, O. Tabata, Microfluidic device to interconnect multiple organs via fluidic circulation: towards body-on-a-chip, in: 2015 Transducers - 2015 18th Int. Conf. Solid-State Sensors, Actuators Microsystems, TRANSDUCERS 2015, 2015, pp. 1549–1552, https://doi.org/10.1109/TRANSDUCERS.2015.7181233.
[41] H. Lee, D.S. Kim, S.K. Ha, I. Choi, J.M. Lee, J.H. Sung, A pumpless multi-organ-on-a-chip (MOC) combined with a pharmacokinetic–pharmacodynamic (PK–PD) model, Biotechnol. Bioeng. 114 (2017) 432–443, https://doi.org/10.1002/bit.26087.
[42] The Uptake of Amino Acids by the, vol. 1 (n.d.).
[43] K.M. Hillgren, A. Kato, R.T. Borchardt, In vitro systems for studying intestinal drug absorption, Med. Res. Rev. 15 (1995) 83–109, https://doi.org/10.1002/med.2610150202.
[44] S.A. Acra, Methods of investigating intestinal transport, J. Parenter. Enteral Nutr. 15 (1991) 93S–98S, https://doi.org/10.1177/014860719101500393S.
[45] L. Barthe, M. Bessouet, J.F. Woodley, G. Houin, The improved everted gut sac: a simple method to study intestinal P-glycoprotein, Int. J. Pharm. 173 (1998) 255–258, https://doi.org/10.1016/S0378-5173(98)00225-7.
[46] J.V. Small, Cell migration, Encycl. Biol. Chem. 769 (2013) 430–435, https://doi.org/10.1016/B978-0-12-378630-2.00420-5, second ed.
[47] N. Kramer, A. Walzl, C. Unger, M. Rosner, G. Krupitza, M. Hengstschläger, H. Dolznig, In vitro cell migration and invasion assays, Mutat. Res. Rev. Mutat. Res. 752 (2013) 10–24, https://doi.org/10.1016/j.mrrev.2012.08.001.
[48] H. Li, J. Li, L. Liu, Y. Zhang, Y. Luo, X. Zhang, P. Yang, M. Zhang, W. Yu, S. Qu, Elucidation of the intestinal absorption mechanism of celastrol using the caco-2 cell transwell model, Planta Med. 82 (2016) 1202–1207, https://doi.org/10.1055/s-0035-1568597.
[49] M. Netzel, G. Netzel, D. Zabaras, L. Lundin, L. Day, R. Addepalli, S.A. Osborne, R. Seymour, Release and absorption of carotenes from processed carrots (Daucus carota) using in vitro digestion coupled with a Caco-2 cell trans-well culture model, Food Res. Int. 44 (2011) 868–874, https://doi.org/10.1016/j.foodres.2010.10.058.
[50] A.M. Marino, M. Yarde, H. Patel, S. Chong, P.V. Balimane, Validation of the 96 well Caco-2 cell culture model for high throughput permeability assessment of discovery compounds, Int. J. Pharm. 297 (2005) 235–241, https://doi.org/10.1016/j.ijpharm.2005.03.008.
[51] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability, Gastroenterology 96 (1989) 736–749, https://doi.org/10.1016/S0016-5085(89)80072-1.
[52] A.A.M. Kämpfer, P. Urbán, S. Gioria, N. Kanase, V. Stone, A. Kinsner-Ovaskainen, Development of an in vitro co-culture model to mimic the human intestine in healthy and diseased state, Toxicol. Vitro 45 (2017) 31–43, https://doi.org/10.1016/j.tiv.2017.08.011.
[53] B. Arnesjö, Å. Nilsson, J. Barrowman, B. Borgström, Intestinal digestion and absorption of cholesterol and lecithin in the human: intubation studies with a fat-soluble reference substance, Scand. J. Gastroenterol. 4 (1969) 653–665, https://doi.org/10.3109/00365526909180651.
[54] G.M. Gray, Carbohydrate digestion and absorption - role of small intestine, Prog. Gastroenterol. 58 (1970) 96–107, https://doi.org/10.1056/NEJM197506052922308.
[55] T.H.E. Human, Method of Werand Flowhetubg Proeing Y , Thelds, Small, 1957, pp. 1521–1536, https://doi.org/10.1172/JCI103549.1522.
[56] T. Higashi, R. Kikuchi, K. Miura, K. Shimada, H. Hiyamizu, H. Ooi, et al., New metabolic pathway of (24r)-24,25-dihydroxyvitamin d3: epimerization of the 3-hydroxy group, Biological & Pharmaceutical Bulletin 22 (7) (1999) 767, https://doi.org/10.1248/bpb.22.767.
[57] J.P.E. Spencer, G. Chowrimootoo, R. Choudhury, E.S. Debnam, S.K. Srai, C. Rice-Evans, The small intestine can both absorb and glucuronidate luminal flavonoids, FEBS Lett. 458 (1999) 224–230, https://doi.org/10.1016/S0014-5793(99)01160-6.
[58] S. Talavéra, C. Felgines, O. Texier, C. Besson, C. Manach, J.-L. Lamaison, C. Rémésy, Anthocyanins are efficiently absorbed from the small intestine in rats, J. Nutr. 134 (2018) 2275–2279, https://doi.org/10.1093/jn/134.9.2275.

[59] P. Marteau, P. Pochart, Y. Bouhnik, J.-C. Rambaud, The fate and effects of transiting, Nonpathogenic Microorganisms in the Human Intestine 74 (2015) 1–21, https://doi.org/10.1159/000422599.

[60] R.B. Sartor, S.K. Mazmanian, Intestinal microbes in inflammatory bowel diseases, Am. J. Gastroenterol. Suppl. 1 (2012) 15–21, https://doi.org/10.1038/ajgsup.2012.4.

[61] M. Montalto, F. D'Onofrio, A. Gallo, A. Cazzato, G. Gasbarrini, Intestinal microbiota and its functions, Dig. Liver Dis. Suppl. 3 (2009) 30–34, https://doi.org/10.1016/S1594-5804(09)60016-4.

[62] Y. Umesaki, H. Setoyama, Structure of the intestinal flora responsible for development of the gut immune system in a rodent model, Microb. Infect. 2 (2000) 1343–1351, https://doi.org/10.1016/S1286-4579(00)01288-0.

[63] Y. Sanz, R. Rastmanesh, C. Agostonic, Understanding the role of gut microbes and probiotics in obesity: how far are we? Pharmacol. Res. 69 (2013) 144–155, https://doi.org/10.1016/j.phrs.2012.10.021.

[64] P.L. Sappington, R. Yang, H. Yang, K.J. Tracey, R.L. Delude, M.P. Fink, HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice, Gastroenterology 123 (2002) 790–802, https://doi.org/10.1053/gast.2002.35391.

[65] J.P. Motta, A. Denadai-Souza, D. Sagnat, L. Guiraud, A. Edir, C. Bonnart, M. Sebbag, P. Rousset, A. Lapeyre, C. Seguy, N. Mathurine-Thomas, H. J. Galipeau, D. Bonnet, L. Alric, A.G. Buret, J.L. Wallace, A. Dufour, E.F. Verdu, M.D. Hollenberg, E. Oswald, M. Serino, C. Deraison, N. Vergnolle, Active thrombin produced by the intestinal epithelium controls mucosal biofilms, Nat. Commun. 10 (2019) 1–12, https://doi.org/10.1038/s41467-019-11140-w.

[66] D. Elshaer, J. Begun, The role of barrier function, autophagy, and cytokines in maintaining intestinal homeostasis, Semin. Cell Dev. Biol. 61 (2017) 51–59, https://doi.org/10.1016/j.semcdb.2016.08.018.

[67] M. Camilleri, K. Madsen, R. Spiller, B.G. Van Meerveld, G.N. Verne, Intestinal barrier function in health and gastrointestinal disease, Neuro Gastroenterol. Motil. 24 (2012) 503–512, https://doi.org/10.1111/j.1365-2982.2012.01921.x.

[68] Y. Wang, Q. Xie, Y. Zhang, W. Ma, K. Ning, J.Y. Xiang, J. Cui, H. Xiang, Combination of probiotics with different functions alleviate DSS-induced colitis by regulating intestinal microbiota, IL-10, and barrier function, Appl. Microbiol. Biotechnol. 104 (2020) 335–349, https://doi.org/10.1007/s00253-019-10259-6.

[69] M. Rosenbaum, Ulcerative colitis, Psychosomatics 24 (1983) 515–519, https://doi.org/10.1016/S0033-3182(83)73183-X.

[70] A. Farhadi, A.L.I. Banan, J. Fields, A.L.I. Keshavarzian, Intestinal barrier : an interface between health and disease basement epithelium membrane coat secretory IgA mucosal M cell unstirred water layer immunoglobulin macrophage endothelium dandrite cell basophil mast cell lymphocyte, J. Gastroenterol. Hepatol. 18 (2003) 479–497.

[71] M.A. McGuckin, R. Eri, L.A. Simms, T.H.J. Florin, G. Radford-Smith, Intestinal barrier dysfunction in inflammatory bowel diseases, Inflamm. Bowel Dis. 15 (2009) 100–113, https://doi.org/10.1002/ibd.20539.

[72] B.P. McNamara, A. Koutsouris, C.B. O'Connell, J.P. Nougayréde, M. S. Donnenberg, G. Hecht, Translocated EspF protein from enteropathogenic Escherichia coli disrupts host intestinal barrier function, J. Clin. Invest. 107 (2001) 621–629, https://doi.org/10.1172/JCI11138.

[73] M.G. Clemente, S. De Virgiliis, J.S. Kang, R. Macatagney, M.P. Musu, M.R. Di Pierro, S. Drago, M. Congia, A. Fasano, Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function, Gut 52 (2003) 218–223, https://doi.org/10.1136/gut.52.2.218.

[74] D. Biran, E.Z. Ron, Extraintestinal pathogenic escherichia coli, Curr. Top. Microbiol. Immunol. 416 (2018) 149–161, https://doi.org/10.1007/82_2018_108.

[75] L.M. Agius, A primary dysregulation in the immunoregulatory role of the intestinal mucosal epithelial cell in inflammatory bowel disease pathogenesis? Biology of inflammatory response as tissue pattern entities in Crohn's versus ulcerative colitis, J. Theor. Biol. 227 (2004) 219–228, https://doi.org/10.1016/j.jtbi.2003.11.002.

[76] J.L. Madara, Intestinal absorptive cell tight junctions are linked to cytoskeleton, Am. J. Physiol. 253 (1987) C171–C175, https://doi.org/10.1152/ajpcell.1987.253.1.C171.

[77] A.T. Blikslager, Intestinal mucosal epithelium: the barrier to sepsis, J. Organ Dysfunct. 2 (2006) 250–253, https://doi.org/10.1080/17471060600765018.

[78] K. Morita, H. Sasaki, M. Furuse, S. Tsukita, Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells, J. Cell Biol. 147 (1999) 185–194, https://doi.org/10.1083/jcb.147.1.185.

[79] M. Saitou, M. Furuse, H. Sasaki, J.-D. Schulzke, M. Fromm, H. Takano, T. Noda, S. Tsukita, Complex phenotype of mice lacking occludin, a component of tight junction strands, Mol. Biol. Cell 11 (2000) 4131–4142, https://doi.org/10.1091/mbc.11.12.4131.

[80] J.-P. Kraehenbuhl, B. Valzasina, M. Francolini, F. Granucci, M. Urbano, M. Rescigno, R. Bonasio, P. Ricciardi-Castagnoli, G. Rotta, Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria, Nat. Immunol. 2 (2002) 361–367, https://doi.org/10.1038/86373.

[81] O. Amano, T. Kanda, T. Ono, S. Iseki, Immunocytochemical localization of rat intestinal 15 kDa protein, a member of cytoplasmic fatty acid-binding proteins, Anat. Rec. 234 (1992) 215–222, https://doi.org/10.1002/ar.1092340208.

[82] S. Kim, R. Goel, A. Kumar, Y. Qi, G. Lobaton, K. Hosaka, M. Mohammed, E. M. Handberg, E.M. Richards, C.J. Pepine, M.K. Raizada, Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure, Clin. Sci. 132 (2018) 701–718, https://doi.org/10.1042/cs20180087.

[83] T. Yang, M.M. Santisteban, V. Rodriguez, E. Li, N. Ahmari, J.M. Carvajal, M. Zadeh, M. Gong, Y. Qi, J. Zubcevic, B. Sahay, C.J. Pepine, M.K. Raizada, M. Mohamadzadeh, Gut dysbiosis is linked to hypertension, Hypertension 65 (2015) 1331–1340, https://doi.org/10.1161/HYPERTENSIONAHA.115.05315.

[84] Y. Leng, M. Yi, J. Fan, Y. Bai, Q. Ge, G. Yao, Effects of acute intra-abdominal hypertension on multiple intestinal barrier functions in rats, Sci. Rep. 6 (2016) 1–9, https://doi.org/10.1038/srep22814.

[85] T.Y. Zhao, L.P. Su, C.Y. Ma, X.H. Zhai, Z.J. Duan, Y. Zhu, G. Zhao, C.Y. Li, L. X. Wang, D. Yang, IGF-1 decreases portal vein endotoxin via regulating intestinal tight junctions and plays a role in attenuating portal hypertension of cirrhotic rats, BMC Gastroenterol. 15 (2015) 1–10, https://doi.org/10.1186/s12876-015-0311-5.

[86] R.M. Insoft, I.R. Sanderson, W.A. Walker, Development of immune function in the intestine and its role in neonatal diseases, Pediatr. Clin. 43 (1996) 551–571, https://doi.org/10.1016/S0031-3955(05)70420-X.

[87] S. Blum, E. Schiffrin, Intestinal microflora and homeostasis of the mucosal immune response: implications for probiotic bacteria? Curr. Issues Intest. Microbiol. 4 (2003) 53–60.

[88] H. Tlaskalová-Hogenová, R. Štěpánková, T. Hudcovic, L. Tučková, B. Cukrowska, R. Lodinová-Žádníková, H. Kozáková, P. Rossmann, J. Bártová, D. Sokol, D. P. Funda, D. Borovská, Z. Řeháková, J. Šinkora, J. Hofman, P. Drastich, A. Kokešová, Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases, Immunol. Lett. 93 (2004) 97–108, https://doi.org/10.1016/j.imlet.2004.02.005.

[89] K.R. Groschwitz, S.P. Hogan, Intestinal barrier function: molecular regulation and disease pathogenesis, J. Allergy Clin. Immunol. 124 (2009) 3–20, https://doi.org/10.1016/j.jaci.2009.05.038.

[90] W.R. Best, J.M. Becktel, J.W. Singleton, F. Kern, Development of a crohn's disease activity index, Gastroenterology 70 (1976) 439–444, https://doi.org/10.1016/s0016-5085(76)80163-1.

[91] R.W. Summers, D.M. Switz, J.T. Sessions, J.M. Becktel, W.R. Best, F. Kern, J. W. Singleton, National cooperative crohn's disease study: results of drug treatment, Gastroenterology 77 (1979) 847–869, https://doi.org/10.1016/0016-5085(79)90385-8.

[92] R.J. Xavier, D.K. Podolsky, Unravelling the pathogenesis of inflammatory bowel disease, Nature 448 (2007) 427–434, https://doi.org/10.1038/nature06005.

[93] L. yan Pei, Y. shi Ke, H. hu Zhao, L. Wang, C. Jia, W. zhi Liu, Q. hui Fu, M. ni Shi, J. Cui, S. chun Li, Role of colonic microbiota in the pathogenesis of ulcerative colitis, BMC Gastroenterol. 19 (2019) 1–11, https://doi.org/10.1186/s12876-019-0930-3.

[94] F.G. Sala, S.M. Kunisaki, E.R. Ochoa, J. Vacanti, T.C. Grikscheit, Tissue-Engineered small intestine and stomach form from autologous tissue in a preclinical large animal model, J. Surg. Res. 156 (2009) 205–212, https://doi.org/10.1016/j.jss.2009.03.062.

[95] D.J. Trott, A.J. McLaren, D.J. Hampson, Pathogenicity of human and porcine intestinal spirochetes in one-day-old specific-pathogen-free chicks: an animal model of intestinal spirochetosis, Infect. Immun. 63 (1995) 3705–3710.

[96] J. Roper, K.E. Hung, Priceless GEMMs: genetically engineered mouse models for colorectal cancer drug development, Trends Pharmacol. Sci. 33 (2012) 449–455, https://doi.org/10.1016/j.tips.2012.05.001.

[97] E. Nuermberger, Using animal models to develop new treatments for tuberculosis, Semin. Respir. Crit. Care Med. 29 (2008) 542–551, https://doi.org/10.1055/s-0028-1085705.

[98] V.K. Singh, V.L. Newman, A.N. Berg, T.J. MacVittie, Animal models for acute radiation syndrome drug discovery, Expet Opin. Drug Discov. 10 (2015) 497–517, https://doi.org/10.1517/17460441.2015.1023290.

[99] S. Wirtz, M.F. Neurath, Animal models of intestinal inflammation: new insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease, Int. J. Colorectal Dis. 15 (2000) 144–160, https://doi.org/10.1007/s003840000227.

[100] H. Komura, M. Iwaki, In vitro and in vivo small intestinal metabolism of CYP3A and UGT substrates in preclinical animals species and humans: species differences, Drug Metab. Rev. 43 (2011) 476–498, https://doi.org/10.3109/03602532.2011.597401.

[101] H. Attarwala, TGN1412: from discovery to disaster, J. Young Pharm. 2 (2010) 332–336, https://doi.org/10.4103/0975-1483.66810.

[102] G.W. Hennig, M. Costa, B.N. Chen, S.J.H. Brookes, Quantitative analysis of peristalsis in the Guinea-pig small intestine using spatio-temporal maps, J. Physiol. 517 (1999) 575–590, https://doi.org/10.1111/j.1469-7793.1999.0575t.x.

[103] R. Yoshikawa, N. Mikami, I. Otani, T. Kishimoto, S. Nishioka, N. Hashimoto, Y. Miyagi, Y. Takuma, K. Sueda, S. ichiro Fukada, H. Yamamoto, K. Tsujikawa, Suppression of ovalbumin-induced allergic diarrhea by diminished intestinal peristalsis in RAMP1-deficient mice, Biochem. Biophys. Res. Commun. 410 (2011) 389–393, https://doi.org/10.1016/j.bbrc.2011.05.141.

[104] J.L. Madara, R. Moore, S. Carlson, Alteration of intestinal tight junction structure and permeability by cytoskeletal contraction, Am. J. Physiol. Physiol. 253 (2017) C854–C861, https://doi.org/10.1152/ajpcell.1987.253.6.c854.

[105] M.J. Karnovsky, Contraction of isolated brush borders from the intestinal epithelium isolation of brush borders morphology of brush borders by light, Microscopy 70 (1976) 541–554.

[106] A.R. Wechezak, T.N. Wight, R.F. Viggers, L.R. Sauvage, Endothelial adherence under shear stress is dependent upon microfilament reorganization, J. Cell. Physiol. 139 (1989) 136–146, https://doi.org/10.1002/jcp.1041390120.

[107] A.A.M. Lopez, H.X. Kim, 5 , 1,2, (2017).

[108] F. De Iorio, C. Malagelada, F. Azpiroz, M. Maluenda, C. Violanti, L. Igual, J. Vitrià, J.R. Malagelada, Intestinal motor activity, endoluminal motion and transit, Neuro Gastroenterol. Motil. 21 (2009), https://doi.org/10.1111/j.1365-2982.2009.01363.x.
[109] A. Szabo, E. Csonka, K. Kucsa, A. Szabó, B. Suki, E. Eszlári, K. Vajda, J. Kaszaki, M. Boros, Flow motion in the intestinal villi during hemorrhagic shock: a new method to characterize the microcirculatory changes, Shock 21 (2004) 320–328, https://doi.org/10.1097/01.shk.0000115824.98274.02.
[110] D.M. Sánchez-Palencia, A. D'Amore, A. González-Mancera, W.R. Wagner, J. C. Briceño, Effects of fabrication on the mechanics, microstructure and micromechanical environment of small intestinal submucosa scaffolds for vascular tissue engineering, J. Biomech. 47 (2014) 2766–2773, https://doi.org/10.1016/j.jbiomech.2014.04.048.
[111] J. Zhang, X. Wei, R. Zeng, F. Xu, X. Li, Stem cell culture and differentiation in microfluidic devices toward organ-on-a-chip, Futur. Sci. OA. 3 (2017) FSO187, https://doi.org/10.4155/fsoa-2016-0091.
[112] F. An, Y. Qu, X. Liu, R. Zhong, Y. Luo, Organ-on-a-chip: new platform for biological analysis, Anal. Chem. Insights 10 (2015) 39–45, https://doi.org/10.4137/Aci.s28905.
[113] H. Kimura, Y. Sakai, T. Fujii, Organ/body-on-a-chip based on microfluidic technology for drug discovery, Drug Metabol. Pharmacokinet. 33 (2018) 43–48, https://doi.org/10.1016/j.dmpk.2017.11.003.
[114] K.J. Pocock, X. Gao, C. Wang, C. Priest, C.A. Prestidge, K. Mawatari, T. Kitamori, B. Thierry, Low-temperature bonding process for the fabrication of hybrid glass–membrane organ-on-a-chip devices, J. Nanolithogr. MEMS, MOEMS 15 (2016), 044502, https://doi.org/10.1117/1.jmm.15.4.044502.
[115] C. Science, Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine Concise Review: Stem Cell Microenvironment on a Chip : Current Technologies for Tissue Engineering and Stem Cell Biology, 2015, pp. 1352–1368.
[116] D. Gao, H. Liu, Y. Jiang, J.M. Lin, D. Gao, H. Liu, Y. Jiang, Recent developments in microfluidic devices for in vitro cell culture for cell-biology research, TrAC Trends Anal. Chem. (Reference Ed.) 35 (2012) 150–164, https://doi.org/10.1016/j.trac.2012.02.008.
[117] D. Huh, H.J. Kim, J.P. Fraser, D.E. Shea, M. Khan, A. Bahinski, G.A. Hamilton, D. E. Ingber, Microfabrication of human organs-on-chips, Nat. Protoc. 8 (2013) 2135–2157, https://doi.org/10.1038/nprot.2013.137.
[118] L. Hajba, A. Guttman, Continuous-flow-based microfluidic systems for therapeutic monoclonal antibody production and organ-on-a-chip drug testing, J. Flow Chem. 7 (2017) 118–123, https://doi.org/10.1556/1846.2017.00014.
[119] A. Polini, L. Prodanov, N.S. Bhise, V. Manoharan, M.R. Dokmeci, A. Khademhosseini, Organs-on-a-chip: a new tool for drug discovery, Expet Opin. Drug Discov. 9 (2014) 335–352, https://doi.org/10.1517/17460441.2014.886562.
[120] K. P, L. D, V. B, S. R, C. P, C. P, B. T, Intestine-on-A-Chip microfluidic model for efficient in vitro screening of oral chemotherapeutic uptake, ACS Biomater. Sci. Eng. 3 (2017) 951–959, https://doi.org/10.1021/acsbiomaterials.7b00023LK. http://resolver.ebscohost.com/openurl?sid=EMBASE&issn=23739878&id=doi:10.1021%2Facsbiomaterials.7b00023&atitle=Intestine-on-A-Chip+Microfluidic+Model+for+Efficient+in+Vitro+Screening+of+Oral+Chemotherapeutic+Uptake&stitle=ACS+Biomater.+Sci.+Eng.&title=ACS+Biomaterials+Science+and+Engineering&volume=3&issue=6&spage=951&epage=959&aulast=Pocock&aufirst=Kyall&auinit=K.&aufull=Pocock+K.&coden=&isbn=&pages=951-959&date=2017&auinit1=K&auinitm=.
[121] M. Kasendra, A. Tovaglieri, A. Sontheimer-Phelps, S. Jalili-Firoozinezhad, A. Bein, A. Chalkiadaki, W. Scholl, C. Zhang, H. Rickner, C.A. Richmond, H. Li, D. T. Breault, D.E. Ingber, Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids, Sci. Rep. 8 (2018) 1–14, https://doi.org/10.1038/s41598-018-21201-7.
[122] A. Grosberg, P.W. Alford, M.L. McCain, K.K. Parker, Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip, Lab Chip 11 (2011) 4165–4173, https://doi.org/10.1039/c1lc20557a.
[123] A. Agarwal, J.A. Goss, A. Cho, M.L. McCain, K.K. Parker, Microfluidic heart on a chip for higher throughput pharmacological studies, Lab Chip 13 (2013) 3599–3608, https://doi.org/10.1039/c3lc50350j.
[124] K. Jang, G.A. Hamilton, L. Mcpartlin, A. Bahinski, H.N. Kim, K. Suh, D.E. Ingber, Human kidney proximal tubule-on-a-chip for drug transporter studies and nephrotoxicity assessment, in: 15th Int. Conf. Minituarized Syst. Chem. Life Sci. 5, 2011, pp. 1502–1504.
[125] M.J. Wilmer, C.P. Ng, H.L. Lanz, P. Vulto, L. Suter-Dick, R. Masereeuw, Kidney-on-a-Chip technology for drug-induced nephrotoxicity screening, Trends Biotechnol. 34 (2016) 156–170, https://doi.org/10.1016/j.tibtech.2015.11.001.
[126] K.H. Lee, S.J. Shin, C.B. Kim, J.K. Kim, Y.W. Cho, B.G. Chung, S.H. Lee, Microfluidic synthesis of pure chitosan microfibers for bio-artificial liver chip, Lab Chip 10 (2010) 1328–1334, https://doi.org/10.1039/b924987g.
[127] C.T. Ho, R.Z. Lin, R.J. Chen, C.K. Chin, S.E. Gong, H.Y. Chang, H.L. Peng, L. Hsu, T.R. Yew, S.F. Chang, C.H. Liu, Liver-cell patterning Lab Chip: mimicking the morphology of liver lobule tissue, Lab Chip 13 (2013) 3578–3587, https://doi.org/10.1039/c3lc50402f.
[128] S. Hassan, S. Sebastian, S. Maharjan, A. Lesha, A. Carpenter, X. Liu, X. Xie, C. Livermore, Y.S. Zhang, A. Zarrinpar, Liver-on-a-Chip models of fatty liver disease, Hepatology (2020), https://doi.org/10.1002/hep.31106.
[129] S.I. Materials, S. Four, Supporting Information (2009) 1–10.
[130] W. Z, Y.S. Z, S.M. B, J. A, S.R. S, K. Y, M. S, J. R, M. D, J. J, R.B. S, D. K, M.R. D, A. A, A. K, Elastomeric free-form blood vessels for interconnecting organs on chip systems, Lab Chip 16 (2016) 1579–1586, https://doi.org/10.1039/c6lc00001k.
[131] M.B. Esch, T.L. King, M.L. Shuler, The role of body-on-a-chip devices in drug and toxicity studies, Annu. Rev. Biomed. Eng. 13 (2011) 55–72, https://doi.org/10.1146/annurev-bioeng-071910-124629.
[132] M.B. Esch, J.H. Sung, J. Yang, C. Yu, J. Yu, J.C. March, M.L. Shuler, On chip porous polymer membranes for integration of gastrointestinal tract epithelium with microfluidic "body-on-a-chip" devices, Biomed. Microdevices 14 (2012) 895–906, https://doi.org/10.1007/s10544-012-9669-0.
[133] K.M. Motsegood, P.P. Elastomer, Three-dimensional micro-channel fabrication in PDMS, J. Microelectromechanical Syst 9 (2010) 76–81, https://doi.org/10.1109/84.825780.
[134] H.J. Kim, H. Li, J.J. Collins, D.E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) E7–E15, https://doi.org/10.1073/pnas.1522193112.
[135] H.J. Kim, J. Lee, J.-H. Choi, A. Bahinski, D.E. Ingber, Co-culture of living microbiome with microengineered human intestinal villi in a gut-on-a-chip microfluidic device, JoVE (2016) 3–9, https://doi.org/10.3791/54344.
[136] S.H. Lee, J.H. Sung, Gut-on-a-chip Microphysiological Systems for the Recapitulation of the Gut Microenvironment, Elsevier Inc., 2020, https://doi.org/10.1016/b978-0-12-817202-5.00010-3.
[137] I. Sekirov, B.B. Finlay, The role of the intestinal microbiota in enteric infection, J. Physiol. 587 (2009) 4159–4167, https://doi.org/10.1113/jphysiol.2009.172742.
[138] L.V. Hooper, A.J. MacPherson, Immune adaptations that maintain homeostasis with the intestinal microbiota, Nat. Rev. Immunol. 10 (2010) 159–169, https://doi.org/10.1038/nri2710.
[139] W. Chen, F. Liu, Z. Ling, X. Tong, C. Xiang, Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer, PloS One 7 (2012), https://doi.org/10.1371/journal.pone.0039743.
[140] J. Penders, E.E. Stobberingh, P.A.V.D. Brandt, C. Thijs, The role of the intestinal microbiota in the development of atopic disorders, Allergy Eur. J. Allergy Clin. Immunol. 62 (2007) 1223–1236, https://doi.org/10.1111/j.1398-9995.2007.01462.x.
[141] K.Y. Shim, D. Lee, J. Han, N.T. Nguyen, S. Park, J.H. Sung, Microfluidic gut-on-a-chip with three-dimensional villi structure, Biomed. Microdevices 19 (2017), https://doi.org/10.1007/s10544-017-0179-y.
[142] S.J. Trietsch, E. Naumovska, D. Kurek, M.C. Setyawati, M.K. Vormann, K. J. Wilschut, H.L. Lanz, A. Nicolas, C.P. Ng, J. Joore, S. Kustermann, A. Roth, T. Hankemeier, A. Moisan, P. Vulto, Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes, Nat. Commun. 8 (2017) 1–7, https://doi.org/10.1038/s41467-017-00259-3.
[143] S. Jalili-Firoozinezhad, R. Prantil-Baun, A. Jiang, R. Potla, T. Mammoto, J. C. Weaver, T.C. Ferrante, H.J. Kim, J.M.S. Cabral, O. Levy, D.E. Ingber, Modeling radiation injury-induced cell death and countermeasure drug responses in a human Gut-on-a-Chip article, Cell Death Dis. 9 (2018), https://doi.org/10.1038/s41419-018-0304-8.
[144] D. Gabryś, O. Greco, G. Patel, K.M. Prise, G.M. Tozer, C. Kanthou, Radiation effects on the cytoskeleton of endothelial cells and endothelial monolayer permeability, Int. J. Radiat. Oncol. Biol. Phys. 69 (2007) 1553–1562, https://doi.org/10.1016/j.ijrobp.2007.08.039.
[145] J. Kahn, P.J. Tofilon, K. Camphausen, Preclinical models in radiation oncology, Radiat. Oncol. 7 (2012) 1–5, https://doi.org/10.1186/1748-717X-7-223.
[146] A.D. Augustine, T. Gondré-Lewis, W. McBride, L. Miller, T.C. Pellmar, S. Rockwell, Animal models for radiation injury, protection and therapy, Radiat. Res. 164 (2006) 100–109, https://doi.org/10.1667/rr3388.
[147] Caco-2- Intestinal Mucosa.pdf, (n.d.).
[148] C. Jumarie, C. Malo, Caco-2 cells cultured in serum-free medium as a model for the study of enterocytic differentiation in vitro, J. Cell. Physiol. 149 (1991) 24–33, https://doi.org/10.1002/jcp.1041490105.
[149] H.Y. Tan, S. Trier, U.L. Rahbek, M. Dufva, J.P. Kutter, T.L. Andresen, A multi-chamber microfluidic intestinal barrier model using Caco-2 cells for drug transport studies, PloS One 13 (2018) 1–23, https://doi.org/10.1371/journal.pone.0197101.
[150] L. Jing, Characterization of tight junction disruption and immune response modulation in a miniaturized Caco-2/U937 coculture-based in vitro model of the human intestinal barrier, Biomed. Microdevices (2016) 1–9, https://doi.org/10.1007/s10544-016-0035-5.
[151] A. Grassart, S. Gobaa, B. Marteyn, P. Sansonetti, Bioengineered Human Organ-On-Chip Reveals Intestinal Microenvironment and Mechanical Forces Impacting Shigella Infection Short Article Bioengineered Human Organ-On-Chip Reveals Intestinal Microenvironment and Mechanical Forces Impacting Shigella Infection, 2019, pp. 435–444, https://doi.org/10.1016/j.chom.2019.08.007.
[152] M.S. Sadabad, J.Z.H. Von Martels, M.T. Khan, T. Blokzijl, G. Paglia, G. Dijkstra, H. J.M. Harmsen, A simple coculture system shows mutualism between anaerobic faecalibacteria and epithelial Caco-2 cells, Nat. Publ. Gr. (2015) 1–9, https://doi.org/10.1038/srep17906.
[153] M.L. Scarino, F. Zucco, A. Stammati, Y. Sambuy, G. Ranaldi, I. De Angelis, The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics, Cell Biol. Toxicol. 21 (2005) 1–26, https://doi.org/10.1007/s10565-005-0085-6.
[154] Shiyin Yee, In vitro permeability across caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man-fact or myth, Pharm. Res. (N. Y.) 14 (1997).
[155] S.R. Finkbeiner, J.R. Spence, A. gutsy task, Generating intestinal tissue from human pluripotent stem cells, Dig. Dis. Sci. 58 (2013) 1176–1184, https://doi.org/10.1007/s10620-013-2620-2.

[156] J.S. Dutton, S.S. Hinman, R. Kim, Y. Wang, N.L. Allbritton, Primary cell-derived intestinal models: recapitulating physiology, Trends Biotechnol. 37 (2019) 744–760, https://doi.org/10.1016/j.tibtech.2018.12.001.
[157] M.J. Workman, J.P. Gleeson, E.J. Troisi, H.Q. Estrada, S.J. Kerns, C.D. Hinojosa, G.A. Hamilton, S.R. Targan, C.N. Svendsen, R.J. Barrett, Enhanced utilization of induced pluripotent stem cell–derived human intestinal organoids using microengineered chips, Cmgh 5 (2018) 669–677, https://doi.org/10.1016/j.jcmgh.2017.12.008, e2.
[158] G.Q. Daley, M.W. Lensch, R. Jaenisch, A. Meissner, K. Plath, S. Yamanaka, Broader implications of defining standards for the pluripotency of iPSCs, Cell Stem Cell 4 (2009) 200–201, https://doi.org/10.1016/j.stem.2009.02.009.
[159] Danielle R. Bogdanowicz, Helen H. Lu, Multifunction Co-culture model for evaluating cell-cell interactions, Methods Mol. Biol. (2014) 29–36, https://doi.org/10.1007/7651.
[160] M.H. Macedo, F. Araújo, E. Martínez, C. Barrias, B. Sarmento, iPSC-derived enterocyte-like cells for drug absorption and metabolism studies, Trends Mol. Med. 24 (2018) 696–708, https://doi.org/10.1016/j.molmed.2018.06.001.
[161] C.L. Watson, M.M. Mahe, J. Múnera, J.C. Howell, N. Sundaram, H.M. Poling, J. I. Schweitzer, J.E. Vallance, C.N. Mayhew, Y. Sun, G. Grabowski, S.R. Finkbeiner, J.R. Spence, N.F. Shroyer, J.M. Wells, M.A. Helmrath, An in vivo model of human small intestine using pluripotent stem cells, Nat. Med. 20 (2014) 1310–1314, https://doi.org/10.1038/nm.3737.
[162] T. Ozawa, K. Takayama, R. Okamoto, R. Negoro, F. Sakurai, M. Tachibana, K. Kawabata, H. Mizuguchi, Generation of enterocyte-like cells from human induced pluripotent stem cells for drug absorption and metabolism studies in human small intestine, Sci. Rep. 5 (2015) 1–11, https://doi.org/10.1038/srep16479.
[163] R.E. Ley, D.A. Peterson, J.I. Gordon, Ecological and evolutionary forces shaping microbial diversity in the human intestine, Cell 124 (2006) 837–848, https://doi.org/10.1016/j.cell.2006.02.017.
[164] I.I. Ivanov, K. Honda, Intestinal commensal microbes as immune modulators, Cell Host Microbe 12 (2012) 496–508, https://doi.org/10.1016/j.chom.2012.09.009.
[165] M. Ventura, F. Turroni, C. Canchaya, V. Ee, O.T. Pw, V.S. D, M. Ventura, F. Turroni, C. Canchaya, E.E. Vaughan, O ' Toole Pw, D. van Sinderen, Microbial diversity in the human intestine and novel insights from metagenomics . Frontiers in Bioscience , 14 ( 1 ) Deposited on : 15 February 2010 CORA Cork Open, Front. J. Women Stud. 14 (2010) (2009) 3214–3221.
[166] A. Nanoparticles, Nanoscale Microlandscaping on Graphene Oxide Film via Localized Decoration of, 2013, https://doi.org/10.1039/b000000x.
[167] J.M. Beekman, Individualized medicine using intestinal responses to CFTR potentiators and correctors, Pediatr. Pulmonol. 51 (2016) S23–S34, https://doi.org/10.1002/ppul.23553.
[168] W. Shin, H.J. Kim, Pathomimetic Modeling of Human Intestinal Diseases and Underlying Host-Gut Microbiome Interactions in a Gut-On-a-Chip, first ed., Elsevier Inc., 2018 https://doi.org/10.1016/bs.mcb.2018.05.006.
[169] C. Beaurivage, A. Kanapeckaite, C. Loomans, K.S. Erdmann, J. Stallen, R.A. J. Janssen, Development of a human primary gut-on-a-chip to model inflammatory processes, Sci. Rep. 10 (2020) 1–16, https://doi.org/10.1038/s41598-020-78359-2.
[170] A. Manuscript, Lab on a Chip, 2018, https://doi.org/10.1039/C8LC00111A.
[171] Y. Fang, C. Wu, Q. Wang, J. Tang, Farnesol contributes to intestinal epithelial barrier function by enhancing tight junctions via the JAK/STAT3 signaling pathway in differentiated Caco-2 cells, J. Bioenerg. Biomembr. 51 (2019) 403–412, https://doi.org/10.1007/s10863-019-09817-4.
[172] M. Chen, Y. Liu, S. Xiong, M. Wu, B. Li, Z. Ruan, X. Hu, Dietary l-tryptophan alleviated LPS-induced intestinal barrier injury by regulating tight junctions in a Caco-2 cell monolayer model, Food Funct 10 (2019) 2390–2398, https://doi.org/10.1039/c9fo00123a.
[173] M. Odijk, A.D. Van Der Meer, D. Levner, H.J. Kim, M.W. Van Der Helm, L. I. Segerink, J.P. Frimat, G.A. Hamilton, D.E. Ingber, A. Van Den Berg, Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems, Lab Chip 15 (2015) 745–752, https://doi.org/10.1039/c4lc01219d.
[174] K. Benson, S. Cramer, H.J. Galla, Impedance-based cell monitoring: barrier properties and beyond, Fluids Barriers CNS 10 (2013) 1–11, https://doi.org/10.1186/2045-8118-10-5.
[175] R. Villa, C. Gutiérrez, X. Illa, A. Guimerà, M. Solé, J. Yeste, Geometric correction factor for transepithelial electrical resistance measurements in transwell and microfluidic cell cultures, J. Phys. D Appl. Phys. 49 (2016) 375401, https://doi.org/10.1088/0022-3727/49/37/375401.
[176] M.W. van der Helm, O.Y.F. Henry, A. Bein, T. Hamkins-Indik, M.J. Cronce, W. D. Leineweber, M. Odijk, A.D. van der Meer, L.I. Segerink, D.E. Ingber, Non-invasive sensing of transepithelial barrier function and tissue differentiation in organs-on-chips using impedance spectroscopy, Lab Chip 19 (2019) 452–463, https://doi.org/10.1039/c8lc00129d.
[177] B. Srinivasan, A.R. Kolli, M.B. Esch, H.E. Abaci, M.L. Shuler, J.J. Hickman, TEER measurement techniques for in vitro barrier model systems, J. Lab. Autom. 20 (2015) 107–126, https://doi.org/10.1177/2211068214561025.
[178] F. Mathieu, M.J. Galmier, J.F. Pognat, C. Lartigue, Influence of different calcic antagonists on the Caco-2 cell monolayer integrity or "TEER, a measurement of toxicity? Eur. J. Drug Metab. Pharmacokinet. 30 (2005) 85–90, https://doi.org/10.1007/BF03226412.
[179] T. Mukherjee, E. Squillantea, M. Gillespieb, J. Shao, Transepithelial electrical resistance is not a reliable measurement of the caco-2 monolayer integrity in transwell, Drug Deliv. J. Deliv. Target. Ther. Agents. 11 (2004) 11–18, https://doi.org/10.1080/10717540490280345.
[180] A.S. Tang, P.J. Chikhale, P.K. Shah, R.T. Borchardt, Utilization of a human intestinal epithelial cell culture system (Caco-2) for evaluating cytoprotective agents, Pharm. Res. An Off. J. Am. Assoc. Pharm. Sci. 10 (1993) 1620–1626, https://doi.org/10.1023/A:1018976804403.
[181] C.F. Lee, T.R. Yan, T.H. Wang, Long-term monitoring of Caco-2 cell growth process using a QCM-cell system, Sensor. Actuator. B Chem. 166–167 (2012) 165–171, https://doi.org/10.1016/j.snb.2012.02.027.
[182] T. Prien, N. Backhaus, F. Pelster, W. Pircher, H. Büe, P. Lawin, Effect of intraoperative fluid administration and colloid osmotic pressure on the formation of intestinal edema during gastrointestinal surgery, J. Clin. Anesth. 2 (1990) 317–323, https://doi.org/10.1016/0952-8180(90)90077-G.
[183] M. Marvola, A. Reinikainen, M.L. Heliövaara, A. Huikari, The effects of some sweetening agents and osmotic pressure on the intestinal absorption of sulfafurazole in the rat, J. Pharm. Pharmacol. 31 (1979) 615–618, https://doi.org/10.1111/j.2042-7158.1979.tb13603.x.
[184] S. Ohigashi, K. Sudo, D. Kobayashi, O. Takahashi, T. Takahashi, T. Asahara, K. Nomoto, H. Onodera, Changes of the intestinal microbiota, short chain fatty acids, and fecal pH in patients with colorectal cancer, Dig. Dis. Sci. 58 (2013) 1717–1726, https://doi.org/10.1007/s10620-012-2526-4.
[185] H. Hayashi, K. Suruga, Y. Yamashita, Regulation of intestinal Cl −/HCO 3 − exchanger SLC26A3 by intracellular pH, Am. J. Physiol. Physiol. 296 (2009) C1279–C1290, https://doi.org/10.1152/ajpcell.00638.2008.
[186] B. Acta, F. Generale, C. Biologica, S. Unioersity, (Received May 3rd, 1976), 455 (1976) 759–766.
[187] X. Wang, J. Xiao, H. Zhou, Y. Qiu, H. Peng, Y. Sun, J. Zhang, W. Qian, L. Sun, Simultaneous determination of eight B-vitamins in rat intestinal perfusate to identify effects of osmotic pressures on absorptions, Biomed. Chromatogr. 31 (2017), https://doi.org/10.1002/bmc.3952.
[188] N.I. McNeil, The effect of PH on rectal absorption in ulcerative proctocolitis, Clin. Sci. 66 (2015), https://doi.org/10.1042/cs066009p, 9P.1-9P.
[189] X.J. Wang, J.H. Feng, M.H. Zhang, X.M. Li, D.D. Ma, S.S. Chang, Effects of high ambient temperature on the community structure and composition of ileal microbiome of broilers, Poultry Sci. 97 (2018) 2153–2158, https://doi.org/10.3382/ps/pey032.
[190] A. Bredel-Geissler, U. Karbach, S. Walenta, L. Vollrath, W. Mueller-Klieser, Proliferation-associated oxygen consumption and morphology of tumor cells in monolayer and spheroid culture, J. Cell. Physiol. 153 (1992) 44–52, https://doi.org/10.1002/jcp.1041530108.
[191] F. Kallinowski, G. Tyler, W. Mueller-Klieser, P. Vaupel, Growth-related changes of oxygen consumption rates of tumor cells grown in vitro and in vivo, J. Cell. Physiol. 138 (1989) 183–191, https://doi.org/10.1002/jcp.1041380124.
[192] Y. Isobe, A. Sudo, T. Endo, Communications to the editor, Macromolecules 39 (2006) 7783–7785.
[193] M. Jie, H. Lin, Z. He, H. Liu, H. Li, J.M. Lin, An on-chip intestine-liver model for multiple drugs absorption and metabolism behavior simulation, Sci. China Chem. 61 (2018) 236–242, https://doi.org/10.1007/s11426-017-9167-0.
[194] A. Prosperini, A. Juan-García, G. Font, M.J. Ruiz, Beauvericin-induced cytotoxicity via ROS production and mitochondrial damage in Caco-2 cells, Toxicol. Lett. 222 (2013) 204–211, https://doi.org/10.1016/j.toxlet.2013.07.005.
[195] K.A. Kim, J.Y. Kim, Y.A. Lee, K.J. Song, D. Min, M.H. Shin, NOX1 participates in ROS-dependent cell death of colon epithelial Caco2 cells induced by Entamoeba histolytica, Microb. Infect. 13 (2011) 1052–1061, https://doi.org/10.1016/j.micinf.2011.06.001.
[196] D. Zhang, W.G. Humphreys, H. Zhang, C.E. Frost, Z. Yu, S. Chen, D. Pinto, S. Bonacorsi, K. He, P.C. Wong, N. Raghavan, Apixaban metabolism and pharmacokinetics after oral administration to humans, Drug Metab. Dispos. 37 (2008) 74–81, https://doi.org/10.1124/dmd.108.023143.
[197] J. Stangier, Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate, Clin. Pharmacokinet. 47 (2008) 285–295, https://doi.org/10.2165/00003088-200847050-00001.
[198] I.A.M. De Graaf, P. Olinga, M.H. De Jager, M.T. Merema, R. De Kanter, E.G. Van De Kerkhof, G.M.M. Groothuis, Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies, Nat. Protoc. 5 (2010) 1540–1551, https://doi.org/10.1038/nprot.2010.111.
[199] G.J. Mahler, M.B. Esch, R.P. Glahn, M.L. Shuler, Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity, Biotechnol. Bioeng. 104 (2009) 193–205, https://doi.org/10.1002/bit.22366.
[200] H. L, Human in vivo regional intestinal permeability: importance for pharmaceutical drug development, Mol. Pharm. 11 (2014) 12–23, https://doi.org/10.1021/mp4003392LK. http://resolver.ebscohost.com/openurl?sid=EMBASE&issn=15438384&id=doi:10.1021%2Fmp4003392&atitle=Human+in+vivo+regional+intestinal+permeability%3A+Importance+for+pharmaceutical+drug+development&stitle=Mol.+Pharm.&title=Molecular+Pharmaceutics&volume=11&issue=1&spage=12&epage=23&aulast=Lennern%C3%A4s&aufirst=Hans&auinit=H.&aufull=Lennern%C3%A4s+H.&coden=MPOHB&isbn=&pages=12-23&date=2014&auinit1=H&auinitm=.
[201] V.N.S.K. Varma, H.G. Shivakumar, V. Balamuralidhara, M. Navya, U. Hani, Development of pH sensitive nanoparticles for intestinal drug delivery using chemically modified guar gum co-polymer, Iran, J. Pharm. Res. 15 (2016) 83–94.
[202] D.F. Williams, On the mechanisms of biocompatibility, Biomaterials 29 (2008) 2941–2953, https://doi.org/10.1016/j.biomaterials.2008.04.023.
[203] J. Lu, M. Liong, Z. Li, J.I. Zink, F. Tamanoi, Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals, Small 6 (2010) 1794–1805, https://doi.org/10.1002/smll.201000538.

[204] R.D. Mcdowall, S. Spector, V.P. Shah, I.J. McGilveray, T. Layloff, C. T. Viswanathan, A. Yacobi, K.A. Pittman, J.P. Skelly, K.K. Midha, S. Dighe, C. Edgar Cook, Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies, J. Pharmaceut. Sci. 81 (2006) 309–312, https://doi.org/10.1002/jps.2600810324.

[205] D.R. Friend, Drug delivery to the small intestine, Curr. Gastroenterol. Rep. 6 (2004) 371–376, https://doi.org/10.1007/s11894-004-0052-z.

[206] M. Bokhari, R.J. Carnachan, N.R. Cameron, S.A. Przyborski, Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge, J. Anat. 211 (2007) 567–576, https://doi.org/10.1111/j.1469-7580.2007.00778.x.

[207] X. Deng, G. Zhang, C. Shen, J. Yin, Q. Meng, Hollow fiber culture accelerates differentiation of Caco-2 cells, Appl. Microbiol. Biotechnol. 97 (2013) 6943–6955, https://doi.org/10.1007/s00253-013-4975-x.

[208] H. Kimura, T. Ikeda, H. Nakayama, Y. Sakai, T. Fujii, An on-chip small intestine–liver model for pharmacokinetic studies, J. Lab. Autom. 20 (2015) 265–273, https://doi.org/10.1177/2211068214557812.

[209] K.L. Audus, M.L. Avery, C.G. Oster, M.M. Mayer, K.A. Foster, Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism, Exp. Cell Res. 243 (2002) 359–366, https://doi.org/10.1006/excr.1998.4172.

[210] I. Maschmeyer, A.K. Lorenz, K. Schimek, T. Hasenberg, A.P. Ramme, J. Hübner, M. Lindner, C. Drewell, S. Bauer, A. Thomas, N.S. Sambo, F. Sonntag, R. Lauster, U. Marx, A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents, Lab Chip 15 (2015) 2688–2699, https://doi.org/10.1039/c5lc00392j.

[211] M.W. Toepke, D.J. Beebe, PDMS Absorption of Small Molecules and Consequences in Microfluidic Applications, 2006, pp. 1484–1486, https://doi.org/10.1039/b612140c.

[212] M.T. Nelson, V. Shukla, W. Zhang, Using a novel microfabricated model of the alveolar-capillary barrier to investigate the effect of matrix structure on atelectrauma, Sci. Rep. (2017) 1–13, https://doi.org/10.1038/s41598-017-12044-9.

[213] A. Datar, P. Joshi, M. Lee, Biocompatible Hydrogels for Microarray Cell Printing, 2015, pp. 647–663, https://doi.org/10.3390/bios5040647.

[214] Polymer Coating on a Micropillar Chip for Robust Attachment of PuraMatrix Peptide Hydrogel for 3D Hepatic Cell Culture, 2018.

[216] Y. Chen, W. Zhou, T. Roh, M.K. Estes, D.L. Kaplan, In vitro enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses, PloS One 12 (2017) 1–20, https://doi.org/10.1371/journal.pone.0187880.