

# Emulating Host-Microbiome Ecosystem of Human Gastrointestinal Tract in Vitro

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**Abstract** The human gut microbiome performs prodigious physiological functions such as production of microbial metabolites, modulation of nutrient digestion and drug metabolism, control of immune system, and prevention of infection. Paradoxically, gut microbiome can also negatively orchestrate the host responses in diseases or chronic disorders, suggesting that the regulated and balanced host-gut microbiome crosstalk is a salient prerequisite in gastrointestinal physiology. To understand the pathophysiological role of host-microbiome crosstalk, it is critical to recreate in vivo relevant models of the host-gut microbiome ecosystem in human. However, controlling the multi-species microbial communities and their uncontrolled growth has remained a notable technical challenge. Furthermore, conventional two-dimensional (2D) or 3D culture systems do not recapitulate multicellular microarchitectures, mechanical dynamics, and tissue-specific functions. Here, we review recent advances and current pitfalls of in vitro and ex vivo models that display human GI functions. We also discuss how the disruptive technologies such as 3D organoids or a human organ-on-a-chip microphysiological system can contribute to better emulate host-gut microbiome crosstalks in health and disease. Finally, the medical and pharmaceutical significance of the gut microbiome-based personalized interventions is underlined as a future perspective.

**Keywords** Gut microbiome · In vitro model · Co-culture · Organoids · Organ-on-a-chip

## Introduction

In 2008, the United States National Institutes of Health (NIH) launched the “Human Microbiome Project (HMP)” to identify and characterize the physiological functions of microorganisms in human body [1]. Although “microbiology” is one of the oldest disciplines in life sciences, we still have not clearly answered questions such as, “Why do we fail to grow ~99% of soil microbes in the laboratory?” or “How can we stably maintain multi-species microbial communities in vitro?”. Here, we add one more tricky question, “What is the role of human the gut microbiome in gastrointestinal (GI) homeostasis and diseases?” Indeed, there have been burgeoning interests in the human gut microbiome and its key functions in metabolic (e.g. diabetes [2]), inflammatory (e.g. Crohn’s disease [3]), and neurodegenerative diseases (e.g. Parkinson’s disease [4]). Specifically, in cellular and molecular context, pivotal modulatory effects of the gut microbiome germane to the regulation of immune system and local barrier functions have not been fully understood [5]. In xenobiotic metabolism, gut microbiome can alter drug absorption, metabolism, and pharmacokinetics, which eventually influences the efficacy and toxicity of the drug [6]. Hence, it is obvious that the study of human gut microbiome is of great significance and impact. Nevertheless, how can we demonstrate host-gut microbiome interactions in the laboratory?

To explore the microbial signatures in human health, microbial metagenomic pools have been predominantly investigated by using high-throughput next-generation sequencing

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(NGS) techniques [7] in coordination with various bioinformatics tools [8]. This approach mostly depends on massive sample collections in epidemiological aspects via cohort studies [9, 10]; but these metagenomic results never distinguish culturable versus unculturable microbes and their symbiotic communities [11]. Hence, although metagenomic databases provide a whole catalog of the species-level microbial signature, this approach is inherently limited in dictating the metabolic function of microbial cells and the connectivity of syntrophic microbial ecosystem [12]. Most importantly, metagenomic approaches only focus on the microbial genomic backgrounds, by which the information of inter-dependent interactions with host tissues is considerably biased [13].

To scrutinize the role of microbial communities in human physiology and pathology, there have been multiple experimental models to recapitulate host-microbiome interactions using in vitro cell/tissue culture techniques, ex vivo explants or biopsies, and in vivo animal surrogates. This review will discuss the pros and cons as well as the relevance and applications of established experimental models in vitro and ex vivo, where we particularly focus on models of the GI tract as they have been predominantly studied (Table 1). Finally, we address the therapeutic potential of recently developed 3D organoid cultures and organ-on-a-chip systems that have been designed to reconstitute host-gut microbiome interactions.

**Table 1** Experimental models that emulate host-microbiome interactions in the human GI tract

Models	Culture format	Target tissues and cells	Microbial species	References	
In vitro	2D model	Well plate	Mouth (gingival epithelium; Ca9–22, HGPEC, OLF6-TERT2)	<i>Fusobacterium nucleatum</i> , <i>Porphyromonas gingivalis</i> , <i>Aggregatibacter actinomycetemcomitans</i> <i>Streptococcus mitis</i>	[20]
		Centrifuge tube	Intestine (Caco-2)	<i>Faecalibacterium prausnitzii</i>	[21]
		Transwell	Intestine (Caco-2)	Non-pathogenic <i>E. coli</i> , <i>Lactobacillus sakei</i> , <i>L. johnsonii</i> , and enteropathogenic <i>E. coli</i>	[44]
	3D model	Rotating wall vessel with microbeads	Intestine (HT29, HT-29-16E)	<i>Salmonella enterica serovar typhimurium</i> , <i>Brachyspira</i> sp., and <i>Lactobacillus</i> sp.	[57]
		Host-Microbiota Interaction (HMI™) module	Intestine (Caco-2)	LGG, fecal microbiome	[137]
		3D silk fibroin protein scaffold	Intestine (Caco-2, HT29-MTX, primary human intestinal myofibroblasts)	<i>Yersinia pseudotuberculosis</i> and LGG	[67]
Ex vivo model	Organoids	Stomach (iPS cells)	<i>Helicobacter pylori</i>	[72]	
	Cell culture dish with sponge or agar disk	Intestine (duodenal mucosal biopsy from peptic ulcer disease patients)	Enterotoxigenic <i>E. coli</i> ,	[80]	
	Transwell	Intestine (ileum biopsy from Crohn's disease patients)	Non-pathogenic <i>E. coli</i> , and <i>Lactobacillus casei</i>	[82]	
	Snapwell	Intestine (duodenal biopsy from intestinal disorder patients)	Enteropathogenic <i>E. coli</i>	[86]	
	Ussing chamber	Intestine (ileum biopsy from colon cancer patients)	<i>E. coli</i>	[89]	
Microfluidics system or Microphysiological system	Microfluidic device	GI tract (HeLa)	<i>E. coli</i> BW25113, and <i>E. coli</i> O157:H7	[101]	
	The HuMiX model	Intestine (Caco-2)	LGG <i>Bacteroides caccae</i>	[102]	
	Human Gut-on-a-chip	Intestine (Caco-2)	LGG, VSL#3, non-pathogenic GFP-labeled <i>E. coli</i> , and enteroinvasive <i>E. coli</i>	[15, 16, 18, 136, 138, 139]	

## Experimental Models of Host-Microbiome Interactions in the GI Tract

### In Vitro Static 2D Culture

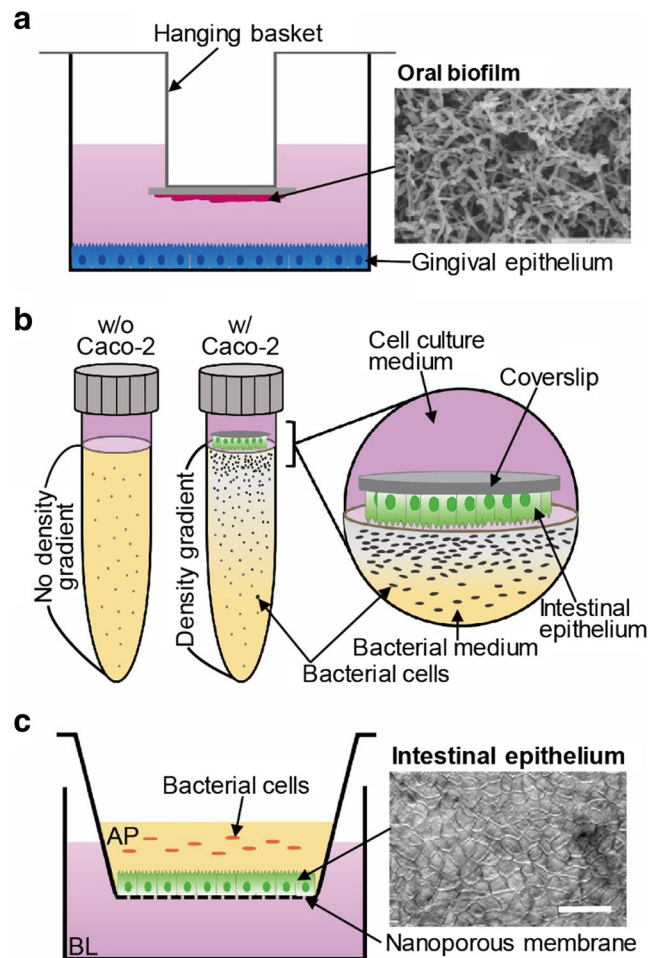
The main advantages of the 2D tissue culture system include its robustness, simplicity, and reproducibility. The 2D tissue culture models typically employ established cell lines and provide an intact monolayer of epithelium with tight junction barriers. This method, however, often fails to induce full cytodifferentiation [14, 15], tissue regeneration [15, 16], and other physiological tissue-specific functions [14, 17, 18]. Most importantly, most in vitro 2D models undergo static culture condition; thus microbial overgrowth cannot be appropriately controlled, which remarkably hampers the long-term maintenance of host-gut microbiome co-cultures. As a result, majority of studies discussed in this section have applied living microbial cells for a limited period of time (for several hours) to cultured human tissues, then eliminated the microbial population by adding bacteriostatic antibiotics [19]. The following sections summarize representative examples.

### Well Plate

In general, a well plate format has not been widely used for demonstrating host-gut microbiome interactions because it only provides the apical side of an epithelial monolayer to microbial cells in the static condition, whereas epithelial basolateral secretion is notably limited. A modified version of the well plate co-culture that uses a hanging basket has been considered to better recapitulate host-microbiome interactions (Fig. 1a), where the distance between gingival epithelium (OKF6-TERT2) and oral biofilm consisting of four pathogenic oral microbes (*Streptococcus mitis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) can be adjusted [20]. To establish a biofilm layer, those four bacterial species are seeded and grown onto a coverslip under anaerobic condition at 37 °C for three days. After a biofilm layer is fully established (Fig. 1a, a right inset), the coverslip containing the biofilm is added to the well plate pre-cultured with oral keratinocytes to induce inflammatory responses. In this study, antimicrobial (e.g. chlorhexidine) and anti-inflammatory chemicals (e.g. polyphenol resveratrol) are tested in the compartment where host-microbiome interactions occur. However, this model neither fully reconstitutes the 3D structure of oral microenvironment nor conveys dynamic cell-cell interactions.

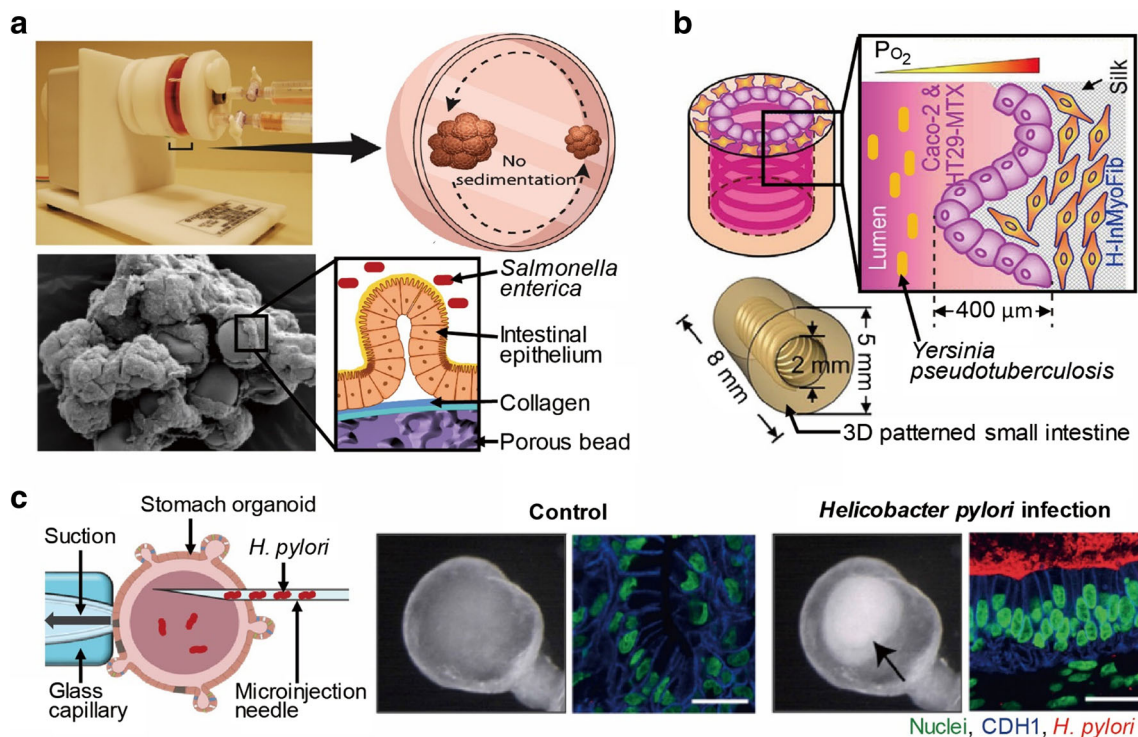
### Centrifuge Tube

A centrifuge tube loosely (air-open) or tightly (air-closed) closed may provide an aerobic-anaerobic interface to mimic host-gut microbiome interactions that happen in the gut by



**Fig. 1** Host-microbiome interactions in static 2D cultures. **a** The hanging basket co-culture model. The biofilm pre-grown on a coverslip is attached on the hanging basket to induce host-biofilm interactions with gingival epithelium up to 24 h. *Right inset* shows a scanning electron microscopic (SEM) image of the biofilm (magnification, 2000 $\times$ ). Reproduced with permission [20], 2014 BioMed Central Ltd. **b** A centrifuge tube is used to co-culture *Faecalibacterium prausnitzii* grown in solid agar medium overlaid with the Caco-2 cells cultured on the coverslip. The presence (a *right tube*) of Caco-2 cells strongly promotes *F. prausnitzii* growth in the upper layer of the bacterial medium, whereas the absence (a *left tube*) of Caco-2 cells does not. A *right inset* displays a close-up of the Caco-2-microbiome interface. **c** The Transwell culture model displaying the co-culture of intestinal epithelium (Caco-2) with bacterial cells seeded into the apical side. A DIC image (a *right inset*) shows the morphology of a Caco-2 monolayer grown on a Transwell nanoporous insert for 4 weeks. AP apical side, BL basolateral side. Bar, 50  $\mu$ m

compartmentalizing the microbial and epithelial cell side in solid agar and liquid medium, respectively (Fig. 2b) [21]. *Faecalibacterium prausnitzii* is an obligate anaerobic bacteria that contribute up to 25% of the gut microbiome in a healthy individual [22, 23]; consequently decreased population of *F. prausnitzii* is often associated with intestinal inflammation [24]. To study its role in vitro, *F. prausnitzii* cells are seeded in the solid agar medium (Fig. 2b, yellow); after 18 h of culture, microcolonies are evenly dispersed throughout the agar



**Fig. 2** The host-gut microbiome interactions demonstrated in 3D cultures. **a** The rotating wall vessel (RWV) cell culture system. A photograph of the RWV bioreactor (left top) containing circulating porous microcarrier beads (right top) lined by intestinal epithelial cells (left bottom; an SEM image) that have the 3D morphology. Continuous rotating fluid induces shear stress and stochastic collision of epithelium, which allows cells forming 3D aggregates. A schematic (right bottom) shows a scheme of infection with *Salmonella typhimurium*. Reproduced with permission [57]. 2010 licensed under a Creative Commons Attribution. **b** A microengineered human intestine model reconstituted on the 3D porous silk scaffolds. The screw patterned 3D porous scaffolds (2 mm in diameter, villi-like features with a height of 400 μm) are trimmed along the axis of the hollow cylinder with dimensions of 5 mm outer diameter, 2 mm inner diameter, and 8 mm length. A mixed

population of intestinal epithelial cells (Caco-2 and HT29-MTX) is seeded and fully polarized inside the scaffold to form intestinal lumen, where *Yersinia pseudotuberculosis* is inoculated to demonstrate infection. Reproduced with permission [67]. 2015 licensed under a Creative Commons Attribution. **c** A 3D organoid model to mimic the host-pathogen interactions. A schematic (left) shows the experimental procedure of the bacterial infection using a microinjector needle. A set of bright field (left) and immunofluorescence (right) images of human gastric organoids in the non-infected ("Control") or the infected group with *Helicobacter pylori* ("Helicobacter pylori infection"; 24 h since onset), respectively. *H. pylori* cells are tightly associated with the apical surface of the human gastric organoid epithelium. *CDH1* Cadherin-1. Bars, 100 μm. Reproduced with permission [72]. 2014 Nature Publishing Group

medium. In contrast, when *F. prausnitzii* cells are co-cultured in a close contact with Caco-2 cells grown on a coverslip (Fig. 2b, pink), clearer and bigger *F. prausnitzii* colonies appear closer to the coverslip-attached Caco-2 cells (Fig. 2b, a zoomed-in inset), both in air-closed and air-open tubes. This finding coincides well with a previous report that *F. prausnitzii* is able to grow close, and even adhere, to the intestinal epithelium, and can also survive within the mucus layer [25]. This system is relatively simple and experimentally convenient; however, the expected culture period is quite limiting because of the quick depletion of nutrient and overgrowth of bacterial cells.

### Transwell

The Transwell is the most popular culture format to grow epithelium either on the nanoporous insert or on the surface of a well plate, by which a monolayer of the polarized

intestinal epithelium (e.g. T84 [26], HT-29 [27], or Caco-2 [28, 29] intestinal epithelium) can provide luminal (i.e. apical membrane side; AP) and abluminal (i.e. basolateral membrane side; BL) compartments (Fig. 1c). After a monolayer with high barrier integrity is established (Fig. 1c, a right inset displaying a monolayer of Caco-2 cells), pre-cultured microbial cells that are probiotic (e.g. VSL#3 [30, 31], *Lactobacillus rhamnosus* GG [32, 33] or *Escherichia coli* Nissle 1917 [34]) or pathogenic (e.g. *Salmonella* sp. [31], *Vibrio cholera* [35], *Shigella* sp. [36, 37], enteroinvasive [38] or enterotoxigenic *E. coli* [39], or *Campylobacter jejuni* [40]) can be introduced into the apical compartment to induce short-term host-microbiome interactions. For quantitative results, enhanced or compromised intestinal barrier function is measured through the transepithelial electrical resistance (TEER; unit, Ω·cm<sup>2</sup>) [30, 41] or the permeability assay [30]. To induce inflammatory responses during host-microbiome interactions, isolated innate immune cells such as neutrophils

[42, 43], macrophages [44], or dendritic cells [45] are added in the basolateral compartment (i.e. abluminal side) in the presence of the microbial stimulation in the apical compartment (i.e. luminal side). Since a confluent cell monolayer grown in the Transwell insert provides apical and basolateral compartments, the secretomes released into apical or basolateral directions can be independently collected and analyzed. While Transwell cultures are robust, easy, and reproducible, they often fail to support tissue-specific differentiations [15, 16]. Furthermore, given its static nature, host-microbiome crosstalk can only be induced for a relatively short period (from a half-hour to several hours) because microbial cells are prone to overgrow under static culture conditions.

### In Vitro 3D Cultures

Some mammalian cells require a specific type of extracellular matrix (ECM) and 3D structural microenvironment because they do not properly grow and function in the conventional 2D culture format. To overcome limitations of the 2D system, several studies have focused on mimicking the tissue microenvironment and understanding the processes of cell growth and differentiation using 3D culture methods [46, 47]. It has been reported that temperature-responsive hydrogels such as polyethylene glycol (PEG) or poly(lactic-co-glycolic) acid (PLGA)-based hydrogels and poly(*N*-isopropylacrylamide) are promising materials for creating a 3D culture matrix in an injectable tissue-engineering system, driving multilineage differentiation from human mesenchymal stem cells [48–52]. By harnessing these functional hydrogel materials, cellular spheroids (spherical cell aggregation) have suggested a simple 3D model utilizing wide range of cell types (mono- or multicellular spheroids). Alternatively, the hanging-drop [53, 54], the self-organization method (e.g. organoid) [55], and the rotating wall vessel culture methods [56] have also been explored. These platforms have improved physiological cogeny by achieving higher levels of cell differentiation, mimicking fluid shear stress, forming polarized exposure of the GI lumen, and extending co-culture possibilities. Here, we review a couple of representative models that leverage these 3D cultures using human tissues to reconstitute in vivo host-microbiome interactions.

### Rotating Wall Vessel

The rotating wall vessel (RWV) is a cell culture bioreactor that consists of a rotating component containing culture medium and epithelial cells grown on the surface of a spherical bead coated with ECM (Fig. 2a) [57, 58]. Similar to a ‘drum washing machine’, the RWV rotates the culture medium to induce continuous surface shear stresses on the epithelial cells adhered to the beads (Fig. 2a, an inset in upper right). This approach presents a semi-3D tissue culture model with

improved cell differentiation [57], as intestinal epithelial HT-29 cells grown in the RWV show 3D morphologies (Fig. 2a, left bottom) with enhanced expression of junctional proteins (E-cadherin,  $\beta$ -catenin, occludin, and zonula occludens (ZO)-1) compared to the cells in a 2D monolayer [58]. Furthermore, a RWV bioreactor enables to demonstrate enteropathogenesis of *Salmonella enterica* serovar *typhimurium* (Fig. 2a, right bottom), where *S. typhimurium* cells show increased adherence to (> 6-fold) but significantly lower invasion (< 9-fold) of the 3D epithelial cells compared to those grown in a 2D monolayer. This result suggests that this culture method better models human enteric salmonellosis and provides relevant pathological insight [58]. Notable advantages of the RWV bioreactor include the tunable shear stress applied to epithelial cells and the maintenance of the steady-state condition that can control the microbial population in situ. However, this system may include high equipment costs and multiple experimental steps for preparing bead-based epithelial aggregates, which are relatively complex. Furthermore, epithelial cells adhered on the bead can not be used to quantitatively measure the barrier functions or to induce basolateral immune stimulation.

### 3D Hydrogel Scaffold

Three-dimensional scaffolds using biocompatible hydrogel such as hyaluronic acid [59], PLGA [48], Calcium-alginate [60], or PEG-peptide [61] have been developed to recreate the microarchitecture of the human GI tract. In addition, 3D printing or bioprinting technologies have emerged to rebuild more accurate and robust structure of human organs and tissues such as skin [62, 63], vasculature [64, 65], and kidney [66]. Potentially, this method can also be applied to study host-microbiome or host-pathogen interactions. A recent study reports that silk fibrin-based 3D scaffolding reproduces the villi-like microarchitecture of the human small intestine, where a mixed population of Caco-2 (absorptive) and HT29-MTX (mucus secretory) epithelial cells successfully attenuates on this artificial gut in co-culture with primary human intestinal myofibroblasts [67] (Fig. 2b). Particularly, this microengineered model replicates physiological conditions such as enhanced mucus production, increases cytodifferentiation and lastly, forms better luminal oxygen gradient when compared to the 2D Transwell system. In addition, this model demonstrates the infection of *Yersinia pseudotuberculosis* or the co-culture with *Lactobacillus rhamnosus* GG (LGG). However, static culture conditions applied in this model strongly restrict the period of host-microbiome co-culture.

### 3D Organoids

Organoid culture is one of the most spotlighted methods to form 3D micro-organ architecture with fully differentiated

epithelial cells [68]. Variety of organoid culture protocols have been proposed to regenerate small intestine [69, 70], colon [71], stomach [72], lung [73, 74], liver [14], prostate [75], pancreas [76], kidney [77], and brain tissues [78] using either primary or induced pluripotent stem (iPS) cells [55]. Organoid culture techniques guarantee high reproducibility and robustness, where stem cell-containing tissue segment can regenerate into highly organized, structurally matured organoid bodies in hydrogel under growth factor signals. Along with the success of 3D organoid cultures of human tissues, there have been multiple attempts to co-culture the human microbiome with organoids. For instance, stomach organoids have been used to study epithelium-microbiome interactions (Fig. 2c), where *Helicobacter pylori* cells that can cause stomach ulcer [72, 79] are injected into the lumen of the gastric organoids (Fig. 2c; a left schematic). Since the lumen is enclosed inside the stomach organoid body, a microinjection step is essential to introduce microbial cells on the apical side of the differentiated epithelium. This approach still holds the aforementioned challenges of employing a stable host-microbiome ecosystem for an extended period of culture. Nevertheless, the organoid cultures have been recognized as a wonderful technology to contribute to personalized precision medicine because of the possible use of iPS cells or tissue biopsies as well as microbiome samples isolated from individual patients.

### Ex Vivo Models

The ex vivo models generally use tissue biopsies obtained from surgery. After washing the biopsy samples with saline or phosphate buffered saline (PBS), biopsies are placed in polarized or non-polarized orientations depending on the purpose of the study. The non-polarized, ex vivo culture method places the biopsies on top of an agar disk or sponge. After mounted biopsies are covered with culture medium, bacterial cells are inoculated on top of the biopsy [80, 81]. For instance, jejunal tissues from pediatric patients who have gastrointestinal disorders are isolated and co-cultured with 5 different enteroaggregative *E. coli* strains to test adhesion properties [81]. However, this approach allows for bacterial cells to access not only the mucosal but also the sub-mucosal sides, which seriously compromises the validity of this model. Another ex vivo method uses the Transwell insert, in which the colonic tissue is placed on top of the porous membrane and then submerged in culture medium [82, 83] with non-pathogenic *E. coli* (ECOR-26) or several *Lactobacillus* strains introduced on the apical side. In response to different bacterial cells, tumor necrosis factor (TNF)- $\alpha$ , transforming growth factor (TGF)- $\beta$ 1, interleukin (IL)-8, and IL-10 productions are significantly changed [83].

A micro-Snapwell system [84–87] is a sandwich-type biopsy culture method, similar to the Transwell approach. First, a duodenal biopsy is open and spread in a planar sheet. Then

using a histoacryl tissue glue [86], the biopsy piece is bonded onto an acrylic glass with a 2 mm hole in the center. Lastly, the glass disc-covered tissue is positioned on a nitrocellulose filter where the lumen side of the tissue is facing upward, and another glass disc is placed at the bottom. In this model, infection of enteropathogenic *E. coli* is performed for <8 h.

The Ussing chamber is also a sandwich-type tissue mounting system originally designed by Hans Ussing to study ion transport across the frog skin [88]. This system is usually used to run ion or nutrient transport assays, but bacterial co-culture can be also applied [89]. An Ussing chamber holds the intestinal biopsy in an open planar sheet conformation, such that the apical and basolateral sides each contain their own compartments. Bacterial cells are introduced to the apical chamber for a limited period (< 3 h) to study infection [90], transmigration [91–93], or immune responses [94]. Anaerobic gas (10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub> mixture) introduced to the apical chamber creates local anoxic gradient; but in general, Ussing chamber requires continuous sparging of oxygen for maintaining the viability of a biopsy tissue. Human small intestinal mucosal biopsies can be viable for up to a day [95] or a bit longer [96, 97], but it is quite challenging to maintain the viability for longer than 2 days. While anaerobic conditioning is theoretically possible, it is technically not plausible. In brief, given all the existing ex vivo models are performed in static conditions, which is a considerable drawback, the additional lack of physical, mechanical stimulation and stable maintenance of the tissue sample notably hampers the robustness of the system in demonstrating mid- and long-term host-microbiome crosstalk.

### Microphysiological System

The development of the microphysiological system, also known as the “Human organ-on-a-chip”, has presented disruptive enabling technologies to create in vitro models with an in vivo relevant tissue microenvironment, direct fluid shear stress (e.g. blood flow in the vasculature [98]) and cyclic mechanical deformations (e.g. peristalsis in the intestine [16]) to induce physiological cytodifferentiation with spatial resolution. By harnessing soft lithography-based microfabrication and microfluidic technologies, 3D tissue microarchitecture has been reconstituted on a microengineered platform that allows co-culturing multiple types of cells as well as the human microbiome. While numerous organ models have been reported, demonstration of viable host-microbiome interactions using organs-on-chips have been remarkably limited.

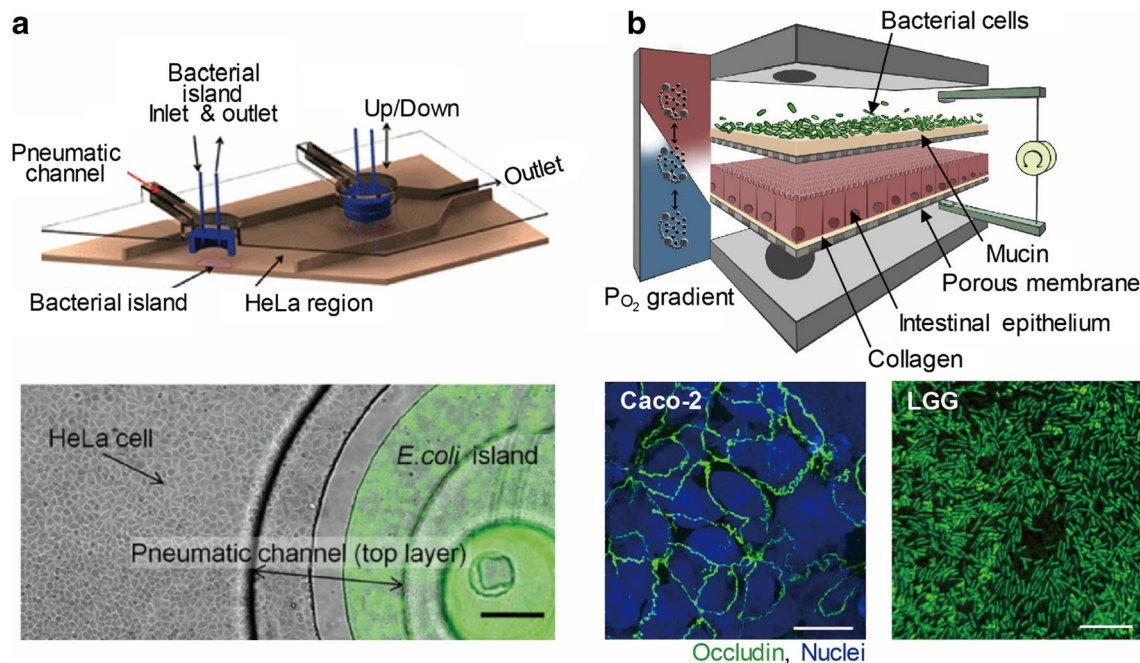
The primary goal with demonstrating host-microbiome interactions in vitro is to maintain viable and functional tissue and microbial components simultaneously in a defined spatial structure. However, it has been evident that microbial cells in the static cell/tissue culture rapidly overgrow, resulting in the depletion of nutrients and oxygen, and the accumulation of

organic wastes (e.g. acetate or lactate) that can potentially damage host cells. Thus, the nascent microfluidic model has attempted to separate the culture compartments of microbial and host cells. Specifically, pneumatic channels separate a compartment containing a “microbial island” and the adjacent compartment lined by epithelial cells (Fig. 3a). Microfluidic valves made by silicone polymer such as polydimethylsiloxane (PDMS) secure the independent growth of host and microbial cells. In this model, epithelial HeLa cells (an immortalized cervical cancer cell line [99, 100]) interact with non-pathogenic *E. coli* biofilm in the presence of enterohemorrhagic *E. coli* (EHEC), where the commensal biofilm protects EHEC infection [101]. However, most of the human microbiome is in direct contact to host cells; thus, spatially segregated growth of host and microbial cells is physiologically questionable.

Another recent model named the “HuMiX (human-microbial crosstalk)” consists of a stacked assembly of elastomeric gaskets separated by a nanoporous membrane for co-culturing human intestinal epithelium (Caco-2) and microbial cells, replicating the host-microbe GI interface (Fig. 3b) [102]. The HuMiX consists of three chambers capable of recreating the internal aerobic and non-aerobic conditions, where generating the gradient of oxygen and biomolecules is feasible. Indeed, the HuMiX system supports to co-culture the Caco-2 epithelium (Fig. 3b, “Caco-2”) with aerobic (e.g. LGG; Fig. 3b,

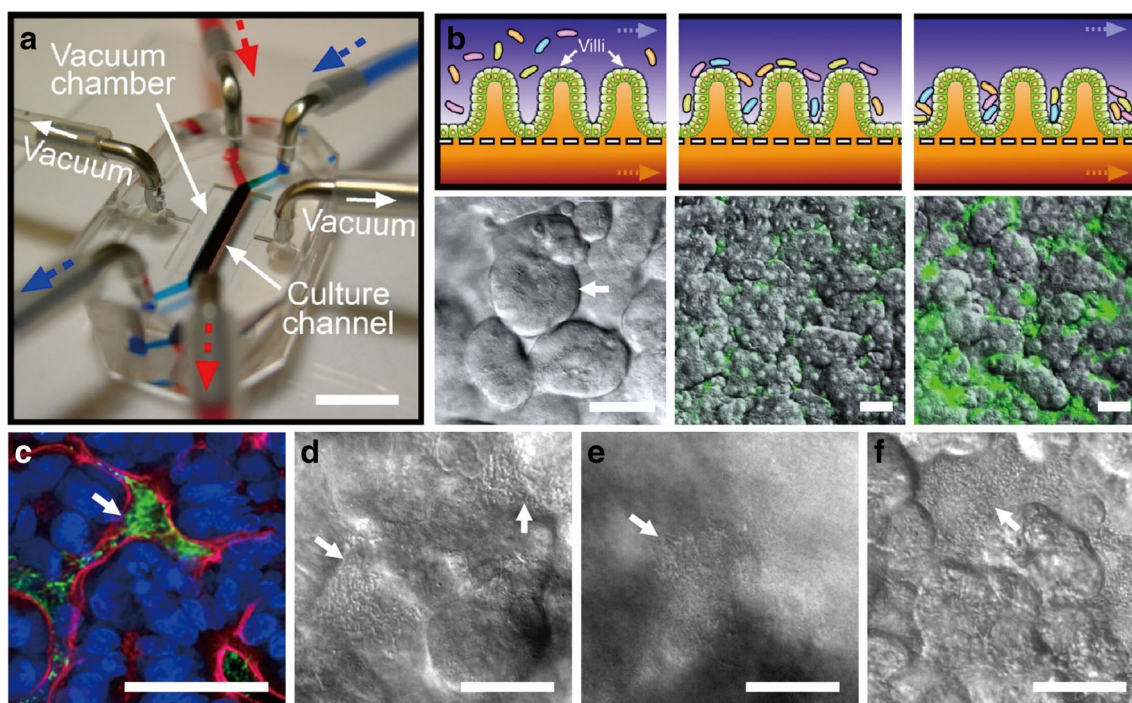
“LGG”) or obligate anaerobic bacteria (e.g. *Bacteroides caccae*). This experimental setup highlights the significant technical advance in manipulating metabolically mismatched (e.g. aerobic vs. anaerobic) microenvironments in vitro. However, the HuMiX lacks both the cyclic mechanical deformations mimicking peristalsis germane to the prevention of microbial overgrowth [18] and the direct contact between microbial and host cells, which reduces the physiological cogency of this model. Furthermore, all host-microbiome co-cultures are carried out in only 24 h, which may not be sufficient to extrapolate their results to quantitatively predict the transcriptional and metabolic characteristics in human host-microbiome crosstalk.

Finally, the microengineered human gut-on-a-chip model [15, 16, 18] provides a 3D lumen-capillary tissue interface in two juxtaposed microchannels separated by an ECM-coated, flexible porous PDMS membrane lined by human intestinal Caco-2 epithelium (Fig. 4a). Besides the central cell channel, two vacuum chambers linked to a vacuum controller exert cyclic physical deformations in response to the repeated suction motions, by which a flexible membrane lined by epithelial cells in the central cell microchannel experiences rhythmical stretching motions mimicking peristalsis. The Caco-2 epithelial cells spontaneously undergo villus morphogenesis (Fig. 4b, left panel) [15, 16], in which



**Fig. 3** Host-microbiome interactions in microfluidic models. **a** A co-culture model in a microfluidic device designed to compartmentalize pneumatically actuated trapping regions for forming bacterial islands around epithelial cells (HeLa). Each bacterial island (1200  $\mu\text{m}$  in diameter; 1000  $\mu\text{m}$  apart) has a separate inlet and outlet ports for providing the nutrient and removing the waste. The overlaid transmitted and green fluorescence images showing the co-culture of HeLa cells and GFP-labeled *E. coli* for 48 h. Bar, 200  $\mu\text{m}$ . Reproduced with permission

[101]. 2010 The Royal Society of Chemistry. **b** A schematic of HuMiX system (top) and the images (bottom) of intestinal epithelial (“Caco-2”) and microbial cells (“LGG”). The immunofluorescent images showing the tight junction protein occludin (green) in Caco-2 cells following 24 h of co-culture with *Lactobacillus rhamnosus* GG (LGG) grown under anaerobic condition. The “LGG” image displays the live (green) and dead (red) cells. Bar, 10  $\mu\text{m}$ . Reproduced with permission [102]. 2016 licensed under a Creative Commons Attribution



**Fig. 4** The microengineered human gut-on-a-chip. **a** A photograph of a gut-on-a-chip microdevice. *Dashed arrows* indicate the direction of the flow of culture medium in the apical (*blue*) and basolateral (*red*) microchannels. Bar, 5 mm. **b** A set of a schematic (*upper*) and corresponding micrographs (*lower*; DIC alone in the left or DICs overlaid with fluorescence images of GFP *E. coli* in both middle and right) displays the germ-free microengineered villi (*left panel*), inoculation (*middle panel*; at 0 h), and colonization of GFP *E. coli* (*right panel*; at 13 h). Cells in the gut-on-a-chip device are grown at

40  $\mu\text{L/h}$  of flow rate and 10%, 0.15 Hz of stretching motions. **c** An immunofluorescence image of the co-culture of intestinal villi with GFP *E. coli*. An arrow indicates a microcolony of GFP *E. coli*. Blue, nuclei; magenta, F-actin. **d** A microscopic image displaying the stochastic microbial niches (*arrows*) of VSL#3 cells on the villi grown in the gut-on-a-chip. **e** Biofilm formation (*an arrow*) of LGG cells in the gut-on-a-chip. **f** Overgrowth of pathogenic EIEC cells in the gut-on-a-chip. Bars, 100  $\mu\text{m}$ . Reproduced with permission [18, 136]. 2016 National Academy of Sciences; ©2016 licensed under a Creative Commons Attribution

the four lineages of the small intestinal epithelial cells (absorptive, goblet, enteroendocrine, and Paneth) are regenerated. Full cytodifferentiation of the intestinal epithelium including mucin expression reprograms the luminal microenvironment much more feasible for microbial cells adherence [15]. The gut-on-a-chip can provide direct contact between microbial cells and host intestinal villi, by which green fluorescence protein (GFP)-labeled *E. coli* cells inoculated on the surface of villus epithelium (Fig. 4b, middle panel; at 0 h of the co-culture) form multiple stochastic microcolonies across the epithelial layer (Fig. 4b, right panel; at 13 h of the co-culture). High power magnification of an immunofluorescence image reveals the microbial niche of GFP *E. coli* between the microengineered villi (Fig. 4c), suggesting that the host-gut microbiome ecosystem established in a gut-on-a-chip system closely emulates in vivo gut microenvironment. The gut-on-a-chip also supports to demonstrate the colonization of spatially distinct microcolonies of multiple microbial cells (Fig. 4d; co-culture with VSL#3), on-chip formation of the biofilm (Fig. 4e; by LGG cells), and the infection and invasion of pathogenic bacteria (Fig. 4f; co-culture with

enteroinvasive *E. coli*, EIEC). The gut-on-a-chip system is analytically compatible to perform conventional assays including microscopy, biochemical assay such as enzyme-linked immunosorbent assay (ELISA), microfluorimetry, live/dead staining, quantitative polymerase chain reactions (qPCR), and even gene microarray [18]. The gut-on-a-chip also provides the potential to co-culture with other host cells such as capillary or lymphatic endothelium or immune cells [18], by which complex inflammatory responses between the gut microbiome and local immune components such as peripheral blood mononuclear cells (PBMC) [18] or gut-associated lymphoid tissues (GALT) can be robustly demonstrated. However, this system has not demonstrated co-culture with anaerobic microbial cells yet. In terms of the materials perspective, the use of PDMS may hamper the accurate measurement of the transport of hydrophobic molecules because the hydrophobic PDMS wall often adsorb introduced test molecules. In addition, replacement of the flexible porous PDMS membrane to functional biomaterials is another important unmet need. In addition, the multi-step microfabrication process could be a possible pitfall.



## Potential Applications and Perspectives of in Vitro Host-Microbiome Ecosystem

### Personalized Precision Medicine

Human iPS cells or tissue-derived organoids can contribute to the development of personalized treatment strategies [103]. Stem cell-derived organoids provide a compelling new class of biological models to serve as both tissue and organ proxies [104]. Patient-derived organoids also present an important resource for discovering a personalized treatment regime [55]. Organoids recapitulate the spatial organization of heterogeneous tissue-specific cells, cell-cell interactions, cell-matrix interactions and certain physiological functions [105]. However, organoid systems have significant limitations. As previously discussed, an enclosed static lumen results in technical challenges in recreating in vivo host-microbiome interactions. Furthermore, organoids embedded in hydrogel have a lack of mechanical cues (e.g. shear stress by blood flow rate, mechanical deformations by organ movement) that are critical for organ formation [106, 107]. To overcome this limit, dissociated organoid cells can be used to form a polarized monolayer in the Transwell, however the static nature never be appreciated to exert mechanical deformations as well as the stable co-culture with microbes, which is a significant limitation [108–110]. Hence, we envision integrating organoid-derived cells with the organs-on-chips platform that offers a more controllable and modulatory platform to independently manipulate microbiome, host cells, and acellular factors one at a time. Ultimately, the successful integration of organoid cultures and organs-on-chips can be a game changer to investigate detailed roles of the human microbiome on orchestrating the disease pathogenesis, where all the microbial and host cells obtained from human individuals can reflect various genetic, demographic, or epidemiological backgrounds. This approach may potentially contribute to the Precision Medicine Initiative [111].

### Drug Metabolism

It has been known that the gut microbiome considerably alters the efficacy of administered drugs directly or indirectly [112, 113]. While both enterocytes in the intestine and hepatocytes in the liver contribute to the first-pass effect and detoxification of orally administered xenobiotics, gut microbiome can also perform drug metabolizing biotransformation via several biochemical reactions such as hydroxylation, oxidation, or deacylation of drug compounds [6, 114]. As a result, microbiome-driven drug metabolism can change the efficacy and toxicity of administered drugs by activating the prodrugs or inactivating the drug actions.

For instance, irinotecan, an anti-cancer drug, undergoes sequential enzymatic transformations during the drug

metabolism, but the gut microbiome can perturb this biocatalytic reactions and causes adverse effect such as diarrhea. After irinotecan is metabolized to the cytotoxic SN-38 (an inhibitor of topoisomerase I) by hepatic or GI carboxylesterases, the intermediate SN-38 is further transformed to a non-toxic SN-38-G via hepatic UDP-glucuronosyl transferases. However,  $\beta$ -glucuronidases in the gut microbiome can deconjugate SN-38-G and restore it to SN-38, suggesting that the gut microbiome remarkably perturb the drug metabolism and potentially induces adverse effect [115, 116]. Another recent study reveals that human gut actinobacterium, *Eggerthella lenta*, inactivates the cardiac drug digoxin through the microbial drug metabolism, which dramatically alters the efficacy of digoxin [117]. This report clearly discloses that the gut microbiome is literally the “forgotten organ [118]” that can greatly alter the first-pass effect in drug metabolism. However, thus far, only about 40 xenobiotic drugs have been investigated to study the impact of the gut microbiome on drug metabolism [119].

Since the biogeography of gut microbiome is dramatically changed by the diet, medication, or environmental factors [120, 121], drug metabolism by the host cells as well as the gut microbiome should be simultaneously contemplated for validating the drug candidates during the drug development process. We envision that a human-predictive in vitro model that provides a stable host-gut microbiome ecosystem will have enormous potential to accurately validate the pharmacokinetics, pharmacodynamics, and pharmacogenomics of orally administered drugs.

### Microbiome-Based Therapeutics

The fecal microbiota transplantation (FMT) is an emerging microbiome-based therapeutic intervention used to cure recurrent infections caused by *Clostridium difficile* [122]. The basic concept of the FMT is to collect fecal samples from an individual who has healthy bowel functions, and proceed to flash-freeze fecal samples in an aseptic condition. After subsequent processes for isolating gut microbiome, these microbiome suspensions are administered to people who suffer from infectious diarrhea, abdominal pain, and mal-digestion or absorption [123]. Currently in the United States, the Food and Drug Administration (FDA) has only approved the FMT application for the treatment of *C. difficile* infections (CDI). However, multiple GI clinicians may envision expanding the application of FMT to other GI disorders, where GI scientists as well as clinicians have tried to find new avenues to validate the efficacy and safety of FMT. Since the role of the gut microbiome is tremendous in terms of digestion [124], mucosal homeostasis [125], drug metabolism [6], immune modulation [126], disease development including cancers [127], or brain development [128], the therapeutic potential of FMT treatment in GI disorders and its prognosis will be of great significance. We prospect that harnessing the in vitro host-gut microbiome

models such as the gut-on-a-chip will provide many opportunities to validate the effectiveness of FMT as well as assess the risk factors of FMT such as the identification of opportunistic pathogens in the donor's fecal sample.

### Gut-Brain Axis

There is a growing emphasis on understanding the relationship between the complexity and diversity of the human GI microbiome in relation to GI health and disease, and recently, in brain development and disorders of the central nervous system [129]. The relationship between the gut microbiome and the brain is believed to be bi-directional, whereby presumably the gut microbiome can potentially modulate the brain's functions and the brain can direct intestinal functions by changing the GI motility and intestinal permeability barrier [130]. However, there have been no clinically relevant models reported to independently control the microenvironmental factors in the gut and brain to study the gut-brain axis [131]. In addition, interactions of drugs with the blood-brain barrier (BBB) and their translocation across the BBB can not be directly studied in the intact human brain or accurately examined by using animal models. This challenge underscores the importance of developing *in vitro* models that mimic the pathophysiological conditions of the brain, particularly for human diseases, where the existing animal models can not specifically recapitulate [132]. Furthermore, majority of the previous studies using *in vitro* model systems neglect these critical factors due to technical difficulties [133]. Hence, by leveraging microengineering approaches, gut-brain axis should be aggressively considered, where a gut-brain network microfluidic device (e.g. integration of a human gut-on-a-chip and a brain-on-a-chip or a BBB-on-a-chip) can be developed as a physiologically relevant platform.

### Disease Models

Intestinal inflammation can be a potential target disease to model using the *in vitro* host-gut microbiome ecosystem. Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is characterized by chronic inflammation in the intestinal mucosa, where abnormal interactions between the gut microbiome and the local immune system seem to be a critical trigger [134]. However, exact mechanisms of the initiation, progression, and optimal therapeutics of IBD are largely unknown [135]. Hence, if an *in vitro* host-microbiome model can spatiotemporally modulate the key interacting factors associated with IBD pathogenesis, the value of this model can be immense since the effect of the gut microbiome and immune components can be independently decoupled and dissected. In addition, recruitment of various human cohort samples obtained from individuals with different ages, race/ethnicities, family histories of IBD, or

therapeutic histories will be a great opportunity to discover better IBD models that can contribute to identify novel IBD therapeutic interventions such as the FMT.

### Conclusion

The host-gut microbiome interaction plays an important role germane to the maintenance of human health and the development of diseases. Specifically, microphysiological systems can be a strong candidate to reconstitute, recapitulate, demonstrate, and validate the functionality and robustness of host-microbiome interactions *in vitro*. Furthermore, these on-chip physiological models provide the tunable versatility by modulating the volumetric flow rate, shear stress, chemotactic chemical gradient, tissue-tissue interface, and tissue-specific mechanical movements that are all critical for sustaining steady-state microbial populations. By encompassing clinical samples, 3D organoids cultures, and translational researches and collaborations, we may further improve the legitimacy of a human surrogate system towards human "Disease-on-a-chip" or "Patient-on-a-chip". Ultimately, personalized human organs-on-chips with physiologically relevant host-gut microbiome interactions can replace the costly inefficient germ-free or conventional animal models and accelerate the drug development process.

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### Compliance with Ethical Standards

**Conflict of Interest** The authors declare no conflicts of interest.

### References

1. Relman, D. A. (2012). Microbiology: learning about who we are. *Nature*, *486*(7402), 194–195.
2. Tremaroli, V., & Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature*, *489*(7415), 242–249.
3. Kamada, N., Seo, S.-U., Chen, G. Y., & Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology*, *13*(5), 321–335.
4. Scheperjans, F., Aho, V., Pereira, P. A., et al. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*, *30*(3), 350–358.
5. Rinke, C., Schwientek, P., Sczyrba, A., et al. (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature*, *499*(7459), 431–437.

6. Wilson, I. D., & Nicholson, J. K. (2017). Gut microbiome interactions with drug metabolism, efficacy, and toxicity. *Translational Research*, 179, 204–222.
7. Cho, I., & Blaser, M. J. (2012). The human microbiome: at the interface of health and disease. *Nature Reviews Genetics*, 13(4), 260–270.
8. D'Argenio, V., & Salvatore, F. (2015). The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta*, 451, 97–102.
9. Gall, A., Fero, J., McCoy, C., et al. (2015). Bacterial composition of the human upper gastrointestinal tract microbiome is dynamic and associated with genomic instability in a Barrett's esophagus cohort. *PLoS One*, 10(6), e0129055.
10. Calle, E. E., & Kaaks, R. (2004). Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nature Reviews Cancer*, 4(8), 579–591.
11. Diaz-Sanchez, S., Hanning, I., Pendleton, S., & D'Souza, D. (2013). Next-generation sequencing: the future of molecular genetics in poultry production and food safety. *Poultry Science*, 92(2), 562–572.
12. Walker, A. W., Duncan, S. H., Louis, P., & Flint, H. J. (2014). Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends in Microbiology*, 22(5), 267–274.
13. Franzosa, E. A., Hsu, T., Sirota-Madi, A., et al. (2015). Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. *Nature Reviews Microbiology*, 13(6), 360–372.
14. Takebe, T., Sekine, K., Enomura, M., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*, 499(7459), 481–484.
15. Kim, H. J., & Ingber, D. E. (2013). Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integrative Biology (Camb)*, 5(9), 1130–1140.
16. Kim, H. J., Huh, D., Hamilton, G., & Ingber, D. E. (2012). Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab on a Chip*, 12(12), 2165–2174.
17. Artursson, P., Palm, K., & Luthman, K. (2001). Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Advanced Drug Delivery Reviews*, 46(1), 27–43.
18. Kim, H. J., Li, H., Collins, J. J., & Ingber, D. E. (2016). Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proceedings of the National Academy of Sciences of the United States of America*, 113(1), E7–15.
19. Payne, A. N., Zihler, A., Chassard, C., & Lacroix, C. (2012). Advances and perspectives in in vitro human gut fermentation modeling. *Trends in Biotechnology*, 30(1), 17–25.
20. Millhouse, E., Jose, A., Sherry, L., et al. (2014). Development of an in vitro periodontal biofilm model for assessing antimicrobial and host modulatory effects of bioactive molecules. *BMC Oral Health*, 14(1), 80.
21. Sadabad, M. S., von Martels, J. Z., Khan, M. T., et al. (2015). A simple coculture system shows mutualism between anaerobic faecalibacteria and epithelial Caco-2 cells. *Scientific Reports*, 5, 17906.
22. Khan, M. T., Duncan, S. H., Stams, A. J., Van Dijk, J. M., Flint, H. J., & Harmsen, H. J. (2012). The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *The ISME Journal*, 6(8), 1578–1585.
23. Tap, J., Mondot, S., Levenez, F., et al. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental Microbiology*, 11(10), 2574–2584.
24. Lopez-Siles, M., Duncan, S. H., Garcia-Gil, L. J., & Martinez-Medina, M. (2017). *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *The ISME Journal*, 11(4), 841–852.
25. Van den Abbeele, P., Van de Wiele, T., Verstraete, W., & Possemiers, S. (2011). The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiology Reviews*, 35(4), 681–704.
26. Zeng, J., Teng, F., Weinstock, G. M., & Murray, B. E. (2004). Translocation of enterococcus faecalis strains across a monolayer of polarized human enterocyte-like T84 cells. *Journal of Clinical Microbiology*, 42(3), 1149–1154.
27. McCracken, V. J., Chun, T., Baldeón, M. E., et al. (2002). TNF- $\alpha$  sensitizes HT-29 colonic epithelial cells to intestinal lactobacilli. *Experimental Biology and Medicine*, 227(8), 665–670.
28. Hilgers, A. R., Conradi, R. A., & Burton, P. S. (1990). Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharmaceutical Research*, 7(9), 902–910.
29. Finlay, B. B., & Falkow, S. (1990). Salmonella interactions with polarized human intestinal Caco-2 epithelial cells. *Journal of Infectious Diseases*, 162(5), 1096–1106.
30. Krishnan, M., Penrose, H. M., Shah, N. N., Marchelletta, R. R., & McCole, D. F. (2016). VSL# 3 probiotic stimulates T-cell protein tyrosine phosphatase-mediated recovery of IFN- $\gamma$ -induced intestinal epithelial barrier defects. *Inflammatory Bowel Diseases*, 22(12), 2811–2823.
31. Madsen, K., Cornish, A., Soper, P., et al. (2001). Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology*, 121(3), 580–591.
32. Johnson-Henry, K., Donato, K., Shen-Tu, G., Gordanpour, M., & Sherman, P. (2008). Lactobacillus rhamnosus strain GG prevents enterohemorrhagic *Escherichia coli* O157: H7-induced changes in epithelial barrier function. *Infection and Immunity*, 76(4), 1340–1348.
33. Gratz, S., Wu, Q., El-Nezami, H., Juvonen, R., Mykkänen, H., & Turner, P. (2007). Lactobacillus rhamnosus strain GG reduces aflatoxin B1 transport, metabolism, and toxicity in Caco-2 cells. *Applied and Environmental Microbiology*, 73(12), 3958–3964.
34. Roberts, C. L., Keita, A. V., Duncan, S. H., et al. (2010). Translocation of Crohn's disease *Escherichia coli* across M-cells: contrasting effects of soluble plant fibres and emulsifiers. *Gut*, 59(10), 1331–1339.
35. Kernéis, S., Caliot, E., Stubbe, H., Bogdanova, A., Kraehenbuhl, J.-P., & Pringault, E. (2000). Molecular studies of the intestinal mucosal barrier physiopathology using cocultures of epithelial and immune cells: a technical update. *Microbes and Infection*, 2(9), 1119–1124.
36. Mounier, J., Vasselon, T., Hellio, R., Lesourd, M., & Sansonetti, P. (1992). *Shigella flexneri* enters human colonic Caco-2 epithelial cells through the basolateral pole. *Infection and Immunity*, 60(1), 237–248.
37. Kazi, M., Paramita, S., Sheikh, I., Chakraborty, S. (2014). Zinc recovers altered intestinal ion-transport and barrier function caused by *Shigella* infection in T84 cells (902.9). *The FASEB Journal*, 28(1 supplement), 902.909.
38. Eckmann, L., Kagnoff, M., & Fierer, J. (1993). Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. *Infection and Immunity*, 61(11), 4569–4574.
39. Roselli, M., Finamore, A., Britti, M. S., et al. (2007). The novel porcine Lactobacillus sobrius strain protects intestinal cells from enterotoxigenic *Escherichia coli* K88 infection and prevents membrane barrier damage. *The Journal of Nutrition*, 137(12), 2709–2716.
40. Hu, L., Tall, B. D., Curtis, S. K., & Kopecko, D. J. (2008). Enhanced microscopic definition of campylobacter jejuni 81-176 adherence to, invasion of, translocation across, and exocytosis from polarized human intestinal Caco-2 cells. *Infection and Immunity*, 76(11), 5294–5304.
41. Hummel, S., Veltman, K., Cichon, C., Sonnenborn, U., & Schmidt, M. A. (2012). Differential targeting of the E-cadherin/

- β-catenin complex by gram-positive probiotic lactobacilli improves epithelial barrier function. *Applied and Environmental Microbiology*, 78(4), 1140–1147.
42. Hidalgo, I. J., Raub, T. J., & Borchardt, R. T. (1989). Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology*, 96(3), 736–749.
  43. Roselli, M., Finamore, A., Britti, M. S., & Mengheri, E. (2006). Probiotic bacteria *Bifidobacterium animalis* MB5 and *Lactobacillus rhamnosus* GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic *Escherichia coli* K88. *British Journal of Nutrition*, 95(06), 1177–1184.
  44. Haller, D., Bode, C., Hammes, W., Pfeifer, A., Schiffrin, E., & Blum, S. (2000). Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut*, 47(1), 79–87.
  45. Rescigno, M., Urbano, M., Valzasina, B., et al. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunology*, 2(4), 361–367.
  46. Park, M. H., Choi, B. G., & Jeong, B. (2012). Complexation-induced biomimetic long range fibrous orientation in a rigid-flexible block copolymer Thermogel. *Advanced Functional Materials*, 22(24), 5118–5125.
  47. Zhang, S., Dutton, J. R., Su, L., Zhang, J., & Ye, L. (2014). The influence of a spatiotemporal 3D environment on endothelial cell differentiation of human induced pluripotent stem cells. *Biomaterials*, 35(12), 3786–3793.
  48. Gentile, P., Chiono, V., Carmagnola, I., & Hatton, P. V. (2014). An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. *International Journal of Molecular Sciences*, 15(3), 3640–3659.
  49. Mellati, A., Kiamahalleh, M. V., Madani, S. H., et al. (2016). Poly (N-isopropylacrylamide) hydrogel/chitosan scaffold hybrid for three-dimensional stem cell culture and cartilage tissue engineering. *Journal of Biomedical Materials Research Part A*, 104(11), 2764–2774.
  50. Park, M. H., Yu, Y., Moon, H. J., et al. (2014). 3D culture of tonsil-derived mesenchymal stem cells in poly (ethylene glycol)-poly (l-alanine-co-l-phenyl alanine) Thermogel. *Advanced Healthcare Materials*, 3(11), 1782–1791.
  51. Park, M. H., Moon, H. J., Park, J. H., Shinde, U. P., Ko, D. Y., & Jeong, B. (2015). PEG-poly (l-alanine) Thermogel as a 3D scaffold of bone-marrow-derived mesenchymal stem cells. *Macromolecular Bioscience*, 15(4), 464–472.
  52. Yan, S., Wang, T., Feng, L., et al. (2014). Injectable in situ self-cross-linking hydrogels based on poly(l-glutamic acid) and alginate for cartilage tissue engineering. *Biomacromolecules*, 15(12), 4495–4508.
  53. Kelm, J. M., Timmins, N. E., Brown, C. J., Fussenegger, M., & Nielsen, L. K. (2003). Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types. *Biotechnology and Bioengineering*, 83(2), 173–180.
  54. Timmins, N., Harding, F., Smart, C., Brown, M., & Nielsen, L. (2005). Method for the generation and cultivation of functional three-dimensional mammary constructs without exogenous extracellular matrix. *Cell and Tissue Research*, 320(1), 207–210.
  55. Fatehullah, A., Tan, S. H., & Barker, N. (2016). Organoids as an in vitro model of human development and disease. *Nature Cell Biology*, 18(3), 246–254.
  56. Castañeda, F., & Kinne, R. K. H. (2000). Short exposure to millimolar concentrations of ethanol induces apoptotic cell death in multicellular HepG2 spheroids. *Journal of Cancer Research and Clinical Oncology*, 126(6), 305–310.
  57. Radtke, A. L., Wilson, J. W., Sarker, S., & Nickerson, C. A. (2010). Analysis of interactions of *Salmonella* type three secretion mutants with 3-D intestinal epithelial cells. *PLoS One*, 5(12), e15750.
  58. Höner zu Bentrup, K., Ramamurthy, R., Ott, C. M., et al. (2006). Three-dimensional organotypic models of human colonic epithelium to study the early stages of enteric salmonellosis. *Microbes and Infection*, 8(7), 1813–1825.
  59. Tan, H., Ramirez, C. M., Miljkovic, N., Li, H., Rubin, J. P., & Marra, K. G. (2009). Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. *Biomaterials*, 30(36), 6844–6853.
  60. Sung, J. H., Yu, J., Luo, D., Shuler, M. L., & March, J. C. (2011). Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model. *Lab on a Chip*, 11(3), 389–392.
  61. Zhu, J. (2010). Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials*, 31(17), 4639–4656.
  62. Koch, L., Kuhn, S., Sorg, H., et al. (2009). Laser printing of skin cells and human stem cells. *Tissue Engineering Part C: Methods*, 16(5), 847–854.
  63. Skardal, A., Mack, D., Kapetanovic, E., et al. (2012). Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Translational Medicine*, 1(11), 792–802.
  64. Norotte, C., Marga, F. S., Niklason, L. E., & Forgacs, G. (2009). Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials*, 30(30), 5910–5917.
  65. Visconti, R. P., Kasyanov, V., Gentile, C., Zhang, J., Markwald, R. R., & Mironov, V. (2010). Towards organ printing: engineering an intra-organ branched vascular tree. *Expert Opinion on Biological Therapy*, 10(3), 409–420.
  66. Adams, F., Qiu, T., Mark, A., et al. (2016). Soft 3D-printed phantom of the human kidney with collecting system. *Annals of Biomedical Engineering*, 45(4), 963–972.
  67. Chen, Y., Lin, Y., Davis, K. M., et al. (2015). Robust bioengineered 3D functional human intestinal epithelium. *Scientific Reports*, 5, 13708.
  68. Sato, T., & Clevers, H. (2013). Growing self-organizing mini-guts from a single intestinal stem cell: Mechanism and applications. *Science*, 340(6137), 1190–1194.
  69. Koo, B.-K., Stange, D. E., Sato, T., et al. (2012). Controlled gene expression in primary Lgr5 organoid cultures. *Nature Methods*, 9(1), 81–83.
  70. Sato, T., Vries, R. G., Snippert, H. J., et al. (2009). Single Lgr5 stem cells build crypt villus structures in vitro without a mesenchymal niche. *Nature*, 459(7244), 262–265.
  71. Sato, T., Stange, D. E., Ferrante, M., et al. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology*, 141(5), 1762–1772.
  72. McCracken, K. W., Cata, E. M., Crawford, C. M., et al. (2014). Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature*, 516(7531), 400–404.
  73. Dye, B. R., Hill, D. R., Ferguson, M. A. H., et al. (2015). In vitro generation of human pluripotent stem cell derived lung organoids. *eLife*, 4, e05098.
  74. Mondrinos, M. J., Jones, P. L., Finck, C. M., & Lelkes, P. I. (2014). Engineering de novo assembly of fetal pulmonary organoids. *Tissue Engineering. Part A*, 20(21–22), 2892–2907.
  75. Karthaus, W. R., Iaquinia, P. J., Drost, J., et al. (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*, 159(1), 163–175.
  76. Boj, S. F., Hwang, C.-I., Baker, L. A., et al. (2015). Organoid models of human and mouse ductal pancreatic cancer. *Cell*, 160(1–2), 324–338.

77. Takasato, M., Er, P. X., Chiu, H. S., et al. (2015). Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature*, *526*(7574), 564–568.
78. Lancaster, M. A., Renner, M., Martin, C.-A., et al. (2013). Cerebral organoids model human brain development and microcephaly. *Nature*, *501*(7467), 373–379.
79. Bartfeld, S., Bayram, T., van de Wetering, M., et al. (2015). In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology*, *148*(1), 126–136 e126.
80. Knutton, S., Lloyd, D. R., Candy, D., & McNEISH, A. S. (1984). In vitro adhesion of enterotoxigenic *Escherichia coli* to human intestinal epithelial cells from mucosal biopsies. *Infection and Immunity*, *44*(2), 514–518.
81. Hicks, S., Candy, D., & Phillips, A. (1996). Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. *Infection and Immunity*, *64*(11), 4751–4760.
82. Carol, M., Borruel, N., Antolin, M., et al. (2006). Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. *Journal of Leukocyte Biology*, *79*(5), 917–922.
83. Borruel, N., Casellas, F., Antolin, M., et al. (2003). Effects of nonpathogenic bacteria on cytokine secretion by human intestinal mucosa. *The American Journal of Gastroenterology*, *98*(4), 865–870.
84. El Asmar, R., Panigrahi, P., Bamford, P., et al. (2002). Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. *Gastroenterology*, *123*(5), 1607–1615.
85. Raffatellu, M., Chessa, D., Wilson, R. P., Dusold, R., Rubino, S., & Bäumlner, A. J. (2005). The vi capsular antigen of *Salmonella enterica* serotype Typhi reduces toll-like receptor-dependent interleukin-8 expression in the intestinal mucosa. *Infection and Immunity*, *73*(6), 3367–3374.
86. Schüller, S., Lucas, M., Kaper, J. B., Girón, J. A., & Phillips, A. D. (2009). The ex vivo response of human intestinal mucosa to enteropathogenic *Escherichia coli* infection. *Cellular Microbiology*, *11*(3), 521–530.
87. Schüller, S., & Phillips, A. D. (2010). Microaerobic conditions enhance type III secretion and adherence of enterohaemorrhagic *Escherichia coli* to polarized human intestinal epithelial cells. *Environmental Microbiology*, *12*(9), 2426–2435.
88. Ussing, H. H. (1949). The active ion transport through the isolated frog skin in the light of tracer studies. *Acta Physiologica Scandinavica*, *17*(1), 1–37.
89. Keita, Á. V., Gullberg, E., Ericson, A.-C., et al. (2006). Characterization of antigen and bacterial transport in the follicle-associated epithelium of human ileum. *Laboratory Investigation*, *86*(5), 504–516.
90. Worton, K., Candy, D., Wallis, T., et al. (1989). Studies on early association of *Salmonella typhimurium* with intestinal mucosa in vivo and in vitro: Relationship to virulence. *Journal of Medical Microbiology*, *29*(4), 283–294.
91. Kurkchubasche, A. G., Cardona, M., Watkins, S. C., et al. (1998). Transmucosal passage of bacteria across rat intestinal epithelium in the Ussing chamber: Effect of nutritional factors and bacterial virulence. *Shock*, *9*(2), 121–127.
92. Amin, I., Douce, G., Osborne, M., & Stephen, J. (1994). Quantitative studies of invasion of rabbit ileal mucosa by *Salmonella typhimurium* strains which differ in virulence in a model of gastroenteritis. *Infection and Immunity*, *62*(2), 569–578.
93. Albanese, C. T., Cardona, M., Smith, S. D., et al. (1994). Role of intestinal mucus in transepithelial passage of bacteria across the intact ileum in vitro. *Surgergy*, *116*(1), 76–82.
94. Grotz, M. R., Deitch, E. A., Ding, J., Xu, D., Huang, Q., & Regel, G. (1999). Intestinal cytokine response after gut ischemia: role of gut barrier failure. *Annals of Surgery*, *229*(4), 478.
95. Browning, T. H., & Trier, J. S. (1969). Organ culture of mucosal biopsies of human small intestine. *Journal of Clinical Investigation*, *48*(8), 1423.
96. Webster, A., Dyer, C. E., Haswell, S. J., & Greenman, J. (2010). A microfluidic device for tissue biopsy culture and interrogation. *Analytical Methods*, *2*(8), 1005–1007.
97. Davies, J. (2012). *Replacing animal models: a practical guide to creating and using culture-based biomimetic alternatives*. Chichester, West Sussex: John Wiley & Sons.
98. Fernandez, C., Yen, R., Perez, S., et al. (2016). Human vascular microphysiological system for in vitro drug screening. *Scientific Reports*, *6*, 21579.
99. Adey, A., Burton, J. N., Kitzman, J. O., et al. (2013). The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line. *Nature*, *500*(7461), 207–211.
100. Gey, G., Coffman, W. D., & Kubicek, M. T. (1952). Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. In Cancer research, AMER ASSOC CANCER RESEARCH PO BOX 11806, BIRMINGHAM, AL 35202.
101. Kim, J., Hegde, M., & Jayaraman, A. (2010). Co-culture of epithelial cells and bacteria for investigating host–pathogen interactions. *Lab on a Chip*, *10*(1), 43–50.
102. Shah, P., Fritz, J. V., Glaab, E., et al. (2016). A microfluidics-based in vitro model of the gastrointestinal human-microbe interface. *Nature Communications*, *7*, 11535.
103. Clevers, H. (2016). Modeling development and disease with organoids. *Cell*, *165*(7), 1586–1597.
104. Lancaster, M. A., & Knoblich, J. A. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science*, *345*(6194), 1247125.
105. Yin, X., Mead, B. E., Safaee, H., Langer, R., Karp, J. M., & Levy, O. (2016). Engineering stem cell organoids. *Cell Stem Cell*, *18*(1), 25–38.
106. Shyer, A. E., Tallinen, T., Nerurkar, N. L., et al. (2013). Villification: how the gut gets its villi. *Science (New York, N.Y.)*, *342*(6155), 212–218.
107. Basson, M. D. (2003). Paradigms for mechanical signal transduction in the intestinal epithelium. *Digestion*, *68*(4), 217–225.
108. VanDussen, K. L., Marinshaw, J. M., Shaikh, N., et al. (2014). Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. *Gut*, *64*(6), 911–920.
109. Ettayebi, K., Crawford, S. E., Murakami, K., et al. (2016). Replication of human noroviruses in stem cell–derived human enteroids. *Science*, *353*(6306), 1387–1393.
110. Foulke-Abel, J., In, J., Kovbasnjuk, O., et al. (2014). Human enteroids as an ex-vivo model of host–pathogen interactions in the gastrointestinal tract. *Experimental Biology and Medicine (Maywood, N.J.)*, *239*(9), 1124–1134.
111. Ashley, E. A. (2015). The precision medicine initiative: a new national effort. *JAMA*, *313*(21), 2119–2120.
112. Hooper, L. V., & Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science*, *292*(5519), 1115–1118.
113. Claus, S. P., Tsang, T. M., Wang, Y., et al. (2008). Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Molecular Systems Biology*, *4*(1), 219.
114. Roy, S., & Trinchieri, G. (2017). Microbiota: a key orchestrator of cancer therapy. *Nature Reviews Cancer*, *17*(5), 271–285.
115. Stringer, A. M., Gibson, R. J., Logan, R. M., Bowen, J. M., Yeoh, A. S., & Keefe, D. M. (2008). Faecal microflora and  $\beta$ -glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. *Cancer Biology & Therapy*, *7*(12), 1919–1925.
116. Takasuna, K., Hagiwara, T., Hirohashi, M., et al. (1996). Involvement of  $\beta$ -glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative

- irinotecan hydrochloride (CPT-11) in rats. *Cancer Research*, 56(16), 3752–3757.
117. Haiser, H. J., Gootenberg, D. B., Chatman, K., Sirasani, G., Balskus, E. P., & Turnbaugh, P. J. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science*, 341(6143), 295–298.
  118. O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, 7(7), 688–693.
  119. Haiser, H. J., & Turnbaugh, P. J. (2013). Developing a metagenomic view of xenobiotic metabolism. *Pharmacological Research*, 69(1), 21–31.
  120. Falony, G., Joossens, M., Vieira-Silva, S., et al. (2016). Population-level analysis of gut microbiome variation. *Science*, 352(6285), 560–564.
  121. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, 474(7351), 327–336.
  122. Kelly, C. R., Kahn, S., Kashyap, P., et al. (2015). Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. *Gastroenterology*, 149(1), 223–237.
  123. Hamilton, M. J., Weingarden, A. R., Sadowsky, M. J., & Khoruts, A. (2012). Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium Difficile* infection. *The American Journal of Gastroenterology*, 107(5), 761–767.
  124. Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology and Hepatology*, 9(10), 577–589.
  125. Sartor, R. B. (2010). Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. *Gastroenterology*, 139(6), 1816–1819.
  126. Fang, H., Elina, T., Heikki, A., & Seppo, S. (2000). Modulation of humoral immune response through probiotic intake. *FEMS Immunology & Medical Microbiology*, 29(1), 47–52.
  127. Zackular, J. P., Baxter, N. T., Iverson, K. D., et al. (2013). The gut microbiome modulates Colon tumorigenesis. *mBio*, 4(6), e00692–e13.
  128. Sampson, T. R., & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell Host & Microbe*, 17(5), 565–576.
  129. Houser, M. C., & Tansey, M. G. (2017). The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *npj Parkinson's Disease*, 3(1), 3.
  130. Dinan, T. G., & Cryan, J. F. (2017). Gut-brain axis in 2016: Brain-gut-microbiota axis [mdash] mood, metabolism and behaviour. *Nature Reviews Gastroenterology & Hepatology*, 14(2), 69–70.
  131. Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain–gut–microbe communication in health and disease. *Frontiers in Physiology*, 2, 94.
  132. Cucullo, L., Marchi, N., Hossain, M., & Janigro, D. (2011). A dynamic in vitro BBB model for the study of immune cell trafficking into the central nervous system. *Journal of Cerebral Blood Flow & Metabolism*, 31(2), 767–777.
  133. Bicker, J., Alves, G., Fortuna, A., & Falcão, A. (2014). Blood–brain barrier models and their relevance for a successful development of CNS drug delivery systems: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, 87(3), 409–432.
  134. Kostic, A. D., Xavier, R. J., & Gevers, D. (2014). The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*, 146(6), 1489–1499.
  135. De Souza, H. S., Fiocchi, C. (2015). Immunopathogenesis of IBD: current state of the art. *Nature Reviews Gastroenterology & Hepatology*, 13(1), 13–27.
  136. Kang, T. H., & Kim, H. J. (2016). Farewell to animal testing: innovations on human intestinal Microphysiological systems. *Micromachines*, 7(7), 107.
  137. Marzorati, M., Vanhoecke, B., De Ryck, T., et al. (2014). The HMI™ module: a new tool to study the host-microbiota interaction in the human gastrointestinal tract in vitro. *BMC Microbiology*, 14(1), 1.
  138. Lee, J., Choi, J. H., & Kim, H. J. (2016). Human gut-on-a-chip technology: will this revolutionize our understanding of IBD and future treatments? *Expert Review of Gastroenterology & Hepatology*, 10(8), 883–885.
  139. Odijk, M., van der Meer, A. D., Levner, D., et al. (2015). Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems. *Lab on a Chip*, 15(3), 745–752.