

## Review Article

# Organoids, organs-on-chips and other systems, and microbiota

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The human gut microbiome is considered an organ in its entirety and has been the subject of extensive research due to its role in physiology, metabolism, digestion, and immune regulation. Disequilibria of the normal microbiome have been associated with the development of several gastrointestinal diseases, but the exact underlying interactions are not well understood. Conventional *in vivo* and *in vitro* modelling systems fail to faithfully recapitulate the complexity of the human host–gut microbiome, emphasising the requirement for novel systems that provide a platform to study human host–gut microbiome interactions with a more holistic representation of the human *in vivo* microenvironment. In this review, we outline the progression and applications of new and old modelling systems with particular focus on their ability to model and to study host–microbiome cross-talk.

## Introduction

The development of novel prognostic tools and effective therapeutic strategies relies upon a profound understanding of the molecular mechanisms involved in health and disease, including influences from the microbiome [1,2]. For example, the microbiome that inhabits the human gut is integral in regulating normal gut homeostasis. Such physiological functions include aiding digestion, producing metabolites from undigested fibre, regulating drug metabolism, adapting immune responses, and protecting the host from pathogens and infections [3–5]. The realisation that commensal and pathogenic bacteria play a role in dysbiosis, disease development, and chronic disorders [3–5] places an emphasis on gaining a deeper understanding of the underlying mechanisms involved in physiological and pathophysiological host–microbiome cross-talk. The complexity of a holistic understanding of these cause-and-effect relationships in humans makes human studies complicated and somewhat limited, most notably because of the challenges in accessing the human gut, making disease-modelling essential. Preclinical animal models and *in vitro* 2D cell culture studies have provided extensive insights into the physiological and pathophysiological processes that underpin disease. However, as they do not faithfully represent the human body, they are not suitable models to evaluate all research questions nor model drug efficacy. Moreover, the high number of therapeutic compounds that fail to translate in clinical trials [6,7] highlights the need and importance for models that are more physiologically relevant to the human body, in order to personalise treatments and better predict patient outcomes. In this review, we discuss the advantages and disadvantages of conventional modelling systems. Furthermore, we review the advantages and contributions of 3D organoid culture and systems such as organ-on-a-chip that better recapitulate the *in vivo* human body and provide a better understanding of cell–cell interactions and the host–gut microbiome cross-talk that occurs in health and disease. The advantages and disadvantages of the systems discussed in this review are outlined in Table 1. Figure 1 provides schematic representation of these systems.

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**Table 1. Advantages and disadvantages of current culturing techniques with respect to host–microbiome interaction**

Part 1 of 3

Type of assay	Advantages	Disadvantages
2D ethos	<ul style="list-style-type: none"> <li>• Inexpensive, easy-to-use, data generated quickly [9,10].</li> <li>• Retain many genetic, epigenetic, and gene expression features of human cancer [9,10].</li> </ul>	<ul style="list-style-type: none"> <li>• Established from metastatic tumours, restricting their use in cancer progression studies and early drug interventions [149].</li> <li>• Not an accurate representation of the <i>in vivo</i> 3D microenvironment [3,11].</li> <li>• Drug doses that are effective in 2D culture often result in poor efficacy when scaled in to humans [10–13].</li> <li>• 2D culture exerts selection pressures on cells that alter morphology and gene expression [10].</li> </ul>
Transwell	<ul style="list-style-type: none"> <li>• Easily implemented, low-cost assay [145].</li> <li>• High throughput [145].</li> <li>• Can be used to compare metastatic potential of cells [145].</li> <li>• Provides thorough analysis of cells ability to migrate and sense a chemo-attractant [145].</li> <li>• Can be used to investigate cell invasion [146].</li> </ul>	<ul style="list-style-type: none"> <li>• Low physiological relevance [79].</li> <li>• Migration and invasion assays can result in conflicting data [82].</li> <li>• Unstirred water layer may decrease the permeability rate of lipid soluble molecules [82].</li> <li>• Cells are grown in a static media, and there is no shear stress forced on the cells [147].</li> </ul>
Organoids	<ul style="list-style-type: none"> <li>• Near-physiological model for epithelial tissue containing heterogeneous cell populations — maintaining integrity of the <i>in vivo</i> structure [49].</li> <li>• Enable analysis of cell–cell and cell–matrix interactions, while maintaining mechanical properties, gene expression, and metabolic profiles [148].</li> <li>• <i>Ex vivo</i> culture bridges gap between cell culture and animal modelling [149].</li> <li>• Can microinject microbes into the lumen of organoids to study host–pathogen interactions [63].</li> <li>• Organoid/enteroids can be grown in 2D monolayers on Transwell inserts such that microbes can be exposed to the apical cell surface simultaneously with immune cells at the basolateral surface, more representative of the <i>in vivo</i> human microenvironment, and permits human host–pathogen studies [42,75–77].</li> </ul>	<ul style="list-style-type: none"> <li>• Reductionist approach — epithelial cells grow without its native microenvironment [49].</li> <li>• Wrong side of epithelium exposed to compounds/microbiome when added in the media [43].</li> </ul>
SynVivo® (SynTumour)	<ul style="list-style-type: none"> <li>• Realistic vasculature morphology and physiology with diffusion transport [81].</li> <li>• SynVivo's inflammatory model has been successfully validated against <i>in vivo</i> studies [85,86].</li> <li>• Excellent correlation with rolling velocities, adhesion patterns, and migratory processes [85,86].</li> <li>• A SynVivo produced chip can partly mimic the <i>in vivo</i> blood–tumour barrier [82].</li> </ul>	<ul style="list-style-type: none"> <li>• Still cannot be used without conjunction of animal testing [150].</li> <li>• Hard-to-remove bubbles can form — rendering the chip unusable [81].</li> <li>• Not yet applicable to studying microbiome interaction.</li> </ul>
Tissue slices	<ul style="list-style-type: none"> <li>• Has the ability to assess the role of the tumour microenvironment on tumour growth and survival [92].</li> <li>• Freshly resected tissue slices are viable for at least 24 h [92].</li> </ul>	<ul style="list-style-type: none"> <li>• Tissues start to deteriorate after 3 weeks in culture (tissue-dependent) [152].</li> <li>• Donor-to-donor variability [152].</li> <li>• Difficulty in monitoring cells beyond the depth of confocal microscopy (unless cells are isolated for analysis) [152].</li> </ul>

Continued

**Table 1. Advantages and disadvantages of current culturing techniques with respect to host–microbiome interaction**

Part 2 of 3

Type of assay	Advantages	Disadvantages
Organ/tissue baths	<ul style="list-style-type: none"> <li>• Contains a higher level of biological organisation, and has cell–cell interaction, which may better reflect the response of the target organ [151].</li> <li>• Can be used to represent all regions within a tissue [91,92].</li> <li>• Effect of microbiome and pathogenic bacteria on epithelial integrity, permeability, and contractility can be examined when tissue slices are used in conjunction with Ussing chambers [95,98,101].</li> </ul>	<ul style="list-style-type: none"> <li>• The system does not reflect the effects of <i>in vivo</i> systemic factors [152].</li> <li>• Further work is required to maintain anaerobic microbes in intestine [94].</li> </ul>
Organ-on-a-chip	<ul style="list-style-type: none"> <li>• Versatile, simple, and reproducible assay that is suitable for all organ sizes [103,104].</li> <li>• Ability to measure concentration-dependent changes to isometric contractions [103].</li> <li>• Experiment is in real time — can rapidly make conclusions and troubleshoot [103].</li> <li>• 3R's — multiple tissues can be prepared from one animal [103].</li> <li>• Scope to study probiotic and pathogenic microbes <i>ex vivo</i> [107].</li> </ul>	<ul style="list-style-type: none"> <li>• Organs/tissues may experience different degrees of damage during surgical removal [103].</li> <li>• Organs/tissues may also have different viability lengths [103].</li> <li>• Responsiveness of tissues may alter — time controls may be necessary [103].</li> <li>• New tissue required for each experiment [105,106] hindering studies that follow genetic backgrounds.</li> </ul>
Quasi-Vivo® (Kirkstall)	<ul style="list-style-type: none"> <li>• Enables research into physiological and pathophysiological responses of tissues, with interaction from microbes [153,154].</li> <li>• Can be exploited to complement animal studies and even bypass animal modelling in the future [153].</li> <li>• Accurately predict pharmacological effects in patients [153,154].</li> <li>• Raw materials used are relatively cheap [153].</li> <li>• Cheap, versatile, precise, and reliable system [114].</li> </ul>	<ul style="list-style-type: none"> <li>• Microengineering complexities [155], cleanrooms, and pumps can be costly [153].</li> <li>• Surface area and roughness of chip and formation of bubbles can affect capillary forces and flow rate of the microfluid, which can damage cells [154].</li> <li>• Matrix degrades when culturing time increases, affecting cell functionality [153].</li> <li>• Cannot reflect chronic diseases, adaptive immune responses, or mirror complicated behaviours of the endocrine, skeletal, or nervous systems [156].</li> <li>• Human testing is still necessary [155,156].</li> </ul>
HuMiX module	<ul style="list-style-type: none"> <li>• Provides a flexible and easy system that enables long-term co-culture of multiple cell types and improves cell viability [157].</li> <li>• Has the ability to apply high flow rates and create high nutrient turnover to cells without imposing high shear stress or turbulent flow [158].</li> <li>• Enables microwell protocols to be transferred directly to the bioreactor modules without the need to redesign cell culture [130].</li> <li>• Control of oxygen tension to visually represent conditions at the surface and within tumours [130].</li> </ul>	<ul style="list-style-type: none"> <li>• Currently has a limited ability to model all tissue types [130].</li> <li>• The allometric aspects of dosage are unknown [130].</li> <li>• Not applicable to microbiome studies.</li> </ul>
HuMiX module	<ul style="list-style-type: none"> <li>• Can simultaneously establish aerobic and anaerobic conditions and measure oxygen levels in real time [133].</li> <li>• Effects of individual and combinations of bacteria on host physiology are possible [134].</li> </ul>	<ul style="list-style-type: none"> <li>• Cell types cannot fully represent the cellular makeup of the entire intestinal tract [133,134].</li> </ul>

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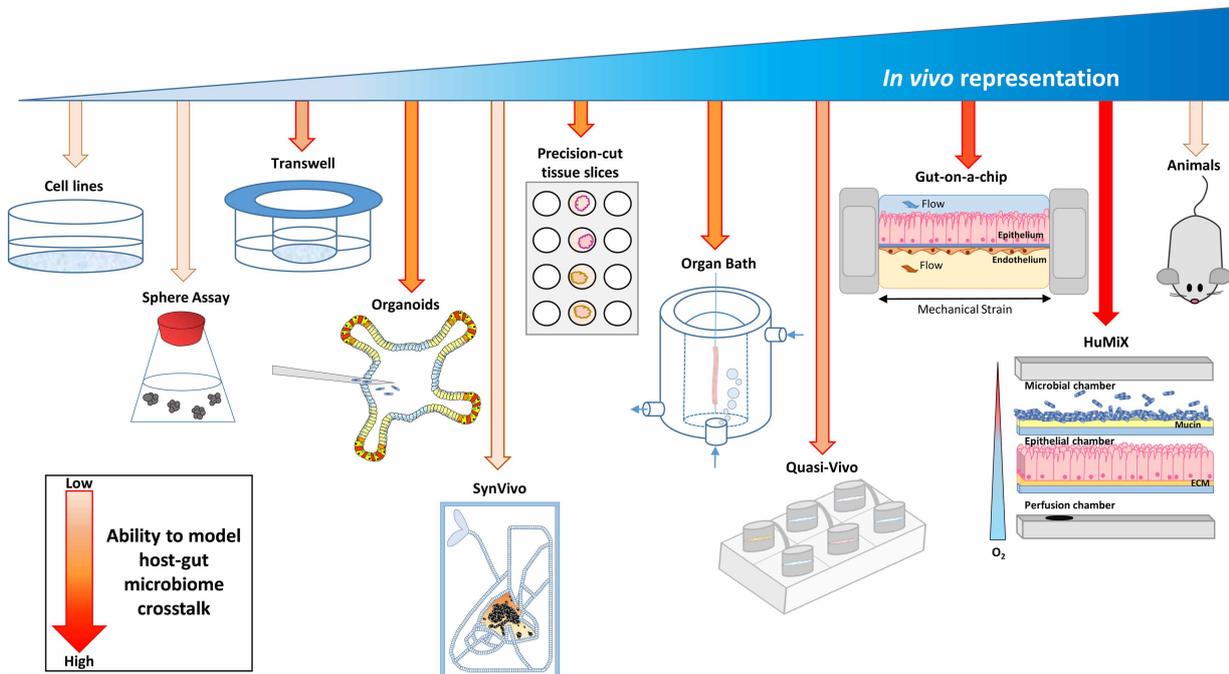
**Table 1. Advantages and disadvantages of current culturing techniques with respect to host–microbiome interaction**

Part 3 of 3

Type of assay	Advantages	Disadvantages
	<ul style="list-style-type: none"> <li>• Can co-culture individual cell lines that are in close proximity to other cell types, yet can be exposed to independent conditions — great for understanding microecology of gut [134].</li> <li>• Ecology of communities can be manipulated, meaning transition states can be studied — potential for identification of early biomarkers in disease linked with dysbiosis [134].</li> </ul>	

## 2D culture and *in vivo* animal models to study human disease

Human cell lines have had a fundamental role in determining the importance of pathways involved in disease and have been a good platform for screening and characterising drug therapeutics [8]. Cell lines retain many genetic, epigenetic and gene expression features of human disease [9,10]. However, they fail to represent the *in vivo* 3D microenvironment, induce cell differentiation [11,12], perform tissue-specific functions [11,13,14], and do not represent the natural cell heterogeneity that compose primary tumours [9]. In addition, it is often found that drug doses that are effective in 2D culture result in poor efficacy when scaled to humans [6,15–17]. Many of these limitations are addressed by genetically engineered mouse models (GEMMs). GEMMs have been an extremely useful tool for studying early and chronic stages of human disease [8], as they share similarities in anatomy, physiology, and genetics [18–22]. However, their use for investigating host–microbiome interactions is limited by the differences in lifespan, variations in diet, and microbiome populations [23]. The inability of these systems to study the cause-and-effect relationships of the host–microbiome interactions in a meaningful way is driving the development of systems to overcome this issue.



**Figure 1. Current cell modelling systems and their potential to model host–microbiome cross-talk.**

Schematic representation of current cell culturing techniques available and their ability to model host–microbiome interactions.

## Cell culturing systems for studying the human microbiome

The gastrointestinal (GI) tract contains a flora of commensal bacteria, known as the microbiome [24]. The living gut microbiome has been of increasing interest in recent years, as its role in the regulation of the immune system, local barrier functions, homeostasis, and tissue regeneration and also in the development of certain diseases (e.g. Crohn's disease, inflammatory bowel disease, and coeliac disease) are not fully understood [3,5,25]. Additionally, it is known that the gut microbiome can influence drug absorption and metabolism [3,26]. Thus, it is paramount that host–gut microbiome interactions are elucidated.

Advancements in techniques for culturing cells have been critical for identifying and understanding cell involvement in disease initiation. *In vitro* sphere-forming assays were among the first techniques adopted to study these interactions. The sphere-forming assays were initially developed to study neural stem cells [27] and were later developed to culture mammary stem cells [28]. In these cultures, cells aggregate together to form 3D spheroid structures, which can then be utilised to determine stem cell behaviour of both human and animal cell populations [29,30]. Typically, the study of human GI disease has relied upon culturing intestinal epithelial cells in static well plate assays. For example, Transwell culture has been used to grow a polarised monolayer of human cell lines [31] on a nanoporous insert [3]. Microbial cells, probiotic [32–35] or pathogenic [36–39], can then be added to the apical cell surface. To gain further insights, immune cells can be simultaneously added to the basolateral side of the monolayer culture [31,40–42]. Transwell culture is limited however, as cultured epithelial cells do not produce functional specialised cell types [12,43]. Consequently, a substantial amount of research has focused on the development of a 3D culturing technique, initially using individual murine intestinal stem cells [44,45] and also patient-derived tissues and human stem cells [46–48] that grow into self-renewing organoids that encompass the structure, and all cell types of the *in vivo* intestinal crypt, providing a more human-relevant model.

## The progression from 2D to 3D organoid culture

Long-term organoid culturing has been a major breakthrough in research and is now an extensively used tool for studying both basic and clinical biology [49–51]. Advancement in technology has enabled culturing of the stomach [52–54], pancreas [55–57], liver [58], prostate [59,60], oesophagus [46,61], gall bladder [62,63], and taste buds [64]. Additionally, CRISPR-Cas9 technology has been exploited to induce gene editing in intestinal organoids that mimics the sequential loss and gain of functions of genes involved in human colorectal cancer [65], which, after in orthotopic engraftment, could metastasise to the liver [66,67]. Thus, organoid models can be used to study advanced aspects of cancer development in a more complexed *in vivo* scenario. Organoid culture has been exploited to investigate host–gut microbiome interactions by microinjection of microbes to the lumen of organoids (apical surface, as this mimics aspects of microbial infection *in vivo*) or basally by the addition of microbes to the media [63]. The potential of organoids as a model for studying host–microbial interactions has been demonstrated using human gastric organoids and the pathogenic microbe, *Helicobacter pylori* [54,68,69], that is known to cause infection, ulceration, and gastric cancer in humans [70]. Apical infection of gastric organoids with *H. pylori* recapitulates known hallmarks of infection, including a marked increase in mucus-secreting cell expression, induction of cytokine production via up-regulation of NF- $\kappa$ B (known to induce gastritis), and production of the *H. pylori* virulence factor CagA (cytotoxin-associated gene A), which is associated with motogenesis and tumour progression [71,72]. Other studies have been able to efficiently infect human-derived organoids in a similar manner with a range of known pathogens, including *Salmonella typhimurium* [63], *Clostridium difficile* [73,74], and rotaviruses [75]. Thus, these studies present a valuable near-physiological modelling system in which to begin deciphering the complex interactions between host epithelium and infectious agents. Additionally, several recent studies have improved the human relevance of Transwell cultures. For example, Transwells have been adapted to grow human small intestinal and colonic organoids in monolayers on Transwell membranes that support microbial (*Escherichia coli* [42,76,77] and norovirus [78]) adherence at the cell surface, as seen *in vivo*. The above studies have provided an informative platform that can be exploited to use patient-derived tissues to study donor-specific disease progression, host responses to enteric pathogens, and provide a platform to develop personalised treatments.

However, like any other system, organoid culture has its limitations. In instances where microbes are added to culture medium, microbial overgrowth often occurs, which can damage the epithelium and can hinder long-term co-culture, this is also the case with co-culturing human cell lines with microbes using Transwells [3,43] and, thus, should be avoided where possible. It is not possible to exert peristalsis-like motions (which is

required *in vivo* to sustain intestinal differentiation and confine microbial overgrowth [14]) to the cells cultured in Transwell or organoid culture. And the reductionist approach of organoid culture limits the full potential of this method as epithelial cells grow without their native microenvironment, and the true contributions from the stroma, extracellular matrix (ECM), oxygen, vasculature, immune system, and the microbiome cannot be truly assessed [49,79]. This ultimately impedes the understanding of the precise molecular mechanisms involved in transition from healthy to disease states and limits the potential efficacy of interventions when translated to clinic.

It is evident that models, which reflect the holistic environment, are crucial to better predict patient outcomes to therapy, understand the mechanisms that underpin disease and the role environmental factors, such as diet, have on physiology and pathophysiology [12]. The integration of microfluidics with living biological systems has paved the way for new techniques such as the exciting ‘organ-on-a-chip’ concepts, which aims at developing advanced *in vitro* models that replicate the key features of human tissues and organs [80]. The culturing methods outlined below are discussed in increasing order of their ability to represent the *in vivo* environment and its suitability to study host–microbe interactions.

## SynVivo®

SynVivo microfluidic models have been developed to study human tissue microvasculature, cell–cell, and cell–drug interactions in a 3D setting, in the context of *in vivo*-relevant flow and pressures [81]. Currently, SynVivo models have four independent assays that represent the blood–brain barrier [82–84], cancer [85], inflammation, [86,87] and toxicology. SynVivo devices utilise polydimethylsiloxane (PDMS) chambers that comprise a microfluidic map of vasculature with spaces that can be lined with ECM. For example, endothelial cells can be cultured on fibronectin-coated channels and activated using cytokines. Endothelial cell interactions with white blood cells can then be assessed in real time by the injection of cells into the system [81]. Synthetic network assays can also recapitulate *in vivo* leaky vasculature associated with tumourigenesis and thus can be utilised to study both basic and applied research [81]. To date, SynVivo chips have not yet been developed to study microbial interactions; however, its ability to recreate a realistic vasculature network surrounding a tissue will make a profound advancement in modelling *in vivo* 3D environments.

## Tissue slices

Precision-cut tissue slices (PCTS) are a useful tool for metabolic, pharmacology, and toxicological studies [88–90]. Predominately used to model solid organs (e.g. liver and kidney) and agarose-embedded non-solid organs such as the intestine [91,92], uniform tissue slices are cut and incubated in media in 6- or 12-well plates and exposed to atmospheric conditions [92]. PCTS are believed to have advantages over organoids and organ-on-a-chip (discussed later), as they represent all regions of the tissue [91]. It has been reported that murine intestine is maintained for 6 days in the presence of normal neuronal, muscular, and mucosal structures with contractibility functions and some immune responses, therefore, representing a more holistic intestinal organ [93]. Additionally, in the absence of antibiotic media, some aerobic commensal microbes can be maintained [93]. Although this system holds great promise to study holistically the complex mechanisms that underpin healthy gut functions and disease in a 3D physiologically relevant environment, further work is required to maintain anaerobic microbes that are predominately found within the intestinal environment [94]. In addition to maintaining tissue slices in media, epithelial integrity, neural activity, ion/nutrient/drug transport [95–97], and the effect of microbiota and pathogenic bacteria on these processes [98–101] can be examined using analytical tools, such as the Ussing chamber. As this tool compartmentalises the apical and basolateral surfaces of the tissue, each surface can be exposed to independent physiological solutions [95], representative of the *in vivo* microenvironment. The potential of the Ussing chamber to investigate host–pathogen interactions was highlighted when co-culture of human cell lines with the infection-causing bacteria, *C. difficile*, extensively impaired epithelial barrier function and elicited cytotoxic immune responses which contributes to *C. difficile*-mediated pathogenesis [101]. One limitation of the Ussing chamber, however, is that intestinal tissue viability is often limited to 3 h which limits long-term optimal tissue function studies [102].

## Organ baths

Organ bath assays play important roles in evaluating concentration–response relationships in a variety of tissues, predominately contractile organs. The primary advantage of organ baths is that live tissue is cultured that continues to function as a whole unit with respect to contractions visualised *in vivo*. By retaining these

physiological functions, pharmaceutical interactions can be examined to predict *in vivo* responses [103,104]. However, because of using relatively large samples, the core of the suspended tissue strips can often become hypoxic, which can hinder contractile readouts [104]. Another caveat with organ baths is the requirement of fresh tissue for each experiment [105,106]. This prevents analysis from a single donor or genetic background, which is achievable through 3D organoid culture. However, organ baths have been used to show that *E. coli* stimulates the contractility of human colonic smooth muscle cells [107]. Thus, there is scope for advancing organ bath techniques to study probiotic and pathogenic microbes *ex vivo*.

## Organ-on-a-chip

To mimic key aspects of living organs, such as the multicellular structures, cell–cell and tissue–tissue interactions, and the native microenvironment, organ-on-a-chip systems have been developed to incorporate microfluidic and microengineering technology [7,108]. The benefit of developing such systems is that it provides a platform to study the complex physiological and pathophysiological responses of tissues at an organ level, to provide patients with quicker access to new medication. For example, microfluidics can be utilised to mimic blood and nutrient flow and maintain mucus transition in lung epithelia [108,109]. In addition, this system can be exploited to complement animal studies and also more accurately predict pharmacological effects in patients and, in the future, could be used to bypass the use of animal testing completely [110]. Organ-on-a-chip has been developed for several organs, including the lung [111,112], heart [113,114], liver [115], neuron [116], kidney [117,118], gut [14,43,119], blood vessel [120,121], tumour-on-a-chip [122], bone marrow-on-a-chip [123], liver-tumour-bone marrow-on-a-chip [124], and liver-skin-intestine-kidney-on-a-chip [125]. These systems can be extended to disease modelling, pharmaceutical analysis, drug development strategies [6,118,122], and understanding host–microbe interactions [14,111,122]. For the purpose of this review, we will discuss the use of organ-on-a-chip to study intestinal dynamics with particular focus on the gut microbiome. The potential of these systems to accurately model drug uptake and metabolism in the human body has recently been shown in the context of products produced by the microbiome. Verneti et al. (2017) demonstrated that a liver-intestine-kidney-on-a-chip system integrated with an intact blood–brain barrier/neurovascular unit sufficiently modelled *in vivo* trimethylamine (a by-product of the microbiome) metabolism. Specifically, trimethylamine, microinjected into intestinal organoids, was found in the basolateral media and was subsequently metabolised to trimethylamine *N*-oxide by the liver module and then secreted into the lumen of the kidney module, which occurs *in vivo*. The study also revealed a novel finding that trimethylamine *N*-oxide crosses the blood–brain barrier [126], emphasising the potential of such systems to further our understanding of human physiology.

## Progression to gut-on-a-chip assays

The applications of gut-on-a-chip systems are still at the early stages of development [80]; however, they hold great promise for studying the interactions between host and microbiome. Initial studies involved culturing a monolayer of human epithelial Caco-2 cells inside a microfluidic device [127]. Although this microchip provided a vascular flow over the cells, the ability of Caco-2 cells to reach confluence was hindered. This was likely caused from inherent faults within the system that did not allow large volumes of medium, and thus nutrients, to be administered at a time, and resulted in cellular damage from dead cell debris, air bubbles, and the inability to maintain a stable medium flow [127]. These problems were circumvented by identifying optimal microchannel dimensions and appropriate Caco-2 cell density, which provided sufficient nutrients and reduced shear pressures that ultimately promoted Caco-2 cellular growth and normal barrier function [127]. This study highlights the importance of chip scaling, and this should be taken into consideration when developing novel microfluidic devices in the future. More advanced microfluidic models have been subsequently developed, based on the culture of Caco-2 cells under physiologically relevant conditions, including peristalsis-like motions, intraluminal fluid flow, the use of 3D scaffolds, and support the growth of the naturally occurring microbiome without diminishing cell viability [43]. These earliest models of gut-on-a-chip were adapted from the advancements in lung-on-a-chip that enabled microfluidic systems to effectively model the cyclic breathing motions of the human lung [111]. In 2012, a biomimetic human-gut-on-a-chip microdevice was developed that consisted of two closely apposed microfluidic channels made from a self-adhesive elastomer, PDMS (that is highly permeable to oxygen), creating a luminal and capillary channel [43]. Between the channels was a thin elastic porous membrane coated with an ECM. A layer of Caco-2 cells was cultured on top of the ECM. Culture medium was allowed to flow through both channels to mimic *in vivo* fluid flow and shear stress.

Peristaltic-like deformations were instigated by cyclic suction patterns to stretch and relax the porous membrane [43]. The group reported that this human-gut-on-a-chip system allowed intestinal epithelial cells to polarise, form 3D structures that closely resembled physical and functional features of human intestine and created a high-integrity barrier against small molecules which was not achieved when cells were cultured using Transwell models [43]. Additionally, this system allows the study of physiological gut functions in the presence of relevant cues and interactions with the microflora. Caco-2 cells have been effectively co-cultured with a naturally occurring intestinal microbe, *Lactobacillus rhamnosus* GG (LGG), at the luminal surface which improved intestinal barrier function [43], which has been previously reported with probiotic bacteria strains in humans [128]. Moreover, differentiated intestinal epithelium supported the growth of naturally occurring microbiota for more than 2 weeks, which is in contrast with Transwell and organoid cultures where cell viability is often lost within a few hours [12,14,43]. Taken together, these studies provide support for the use of gut-on-a-chip as a tool for studying the mechanisms involved in intestinal function and the involvement of the normal gut microbiome. This system also has the potential to be exploited to study the development of disease in a more human-relevant holistic environment, become a novel platform for drug development, and better predict responses to therapy in clinical trials.

The potential of gut-on-a-chip was proved in a proof-of-principal study in 2016 when Kim et al. studied the contribution of the microbiome to gut pathophysiology. Specifically, Caco-2 cells were co-cultured, with eight living human commensal microbes that have previously been used in clinical trials of chronic inflammatory bowel diseases [129]. Caco-2 cells displayed distinct gene expression profiles and phenotypes when cultured in the presence of commensal microbes, which closely resembled the normal human ileum. Next, the group investigated whether gut-on-a-chip was sufficient to model human intestinal inflammation, and what contribution the gut microbiome played. Culturing Caco-2 cells with immune cells and non-pathogenic bacteria, to induce pro-inflammatory cytokines, resulted in injury to the villus structure and impaired barrier function. By independently controlling the addition of each cytokine to the system, the authors were able to elucidate that only in the presence of interleukin-8 were the cytokines able to induce disease-promoting effects, which is not easy to model using GEMMs. Furthermore, the extent of villus injury and compromised barrier function was reduced and delayed by the addition of commensal probiotic microbes within the chip. Subsequently, antibiotic therapies that are commonly used in clinic to treat intestinal inflammatory disease were added to protect the epithelial cells from damage [14]. This provides direct evidence that gut-on-a-chip is efficient in mimicking the suppression of injury responses from bacteria and the immune system that are observed clinically [14]. More importantly, the gut-on-a-chip assay provides a robust platform to identify the individual and combinational effects of immune cells, commensal and pathogenic microbes, and epithelia and endothelial cells in a system that closely resembles the *in vivo* environment. Although still not foolproof, this system enables the study of intestinal physiology to identify mechanisms involved in gut pathophysiology and could be used to find novel therapeutics in a patient-specific manner in the future.

## Quasi-Vivo® (Kirkstall)

Kirkstall have designed a system based on organ-on-a-chip models to produce a ‘system-on-a-plate’ array, named Quasi-Vivo [130]. Quasi-Vivo technology aims at producing a down-scaled system of the human body (through the use of allometrics, [131,132]), such that *in vivo* biochemical and physical stimuli, metabolic, volumetric and exchange rate relationships are retained *in vitro* [130]. Advantages of Quasi-Vivo over organ-on-a-chip devices include limited cellular damage from high wall shear stresses [130,133] and the inherent issue with bubble formation, which occurs in microfluidic systems, is overcome due to their intelligent design features and size [130]. Preliminary findings from Kirkstall show that *E. coli* is viable within the Quasi-Vivo system and, thus, has scope for future microbial analysis.

## HuMix module

The HuMiX, human–microbial cross-talk module, composes three microfluidic chambers that are parallel to one another and separated by semipermeable membranes. The alignment of these chambers provides a microbial, epithelial, and perfusion chamber. The specific outlets and inlets of each chamber allow for the inoculation of cell lines and, through the perfusion of cell growth media, can precisely control the physiochemical conditions each chamber receives [24,134,135]. This set-up builds on previous systems such that it (1) has the ability to simultaneously establish aerobic and anaerobic conditions and (2) can monitor oxygen levels in real time [134].

Several proof-of-concept studies have highlighted the potential HuMiX to enhance our understanding of host–microbe cross-talk [134,135]. Specifically, Eain et al. report that HuMiX was sufficient to co-culture Caco-2 cells and microbial cells (LGG and *Bacteroides caccae*) under conditions that represented the *in vivo* human GI tract, i.e. the maintenance of oxygen gradients either side of the epithelium. The arterial blood supply was mimicked by the addition of an oxygen-rich media in the perfusion chamber, which provided epithelial cells with oxygen from the basal surface. In doing so, the epithelium could grow in a shear-free environment, while anaerobes were present [135]. Additionally, the group were able to co-culture immune cells via the perfusion chamber and are now focusing on developing a system, Immuno-HuMiX, to study the interactions between the immune system and the intestinal microbiota in the human gut. However, the authors note that despite the success in co-culture of human and microbial cells and the potential to extend this system to study diet, drug screening, discovery, and delivery, these cell types cannot fully represent the complex cellular makeup of the entire intestinal tract [134,135].

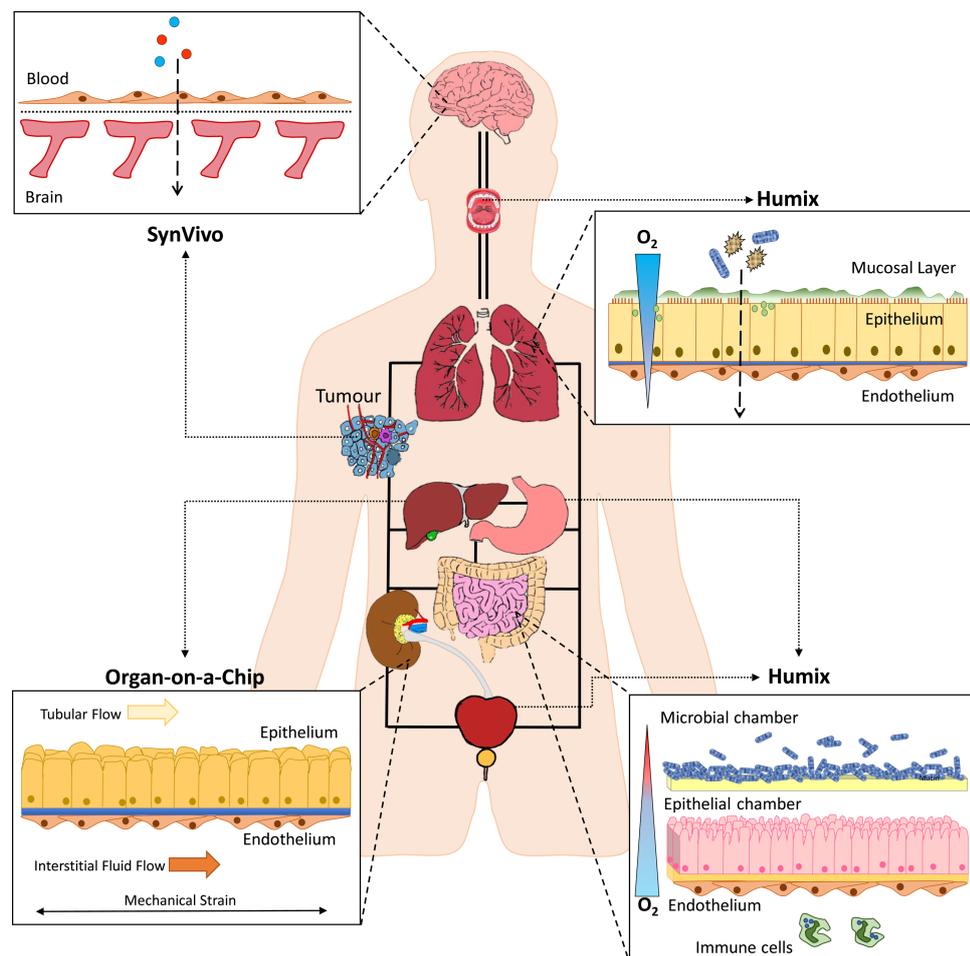
## Bringing cell culture techniques closer to *in vivo*: future directions

There is an abundance of unknown information with regard to the complexity of the gut microbiome and its effect on the human host, which precludes the development of novel therapeutics for disease states. Recently, significant advancements in the scientific field have attempted to unravel these host–gut microbiome interactions. Although the reductionist approach of previous conventional modelling systems, such as static 2D and 3D cell culture, has improved our understanding of how the gut environment is relevant to both human physiology and pathophysiology, advancements in technology have paved the way to microfluidic-based devices that provide a more accurate representation of the *in vivo* microenvironment.

Microfluidic platforms, such as organ-on-a-chip, HuMiX, and other devices, have significant potential in advancing diagnostic, pharmaceutical, and nutritional industries with respect to earlier diagnosis, expedition of drug development, and understanding the mechanisms that underpin dietary involvement in health and disease. As these systems permit 3D co-culture of epithelial cells with underlying endothelium, immune cells, and microbes with the influence of flow and shear effects, they will be fundamental for further improving our understanding of the host–microbiome interactions holistically. Having said that, there are many challenges microfluidic technology still face today which require tweaking to better recapitulate the *in vivo* host–microbiome environment. Firstly, most systems heavily rely on the use of Caco-2 cells as a model of human intestinal epithelium. While this cell line has been crucial in expanding our knowledge, Caco-2 cells were originally from a human colorectal adenocarcinoma [7,24] and thus may have attributes that distinguish them from the normal gut epithelium and any cross-talk between the host–gut microbiome may not be a true representation of the native intestine. Specific issues of using Caco-2 cells to model gut epithelium are (1) Caco-2 cells, a colon cancer-derived cell line, especially in the context of gut-on-a-chip assays, are used to study small intestinal villus formation [12,14,43]. It is necessary to point out that the human colonic epithelium is not composed of villi [136] and that cancer cells can behave differently from normal epithelial cells. (2) Only loose observations can be made about gut physiology when using Caco-2 cells, as it is not clear which section of the intestine, i.e. duodenum, ileum, jejunum or colon, is being modelled. It is important to know exactly which region of the intestine is being modelled such that it mimics the architecture, cellular makeup and immune responses that differs throughout the intestine. For example, the small intestine tends to be more acidic and contains a single mucosal layer, whereas the colon consists of a protective thick double-layered mucus [137] that harbours a higher bacterial density than the small intestine [136]. Therefore, without knowing which section of intestine is being modelled, informed conclusions cannot be made. And (3) interactions between the host and microbes that primarily inhabit the human colon are analysed in the context of epithelial villus formation and barrier integrity. Again, this is not a true representation of human gut physiology and thus emphasises that Caco-2 studies should be limited when evaluating gut physiology. The progression in generating primary epithelium and organoids from stem cells holds great promise for advancing these techniques to recreate the cellular makeup and environmental conditions prevalent in the human gut, especially in light of the available organoid monolayer Transwell culturing methods that utilises donor-specific tissue and enables co-culture with microbes [42,76,77] and which has the potential to be translated to organ-on-a-chip and HuMiX modelling systems. Secondly, most microfluidic devices have been sufficient only in culturing aerobic microbes, which does not reflect the anaerobic nature of the intestinal lumen [24,94]. The development of the state-of-the-art

HuMiX system has provided a platform to culture aerobic-dependent epithelium in the presence of anaerobic microbes, but further refinement of this system is required to decipher the complex interactions between the holistic gut environment (stroma, immune system, diet, microbiome, and metabolome) and the roles they play in maintaining intestinal physiology and initiating and preventing intestinal diseases. The third challenge is a technical issue. The material, PDMS, which is currently used to generate organ-on-a-chip microfluidic chambers does not faithfully represent the physicochemical properties of the ECM that occur *in vivo*, and can often preclude drug studies as small hydrophobic molecules are absorbed by the material [138,139]. In addition, PDMS poses challenges for large-scale reproduction of microfluidic chambers. However, studies have been performed to identify suitable alternative materials or surface coating techniques to combat this issue [140–142].

The challenge for the future is to develop a human-on-a-chip system [143,144], through the amalgamation of the technologies described herein, in which several chambers can be interconnected to represent all aspects of the human body (Figure 2), including factors such as the blood–brain barrier, aerobic–anaerobic nature of the gut epithelium, and microbiome and disease states, i.e. tumours, to functionally mimic and study the complex interactions between the circulatory, endocrine, digestive, immune, lymphatic, nervous, respiratory, and urinary systems. Moreover, such a system would provide a holistic scope to investigate pathophysiological conditions through subtle manipulations within chambers and will also provide a basis to systemically assess drug interventions in the future.



**Figure 2. A proposed microfluidic chip to model host–microbiome cross-talk.**

An amalgamation of SynVivo, organ-on-a-chip, and HuMiX culturing systems has the potential to model the host–microbiome interactions within the entire human body, in the presence of flow and shear stresses (representative of a circulatory system).

## Summary

- Understanding, holistically, the mechanisms that underpin human physiology and pathophysiology are hindered due to the difficulty in accessing organs, and a paucity of information on the interactions between the host and microbiome.
- Conventional modelling systems such as 2D cell culture and Transwells fail to faithfully represent the *in vivo* human environment, especially in the context of studying host–microbiome cross-talk. Emphasising a need for novel modelling systems that better recapitulate the human body.
- Technological advancements involving microfluidic techniques has paved the way for exciting organ-on-a-chip based culturing methods.
- Novel *in vitro* and *ex vivo* culturing techniques such as patient-derived organoid culture, SynVivo®, Quasi-Vivo®, organ-on-a-chip and HuMiX systems, are beginning to provide more clinically relevant platforms in which to better model and study human host-microbiome interactions.

## Abbreviations

CagA, cytotoxin-associated gene A; CRISPR-Cas9, clusters of regularly interspaced short palindromic repeats-CRISPR associated protein 9; ECM, extracellular matrix; GEMMs, genetically engineered mouse models; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; PCTS, precision-cut tissue sections; PDMS, polydimethylsiloxane.

## Author Contribution

S.M. and S.E. designed and wrote the manuscript. L.P. provided guidance, editing, and final approval of manuscript.

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## Competing Interests

The Authors declare that there are no competing interests associated with the manuscript information.

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