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Intestine-on-a-chip: Next level *in vitro* research model of the human intestine

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Abstract

Over the past decade, microfluidic intestine-on-a-chip models have been emerging as a novel platform to study intestinal function in health and disease. These microphysiological systems surpass conventional *in vitro* intestinal model systems, as they add microenvironmental context in the form of mechanical cues or by the incorporation of multiple cell types and/or gut microbiome, thereby better reflecting intestinal architecture and physiology. This review summarizes the current intestine-on-a-chip models with a distinction between cell- or organoid-based models and models that apply *ex vivo* tissue biopsies, as well as describing the progress and hurdles to overcome when applying intestine-on-a-chip models to study host-microbe interactions and intestinal diseases.

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Keywords

Intestine-on-a-chip, Organ-on-a-chip, Microfluidics, Host-microbe, Inflammatory bowel disease.

Abbreviations

CYP3A4, cytochrome P450 3A4; DSS, dextran sodium sulfate; ECM, extracellular matrix; FITC, fluorescein isothiocyanate; IBD, inflammatory bowel disease; iPSC, induced pluripotent stem cells; PBMCs, peripheral blood mononuclear cells; PDMS, polydimethylsiloxane; TEER, transepithelial electrical resistance.

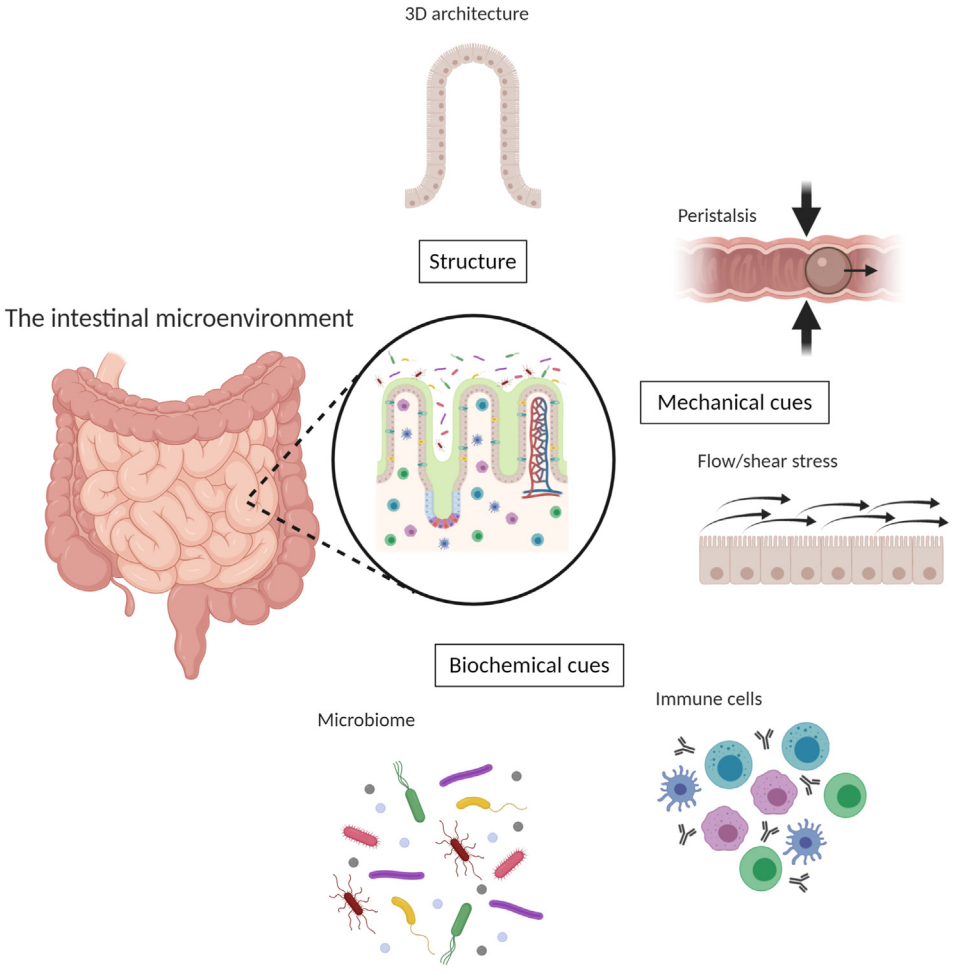
Introduction

The digestive environment of the gut lumen is separated from the body by a protective epithelial barrier that consists of multiple cell types with specialized functions in nutrient and drug absorption, transport and metabolism, mucus production, and the secretion of hormones or cytokines in response to food or environmental factors [1]. Furthermore, a large community of microbes inhabits the gut wall which contributes significantly to intestinal homeostasis by producing factors such as vitamin K and short chain fatty acids, and they can also play an important role in the metabolism of certain drugs [2]. To understand the intestinal barrier function and be able to model drug absorption, a diverse range of *in vitro* and *ex vivo* models are being used. Models applying *ex vivo* tissue, such as the everted sac model [3], Ussing chamber [4,5], and InTESTine™ [6,7] are physiologically highly relevant, but these models often have limited throughput and lifespan (maximum of 6–8 h). Therefore, most *in vitro* research into intestinal barrier function and drug absorption is performed using human intestinal epithelial cell lines, such as Caco-2 and HT-29 cells [8,9], or intestinal 3D-organoid structures derived from primary intestinal crypts [10]. Although the latter better reflects the intestinal architecture and cell type diversity than the monocellular cell line cultures, supporting cells and tissue parts of the normal intestinal wall, such as blood vessels and immune cells, are lacking in intestinal organoids. Moreover, access to the lumen of the organoid is technically challenging, therefore limiting the ability to study critical intestinal barrier functions [10]. Furthermore, the static nature of traditional cell culture on a dish or Transwell is in sharp contrast to the living intestine which endures peristaltic movements and has a continuous supply of nutrients and oxygen and removal of waste products [11,12]. Microfluidic organ-on-a-chip models overcome (some of) these limitations and provide relevant microenvironmental context that brings *in vitro* intestinal research to a next level (Figure 1). This review describes the current intestine-on-a-chip models and reflects on novel developments to use these microphysiological systems to study gut barrier function, intestinal diseases, and host-microbiome interactions.

Cell- vs. tissue-based models

First designs of intestine-on-a-chip microfluidic devices were fabricated using multilayer soft lithography to create PDMS (polydimethylsiloxane, a silicon-based polymer) chips consisting of two chambers separated

Figure 1



Key aspects of the intestinal microenvironment. In order to represent the physiology of the intestine in health and disease *in vitro* it is important to stimulate the physical properties of the intestinal microenvironment, the 3D architecture and mechanical cues, and to provide cellular and biochemical cues normally present in the vicinity of the intestinal wall.

by a porous semipermeable membrane on which seeded epithelial cells formed a barrier to separate the apical and basolateral compartments [13–15]. Medium flow ensures appropriate nutrient distribution and the removal of waste products, as well as providing mechanical cues essential for normal intestinal function [16]. Although these first designs permitted polarized, apical to basolateral, membrane transport of (fluorescently labeled) molecules [13–15], subsequent designs focused on the incorporation of more environmental context to mimic the *in vivo* setting more closely. For example, Sung et al. [17] designed a 3D-hydrogel villi-scaffold using calcium alginate and collagen and integrated it into a microfluidic device with a gravity-driven flow [17,18]. They showed that Caco-2 cells grown on this scaffold have enhanced metabolic enzymatic activity of CYP3A4 and aminopeptidase compared to 2D-cultures in Transwell or the chip without villi-scaffold [19]. Interestingly, Caco-2 cells, biopsy-derived organoid structures, and iPSC-derived intestinal organoids could also spontaneously form villi-like structures when exposed to mechanical cues. These villi structures consist of different types of differentiated epithelial cells (absorptive, mucus-secretory, entero-endocrine, and Paneth cells) along a traditional crypt-villus axis with proliferative cells located at the bottom of the crypt and differentiated cells located along the side and top of the villus [11,20–24]. Mechanical cues provided by the fluid flow were indispensable for the villi formation and also shortened the time needed for Caco-2 cells to differentiate [11]. Furthermore, with microfluidics barrier integrity and proper tight junction functioning, measured by trans-epithelial electrical resistance (TEER) or permeability of compounds transported via the paracellular route, were well-established and improved compared to static

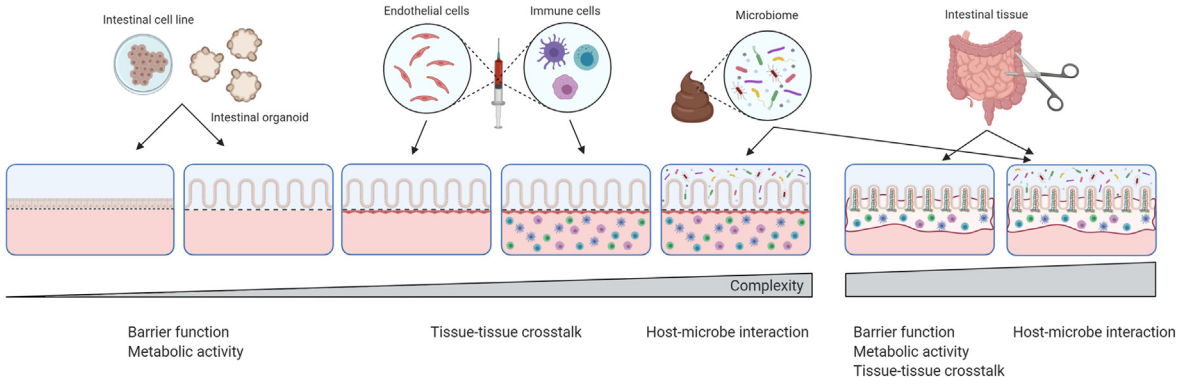
Caco-2 Transwell cultures, as well as other normal intestinal functions such as mucus production and enzymatic activity of CYP3A4 [11,22–24]. The impact of fluid shear stress on cell, and in particular Caco-2 cell, differentiation was recently investigated in more detail using a Hele-Shaw microfluidic device [25,26]. In this device the flow shear stress in a single fluidic channel linearly decreases from the inlet to the outlet, thereby creating 5 different sections with different levels of shear stress. Five days post-seeding, Caco-2 cells demonstrated the highest level of mucus production, metabolic enzyme expression, mitochondrial activity, microvilli, vacuoles, and a denser actin-network at the highest level of shear stress. However, tight junction protein expression was highest at the second and third level of shear stress. These results indicate that the optimal flow shear stress in intestine-on-a-chip models can vary depending on the intestinal function that is aimed to be studied. A second mechanical cue was provided by the PDMS chip designed by the Ingber group, by inducing peristaltic-like motions of a porous membrane by cyclic application of vacuum to hollow side chambers [11,27]. The addition of cyclic strain had no additional effect on intestinal cell maturation/differentiation, but did increase the expression of proteins in lipid and carbohydrate metabolism as well as specific signaling pathways [28]. In addition, increased expression levels of proteins involved in apoptosis and oxidative phosphorylation might hint toward fluid flow–induced injury. The most recent designs of this PDMS chip also incorporate human intestinal microvascular endothelial cells to create an organ-level model with a tissue-tissue interface [22,23,29,30]. So far with this technology, the Gut Chip using Caco-2 cells [11,20], the (Small) Intestine Chip using duodenum-derived organoids [22,29], the Jejunum-Intestine-Chip using jejunal human enteroids [28], and the Colon Chip or Colon Intestine-Chip using biopsy specimens from the sigmoid and ascending colon have been presented [[31–33]] (Table 1). The value of combining the intestinal epithelium cells with an endothelium model is also recognized by other research groups, with different sources of endothelial cells [34]. In contrast to the artificial porous membrane used to seed cells or organoid-like structures in many of the microfluidic intestine-on-a-chip devices, extracellular matrix (ECM) hydrogels were used to separate Caco-2 cells or iPSCs differentiating into a gut monolayer from a flow channel in a multi-well plate with a gravity-driven flow and the capacity to culture 40 microfluidic cell culture structures in parallel, called the OrganoPlate [35,36]. Under flow, the cells formed tubes lining all surfaces of the perfusion channel which showed proper barrier integrity that was prone to disruption by drugs, and responsiveness to an inflammatory trigger [35,36]. In an alternative approach to create tube-like structures, Caco-2 cells were cultured in a hollow fiber membrane setup [37,38]. With a high emphasis on shear stress, these researchers showed that pump-driven unidirectional flow improved differentiation and shortened the time needed for the cells to form a tight monolayer. Moreover, it was shown that this unidirectional flow resulted in tighter monolayers compared to gravity driven bidirectional flow which on itself already induced the formation of villi-like structures. Like the other cell-based intestine-on-a-chips, intestinal functions such as barrier integrity and metabolic capacity could be assessed on these hollow fiber membrane devices.

These studies show the importance of mimicking the architectural structure and dynamic microenvironment of the *in vivo* intestine to study normal intestinal function *in vitro*. Nevertheless, cell- or organoid-based models represent only the intestinal epithelium and therefore miss a broad range of other cell types, such as immune and connective tissue cells, that are important for normal intestinal physiology [39]. Although much closer to the *in vivo* structure and architecture, only a handful of microfluidic models use *ex vivo* intestinal tissue over the past decade. Midwoud et al. [40] applied microfluidics to rat intestinal tissue slices which retained their viability and metabolic activity up to 8 h, but did not provide a separate medium flow to the apical and basolateral side of the tissue. Sustained viability

Table 1 Intestine-on-a-chip names explained.

Model	Intestinal cell or tissue type	Founding institute or company	First citation
Gut Chip	Caco-2	Wyss Institute at Harvard University	[11]
(Small) Intestine Chip	Duodenum organoids	Wyss Institute at Harvard University	[22]
Jejunum-Intestine-Chip	Jejunal enteroids	John Hopkins University School of Medicine	[28]
Colon Chip	Colon organoids	Wyss Institute at Harvard University	[31]
Colon Intestine-Chip	Colon organoids	Emulate Inc.	[33]
OrganoPlate®	Caco-2, HT29-MTX-E12, iPSC-derived	MIMETAS	[35]
HMI™	Caco-2	Ghent University	[50]
HuMiX	Caco-2	University of Luxembourg	[51]
PMI Chip	Caco-2	The University of Texas at Austin	[53]
GuMI	Colon organoids	Massachusetts Institute of Technology	[54]

Figure 2



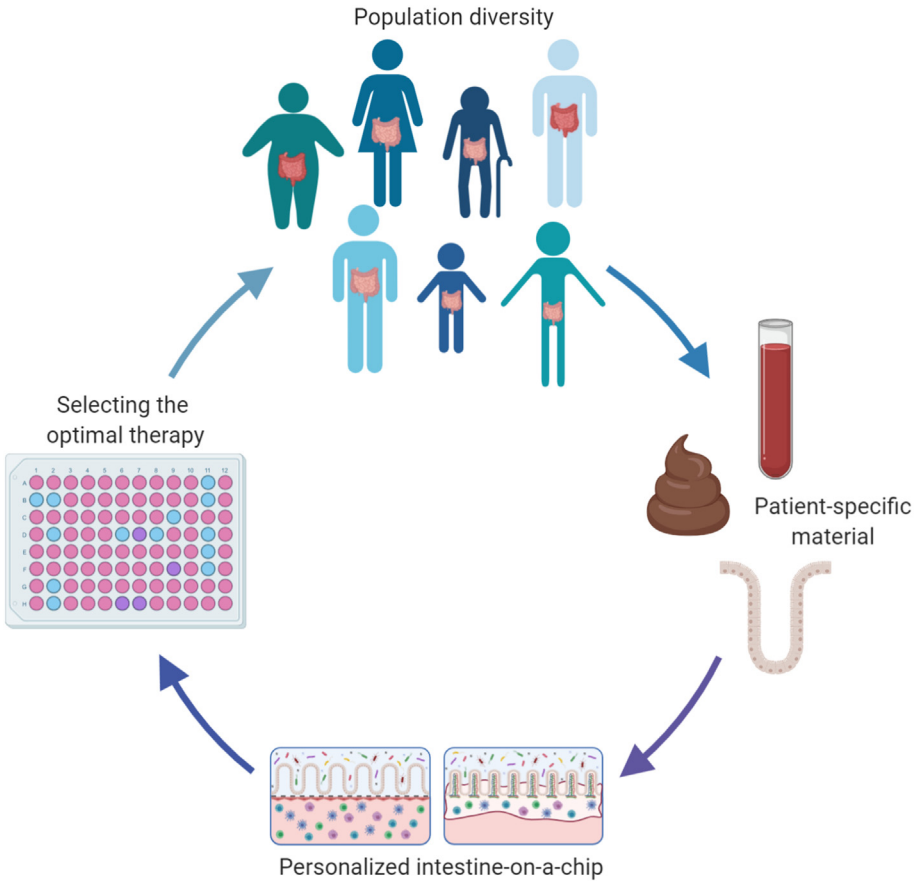
Cell- vs. tissue-based intestine-on-a-chip models. From a relatively simple cell- or organoid-based intestine-on-a-chip model with intestinal cells cultured in a 2D monolayer toward a more complex one with 3D architecture of the intestinal cells, co-culture with multiple cell types and including the gut microbiome. Tissue-based intestine-on-a-chip models have an intact tissue architecture, which include vasculature and multiple cell types, and by adding the gut microbiome even more complexity can be achieved. Additional intestinal functions can be studied by increasing the complexity of the intestine-on-a-chip models.

≥24 h was shown in subsequent studies by research groups that used one or multiple tissue biopsies loosely placed on a porous membrane in a microfluidic device perfused with culture medium from the basolateral side only [41,42]. By design, these *ex vivo* tissue models are not suitable to study intestinal permeability, in contrast to cell-based microfluidic models with a main focus on the formation of a tight intestinal barrier. Proper barrier integrity is not only needed to study intestinal permeability of drugs or nutrients, but is also crucial in studies evaluating the impact of the microbiome, which is only present in the gut lumen. Therefore, microfluidic devices were developed that keep the tissue in place as a barrier between the apical and basolateral compartment with adhesives such as glue or tape [43,44]. Both models provide perfusion to both sides of the tissue biopsy and show an intact barrier by using FITC-labeled molecules of high molecular weight. In another approach short (3–4 cm) intact intestinal fragments were mounted on needles fixed on holders that could facilitate dual-perfusion [45]. Although an interaction was shown between two different microbes, that were administered to the gut lumen with neuronal and immune cells, the device was developed for intestinal fragments of young mice (day 18 prenatal to 14 days old) and therefore not applicable for intestinal tissue from humans or other species.

Interaction with the gut microbiome

Dysbiosis of the commensal gut microbiome is associated with the development of disorders such as inflammatory bowel disease (IBD), colorectal cancer, diabetes, and obesity [46,47]. Despite this knowledge, understanding the molecular crosstalk between microbes and their host has remained largely elusive. Whereas bacteria do overgrow epithelial cells in static *in vitro* models within a few hours, the dynamic microenvironment of intestine-on-chips stimulates the formation of a protective mucus layer and supports co-culture with vascular and immune cells in adjacent channels [20,22]. In successful long-term co-cultures of Caco-2 cells and probiotic bacteria, inflammation could be triggered by introducing peripheral blood mononuclear cells (PBMCs) in the vascular channel and pathogenic bacteria in the upper channel of the chip [11,30,48]. To study *Shigella flexneri* infection *in vitro*, the Intestine Chip microenvironment with mechanical forces and crypt-villus architecture proved to be indispensable for infecting Caco-2 cells [49]. Nevertheless, the aerobic environment in these experiments contrasts with the low oxygen levels *in vivo* (<1% in human colon) and does not facilitate the study of the strict anaerobic bacteria that are a big part of our gut microbiome. The HMI module [50] and the HuMiX device [51] were first examples of microfluidic chips with an aerobic-anaerobic interface in which the anaerobic medium containing facultative or strict anaerobic bacteria was separated from the Caco-2 cells and aerobic cell culture medium with a semi-porous membrane was coated with mucus. An even more direct interaction between bacteria and Caco-2 cells, using the Intestine Chip, was recently achieved by Jalili-Firoozinezhad [52] who co-cultured obligate anaerobes directly on top of Caco-2 cells, covered with their self-produced protective mucus layer, up to 5 days. Efficacious aerobic-anaerobic interface co-cultures were also established using a complex human gut microbiota isolated from fresh human stool samples and primary human intestinal epithelial cells [52] or patient-derived organoids [53]. In a different device called GuMI (gut microbiome), Zhang et al. [54], were the first to show long-term (2 days) co-culture of primary human colon cells with the super oxygen-sensitive

Figure 3



Precision medicine based on personalized intestine-on-a-chip models. In this approach, patient-derived blood and intestinal tissue biopsies will be used to create personalized intestine-on-a-chip models with patient-specific immune, vascular, and intestinal cells in co-culture with microbiome isolated from the patient's stool. Effective therapeutic strategies can subsequently be determined by screening selections of candidate drugs. The lead compound will then be sent back to the clinic for use in the patient.

commensal anaerobe *Faecalibacterium Prausnitzii* and its anti-inflammatory effects on colon epithelium gene expression. Although without aerobic-anaerobic interface, the first fungal host-microbe interaction was recently demonstrated in an immunocompetent model with 4 different cell types (i.e., endothelial and Caco-2 cells, macrophages, and PBMCs) [55]. Interestingly, host-to-microbe effects and microbe-to-host effects were demonstrated in another recent publication to enterohemorrhagic *Escherichia coli* (EHEC) bacteria incubated with human or mouse microbial metabolites in the Colon Chip [[31]]. So by selecting specific microbes and studying their impact on host cells, and in some cases also the other way around, these studies reveal first hints toward pathways in intestinal host-microbe interactions important for a healthy, but also diseased, intestine. However, as in most intestinal diseases more factors than microbiome dysbiosis play a role, these disease-specific features have to be incorporated in intestine-on-a-chip models as well.

Towards models of inflammatory bowel disease

Thus far, most intestine-on-a-chip models find common ground in mimicking the *in vivo* gut as closely as possible by establishing an effective leak-tight barrier and creating the right microenvironment. However, in many intestinal diseases this barrier is impaired leading to a so-called "leaky" gut and intestine-on-a-chip models used to study such disorders need to adjust accordingly. Administration of pro-inflammatory cytokines to induce inflammation-induced intestinal barrier leakiness seems the preferred option of many research groups to create an intestine-on-a-chip model of IBD. Cytokine TNF-α seems to be the common denominator in these so-called pro-inflammatory cytokine cocktails, in combination with IL-1β [56], with IL-1β, IL-6, and IL-8 [30], with IL-1β and IFN-γ [57], or in combination with lipopolysaccharide (LPS) [58]. Whereas initial studies were performed with monocultures of Caco-2 cells [30,57,58], the more recent models have incorporated

immune cells in the basolateral channel or ECM or use co-cultures of Caco-2 cells with other intestinal cell types. In combination with THP-1 and MTZ-3 immune cells, pro-inflammatory cytokine treatment (TNF-α and IL-1β) in the apical and basolateral channel of the OrganoPlate decreased barrier and inflammation in Caco-2/HT29-MTX-E12 tubuli [56]. Decreased barrier integrity of Caco-2 tubules in the OrganoPlate was also achieved when co-cultured with LPS-activated THP-1 cells in the ECM, leading to neutrophil infiltration [59]. Shin and Kim [48] adopted a different strategy and followed the use of dextran sodium sulfate (DSS) to induce colitis, a type of IBD, in mouse models. Two-day treatment of Caco-2 villi in a microfluidic chip with 2% DSS significantly decreased barrier integrity as measured by TEER and FITC-dextran permeability as well as decreasing villus height and disrupting the mucus layer without causing epithelial cytotoxicity [48]. Pretreatment with 8 probiotic bacterial strains could maintain the intestinal barrier during DSS administration, but not when the epithelium was challenged with DSS before adding the probiotic bacteria, consequently leading to translocation of bacteria through the damaged epithelial layer [48]. Patient-specific biopsy-derived organoids or iPSCs, which retain their diseased morphology *ex vivo*, are also used to recreate the IBD-phenotype on-a-chip [53,60]. Using their physiodynamic mucosal interface-on-a-chip (PMI Chip) cultured with biopsy-derived intestinal epithelial organoids from Crohn disease (CD) and ulcerative colitis (UC) patients, Shin et al. [53] successfully demonstrated that the formed epithelial layers of these two IBD types retained their 3D morphology and expression of epithelial markers such as MUC2, a mucus protein, in agreement with IBD patient data. Up to date, the most complex model of IBD-on-a-chip was established by Trapecar et al. [60] who created a gut–liver–immune axis in a multi-organ chip with primary liver, gut, and circulating immune cells and studied the effect of short chain fatty acids (SCFA) on IBD-related inflammation. In the absence of adaptive immune cells, administration of SCFA to the apical side of an UC-derived intestinal epithelial layer in the gut compartment of the multi-organ chip reduced the intestinal innate immune response and improved hepatic metabolism. Interestingly, however, is that increased inflammation, gut barrier disruption, and decreased hepatic function were noted when activated CD4+ T cells were co-administered with SCFA in all compartments of the multi-organ chip [60]. These opposing results for SCFA show the usefulness of such a complex system that explores context-dependent interactions in the gut–liver–immune axis.

Multi-organ chips

Besides studying organ crosstalk in health and disease conditions, the combination of liver and intestinal cells on a multi-organ chip can also be attractive for pharmacokinetic profiling of drugs, as the combination of the main organs involved in drug absorption and metabolism will increase *in vitro* to *in vivo* translation. However, so far only a few research groups that combined intestine and liver cells on multiple-organ chips assessed the pharmacokinetic profile of the administered drug [61–63]. In fact, most studies combining intestinal cells with other organs on multi-organ chips primarily focus on the treatment of tumor cells with anti-cancer reagents [61,64–66]. Although these studies provide promising data, the complexity of co-culturing and connecting multiple organ-compartments on one microfluidic device seems to attenuate wide-scale adoption of multi-organ chips in the organ chip research field.

Conclusions and future perspectives

With the ability to mimic the *in vivo* intestinal architecture and microenvironment more closely than the conventional *in vitro* culture systems, microfluidic intestine-on-a-chip devices offer a novel and more realistic approach to study intestinal function and pathology (Figure 2). Although each intestine-on-a-chip model has its own advantages and limitations, there are some common hurdles that need to be considered. Firstly, correct representation of the intestinal wall remains a challenge as cell- or organoid-based intestine-on-a-chip systems lack diverse cell types and supportive tissue structures. While several researchers have shown that the addition of multiple cell types is possible, their platforms remain complex, labor-intensive and by choosing one cell type over the other cell-based models still miss out important factors. Using *ex vivo* tissue will bypass this problem, but as it is difficult to apply in a leak-tight manner and highly limited by available (human) donor material, so far not many *ex vivo* intestine-on-a-chip models have been developed. Secondly, the chip material can have a big impact when the intestine-on-a-chip models are used for drug response bioassays. Used in many organ-on-a-chip models and loved for its flexibility, transparency, and easy manufacturing, PDMS adsorbs a wide range of molecules [67,68] and intestine-on-a-chip models made of this material are therefore less attractive for studying compound transport or toxicology studies [69]. Hence, investments in alternative materials such as glass [70] or coatings [71] may be useful. Besides the chip material, using ECM in organ-on-a-chip devices can alter the efficacy or mechanism of action of a drug by the composition and stiffness of the matrix [72]. Thirdly, it is an open secret that the throughput of almost all the models is currently limited to running a few chips in parallel and users need training to learn highly specific and delicate techniques. This is an aspect that requires attention in the coming period. Conclusively, although it can take some time before the current or future intestine-on-a-chip models find their way to research labs world-

wide, the great potential of these microphysiological systems of the human intestine has already become clear. We believe that novel developments in this field will direct future research into intestinal function and provide a platform to yield precision medicine based on patient-derived cells or tissue biopsies and their microbiome (Figure 3).

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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* of special interest
** of outstanding interest

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