Research
Microecology—Perspective

Engineering Solutions for Representative Models of the Gastrointestinal Human-Microbe Interface

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ABSTRACT

Host-microbe interactions at the gastrointestinal interface have emerged as a key component in the governance of human health and disease. Advances in micro-physiological systems are providing researchers with unprecedented access and insights into this complex relationship. These systems combine the benefits of microengineering, microfluidics, and cell culture in a bid to recreate the environmental conditions prevalent in the human gut. Here we present the human-microbial cross talk (HuMiX) platform, one such system that leverages this multidisciplinary approach to provide a representative *in vitro* model of the human gastrointestinal interface. HuMiX presents a novel and robust means to study the molecular interactions at the host-microbe interface. We summarize our proof-of-concept results obtained using the platform and highlight its potential to greatly enhance our understanding of host-microbe interactions with a potential to greatly impact the pharmaceutical, food, nutrition, and healthcare industries in the future. A number of key questions and challenges facing these technologies are also discussed.

1. Introduction

Microorganisms, comprising bacteria, archaea, and microeukaryotes, represent the smallest living organisms on the planet. Despite their small size, they play a crucial role in the governance of numerous systems, including the human body. In terms of numbers, the abundance of bacterial cells inside and on the human body are thought to be of the same order of magnitude as the number of human cells that actually make up the body [1]. Human microbiota colonize the surfaces of different human tissues. The majority resides within the gastrointestinal tract (GIT), where it is the main constituent of this extremely diverse and dynamic ecosystem. The status of this system depends on a myriad of factors, including host genetics, immune status, and diet [2].

Numerous studies have highlighted the key role that the gastrointestinal microbiome plays in processes such as digestion, nutrition, metabolism, and, most importantly, in the pathogenesis of disease [3–7]. More specifically, modern high-resolution molecular analyses have implied a link between dysbiosis (a disequilibrium in the microbial ecology of the GIT) and a range of idiopathic diseases, including obesity [8], diabetes [9], colorectal cancer [10], neurological conditions [11], and allergies [12]. One key method is metagenomics, which involves the sequencing of genomic DNA extracted from the gut microbiome. This method provides a rapid and precise means of taxonomy and identification of individual microbes within the gut. Besides metagenomics, functional omic approaches, such as metatranscriptomics, metaproteomics, and metabolomics, provide qualitative and quantitative information on transcripts, proteins, and metabolites present in microbial communities at specific points in space and time [13–15]. These meta-omic analyses provide a useful means of identifying, quantifying, and functionally characterizing the microbes present

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within the gut. However, to causally link the identified differences in the human microbiota with distinct human diseases, experiments allowing the testing and validation of results derived from meta-omics studies are essential [16]. In other words, beyond associations derived from analyses of samples collected *in vivo*, it is now critical to develop a detailed mechanistic understanding of the fundamental molecular mechanisms at play in host-microbe interactions and their role in immune regulation, infection, and metabolism.

While it is possible to study host-microbe interactions *in vivo* using animal models [17] and *in vitro*, for example using Transwell systems [18], it has been well established that these models are not physiologically representative of the conditions within the human GIT [19]. In addition, animal models are very costly, both from financial and temporal standpoints [20]. Hence, there is a significant need to develop *in vitro* solutions that recreate the physiological conditions present inside the human GIT for rapid, reproducible, and high-throughput experimentation.

Recent advances in microengineering, biomaterials, and electronics have provided an alternative means to study molecular interactions at the gastrointestinal human-microbe interface. More specifically, by applying the microfabrication techniques developed for the semiconductor and electronics industries to fluidic automation, it is now possible to construct *in vitro* systems that more closely approximate those conditions present within the gut and other organs on scales identical to those encountered *in vivo*. Such platforms are referred to as an "organ-on-a-chip," and, when seeded with the relevant host cells, make use of microfluidics to mimic the specific physiological conditions seen *in vitro*. These microfluidics-based systems offer numerous advantages over traditional cell-culturing techniques, including a 3D culture environment, provisions for long-term experiments, greater experimental flexibility, the ability to deliver nutrients and other chemical cues to cells in a controlled manner, much-reduced reagent requirements, the ability to precisely tune spatiotemporal oxygen and pH gradients, low shear environments, and the ability for high-throughput experimentation.

A number of microfluidics-based *in vitro* intestinal models have been developed. These involve the culture of human intestinal epithelial cells in hydrogels [21], in polydimethylsiloxane (PDMS)-based chips [22,23], or on polyamide membranes [24]. However, these systems are not representative of the *in vivo* situation, as they fail to reproduce the dynamic microenvironment of the intestine—most notably, the distinct flow regimes that are prevalent at the human-microbe interface. In addition, these approaches fail to recreate the anaerobic conditions for culturing representative bacterial species from the gut (they only allow the culturing of aerobically growing probiotic strains); they do not provide a constant supply of nutrients to the basal interface of the epithelial cells, as is the case *in vivo* through arterial blood supply; and they are not modular, thereby not allowing rapid extraction of the cells post co-culture for detailed molecular analyses [25]. The following section presents the human-microbial cross talk (HuMiX) platform, a modular microfluidics-based human-microbial co-culture system. This platform overcomes a number of the limitations of the previously described *in vitro* models and provides a more representative model of the human gastrointestinal-microbe environment, as validated by an extensive suite of proof-of-concept experiments.

2. Human-microbial cross talk (HuMiX)

The HuMiX platform (Fig. 1) [25] consists of three parallel microfluidic channels. These stacked and aligned channels act as microchambers, and are referred to as the microbial, epithelial, and perfusion microchambers, respectively (Fig. 1(c)). Each chamber has dedicated inlets and outlets, which allow for the inoculation of the relevant cell lines and the precise control of the physiochemical conditions within each microchamber through the perfusion of dedicated cell growth media. Furthermore, the dedicated outlets allow for the collection of eluates from the individual chambers for subsequent analyses. The channels (200 mm × 4 mm × 0.5 mm) are laser cut from polymer gaskets and follow a distinct spiral pattern that optimizes the footprint of the system. The channels are separated from one another by semipermeable polycarbonate membranes. The pore sizes of the membranes (50 nm and 1 µm) are distinct according to their particular function. The 50 nm pore size membrane separates the microbial and epithelial chambers and prevents infiltration of microorganisms into the epithelial chamber, while the 1 µm pore size membrane separates the perfusion chamber from the epithelial chamber and allows diffusion of the cell growth medium to the epithelial chamber (Fig. 1(c)). In addition, the membranes are coated with mucin (50 nm pore size membrane) and collagen (1 µm pore size membrane) during co-culture, which aids in the adherence of the cells to the membranes. During incubation, Dulbecco's Modified Eagle Medium (DMEM) is perfused through the microbial and perfusion chambers via a peristaltic pump to simulate the peristaltic motions and intraluminal fluid flow present within the gut, thereby creating an environment that is
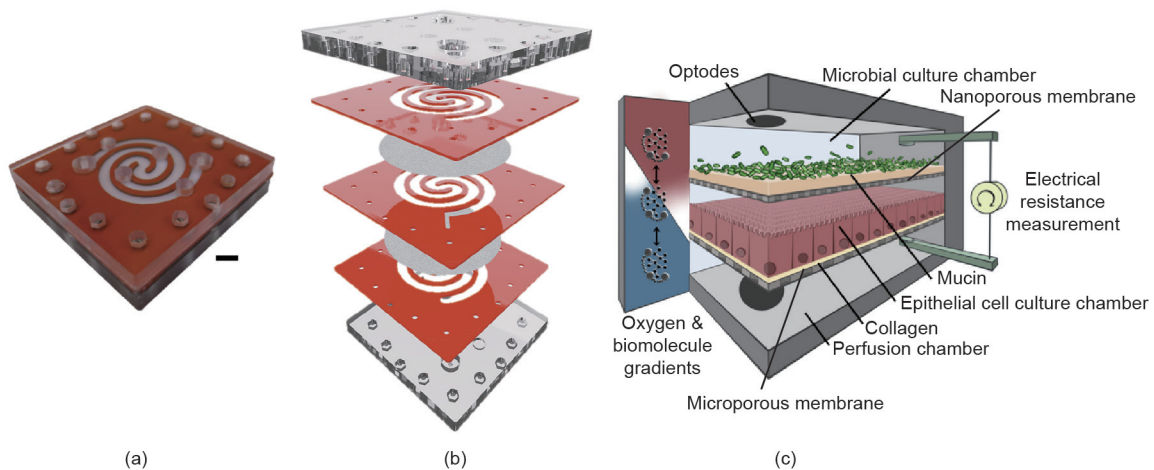

Fig. 1. The HuMiX platform [25]. (a) Image of the assembled HuMiX platform (the scale bar is equivalent to 1 cm); (b) exploded view of the HuMiX platform; (c) annotated schematic illustration of the key features in the HuMiX platform.

representative of a healthy intact epithelial barrier. The environment within the device is routinely monitored through a series of non-invasive oxygen sensors, while the cell growth and differentiation within the device are measured using the standard transepithelial electrical resistance (TEER) measurement electrode, which can be inserted into the device. A more detailed description of the platform and its operating parameters can be found in Ref. [25]. The following section details the co-culture of human and bacterial cells within the HuMiX platform and illustrates the ability of HuMiX to successfully recreate the microenvironment that exists within the human GIT.

2.1. Proof of concept

To validate the ability of HuMiX to successfully co-culture human and microbial cells and to establish environmental conditions closer to those prevalent in the human GIT, the epithelial chamber was inoculated with the human epithelial colorectal adenocarcinoma cell line Caco-2, while the microbial chamber was inoculated with the probiotic *Lactobacillus rhamnosus GG* (LGG). The human cells grew on the collagen-coated microporous membrane and were supplied from their basal side with oxygen-rich DMEM via the perfusion chamber, thereby fluidically mimicking the arterial blood supply and providing a shear-free environment in the epithelial microchamber for the cells to grow. The differentiation of the epithelial cells was evaluated by measuring the TEER of the Caco-2 cell layer (Fig. 2(a)) [25], and through microscopic observation (Fig. 2(b) and (c)). Once a confluent cell layer was established (this typically occurs six days after inoculation), the microbial chamber was seeded with anaerobically grown LGG bacterial cells. The microbial chamber was perfused with bacterial culture medium, in which the O_2 concentration was reduced to below 1% by N_2 gas. Importantly, the modular architecture of HuMiX facilitates access to the individual cell contingents following a particular co-culture regimen. This allows for the extraction of the intracellular biomolecules from the cultured cells, and the obtained DNA, RNA, and protein fractions are subsequently subjected to high-resolution omic analyses [26]. The viability of the co-cultured cells was determined via fluorescence microscopy (Fig. 2(c) and (d)), and such analyses demonstrated that no cytotoxic effects were induced in either cell contingents following 24 h of co-culture. Visible differences in the eluates from the three chambers support the hypothesis of distinct microenvironments within each chamber (Fig. 2(e)). The simultaneous and continuous perfusion of both oxic (21% dissolved O_2) and anoxic (0.1% dissolved O_2) DMEM through the perfusion and microbial chambers, respectively, allows for the establishment and maintenance of the oxygen gradients present *in vivo*. The measured levels, 5.43% ± 0.14% and < 0.8%, are comparable to those recorded in human intestinal tissues (i.e., approximately 4.6% [27]), and in the lumen (i.e., approximately 0.2% [28]), respectively. The data in Fig. 2 [25] illustrates the ability of HuMiX to successfully co-culture human and microbial cells in an environment similar to that encountered within the GIT. To further demonstrate the validity of the platform, studies were performed with additional bacterial and human cell inocula, most notably *Bacteroides caccae* (*B. caccae*) and CCD-18Co, respectively. Furthermore, co-culturing was also performed with primary CD4+ T cells present within the perfusion microchamber. More explicit details of these studies, including results, can be found in Ref. [25]. While the results demonstrate the capability of HuMiX to successfully co-culture different human cell types and microbial cells, it is worth noting that individual cell types only partially represent the complexity of the whole gastrointestinal tissue.

As part of our proof-of-concept experiments, we established different culture regimes to determine if HuMiX-based cellular read-outs were in agreement with previous *in vivo* data. Our results demonstrate a high degree of congruence between the
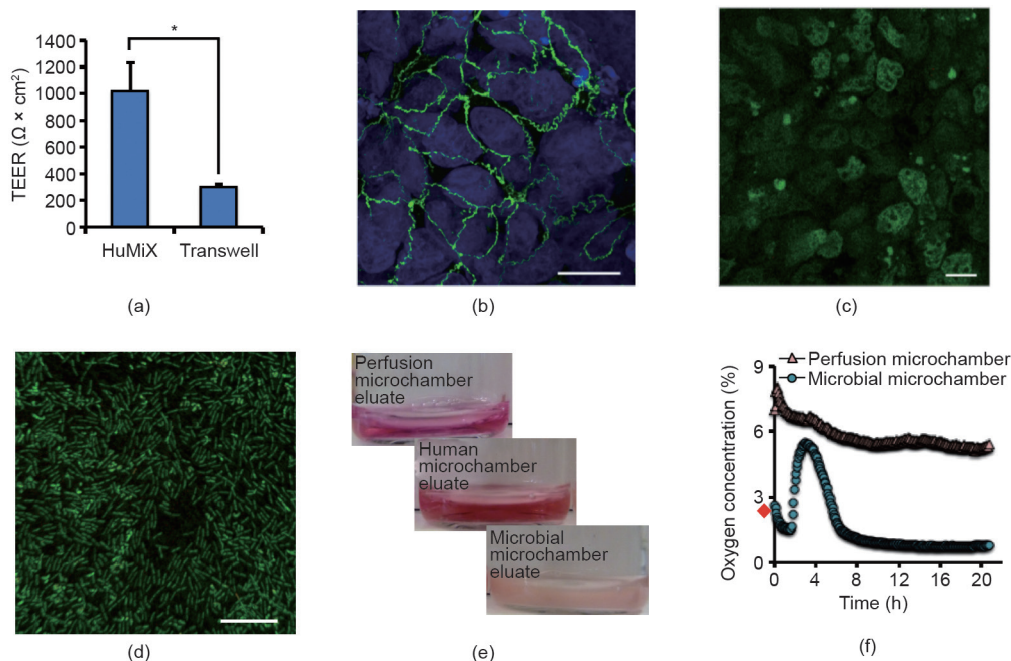

Fig. 2. Data illustrating the ability of the HuMiX platform to sustain the co-culture of human and microbial cells [25]. (a) TEER measurements of the epithelial cell layer formed in HuMiX benchmarked against measurements from a standard Transwell system with error bars indicating the standard error of the mean (n = 3); (b) immunofluorescent microscopic image of the tight junction protein occluding (green) in Caco-2 cells following 24 h of co-culture with LGG grown under anaerobic conditions (the cell nuclei are stained with 4,6-diamidino-2-phenylindole and appear in blue); (c) fluorescent microscopic image of the live-dead stain of the Caco-2 cells 24 h post-culture; (d) fluorescent microscopic image of the live-dead stain of the LGG 24 h post-culture (the live cells appear in green whereas dead cells appear in red, and the scale bar is equivalent to 10 μm); (e) sample eluates from the three separate microchambers in HuMiX following 24 h of co-culture; (f) dissolved oxygen concentration levels (%) present within the perfusion and microbial chambers following inoculation of LGG (♦ denotes pre-inoculation levels in the microbial chamber).

HuMiX experiments and previous *in vivo* data obtained from both humans and animals [25], thus further illustrating the validity of the HuMiX platform as a representative model of the gastrointestinal human-microbe interface. Furthermore, interesting differences in terms of cellular responses were observed under different experimental conditions. More specifically, several molecular hallmarks of the co-cultured human cells were found to be significantly altered following their co-culture with LGG growing under anaerobic conditions. These included a reduction in the expression of pro-inflammatory cytokines (e.g., CCL20 and IL-8), differential expression of genes of known relevance in the context of gastrointestinal cancers (e.g., *Sox4* and *TPD52*), miRNAs with so-far unknown functions but previously found to be of interest in the context of cancer research (e.g., miR 483-3p and miR 1229-3p), and increases in the abundance of tricarboxylic acid (TCA) cycle intermediates (e.g., fumarate and citrate), which suggest a role for the microbiome in regulating primary energy metabolism (Fig. 3).

The results summarized here and presented in Ref. [25] illustrate the capability of HuMiX to establish physiological conditions similar to those present within the human GIT and provide a means to further our understanding of the fundamental mechanisms at play in host-microbe interactions. The flexibility and robustness of the platform allow it to mimic a plethora of host-microbe interactions that occur within the gut. As such, the platform has the potential to be used in a variety of application areas, especially those with relevance to the pharmaceutical, food, and nutrition industries.

The following subsections provide examples of how the platform will be used in the future to study the immunological response of the host to commensal microorganisms/pathogens present within the gut, as well as to determine the effects of diet (via different combinations of prebiotics and probiotics) on host-microbe dynamics, particularly within the context of disease.

2.2. Immuno-HuMiX

The intestinal microbiota is in a constant, homeostatic relationship with the immune system of the gut, and disruption of this balance has been implicated in disease pathogenesis [29]. Thus, the establishment of cause-effect relationships in the complex interactions between the gut microbiota, the intestinal epithelium, and the immune system are a crucial step in the development of successful microbiome-based strategies for maintaining human health. Based on our initial co-culture experiments using CD4^{+} T cells [25], we are now expanding the HuMiX platform to analyze the interactions between the immune system and the intestinal microbiota in the human gut. In the immuno-HuMiX model, we will be able to co-culture gastrointestinal microbes, human epithelial cells, and primary human immune cells isolated from healthy human volunteers, in three distinct chambers, separated by semipermeable membranes (Fig. 4). The first steps have been undertaken to integrate human peripheral blood mononuclear cells (PBMCs) isolated from fresh blood from healthy volunteers into the perfusion microchamber, in order to monitor the interplay of bacterial and epithelial cells on immune cells, and vice versa. Multiple subsets of CD4^{+} T cells have been described in the intestine, which represents an important site for the generation and regulation of cells involved in immune responses both within and outside of the GIT. Intestinal Th1 and Th17 cell populations are required for adequate responses to intracellular bacteria and viruses. We are now investigating the interplay between T cell populations that are important in gut immunity, such as Th1 and Th17 cell, and representative gut bacterial strains. As such, immuno-HuMiX represents a new approach to study the microbiome-immune system interaction and should greatly enhance our understanding of how individual bacterial strains might influence human health and disease.

2.3. Nutri-HuMiX

To understand the role of dietary components and gut bacteria on human physiology, a thorough mechanistic understanding of the interplay between diet, microorganisms, and human cells is required. Being a representative model of the human gastrointestinal interface, the HuMiX model is uniquely suited to answer these questions, as it allows the co-culture of bacterial strains with human intestinal cells for prolonged periods of time under specific experimental conditions. To study the effects of dietary components on the gut microbiome, in addition to its potential synergistic or additive effects on human physiology, we have successfully implemented the HuMiX model to simulate different dietary interventions. For the proof of concept of nutri-HuMiX (Fig. 4), two probiotic strains, LGG and *B. caccae*, were perfused with standard illegal efflux medium (SIEM), supplemented with a soy prebiotic and co-cultured with human Caco-2 cells in the epithelial chamber. Preliminary results show that gene expression patterns in the human epithelial cell line Caco-2 are altered after 24 h of co-culture. More specifically, we found that potential tumour suppressors are up-regulated under these conditions, compared with experiments in which the cells are supplemented with DMEM alone. We are
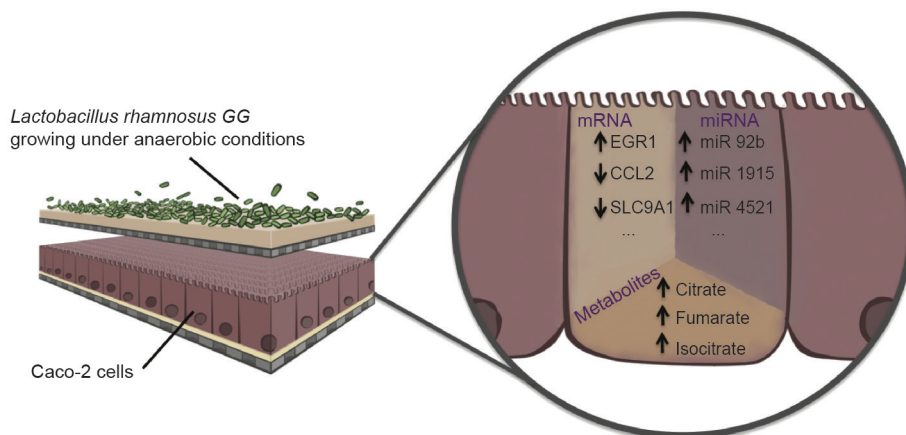

Fig. 3. Examples of genes, miRNAs, and metabolites found to be differentially abundant in Caco-2 cells following 24 h of co-culture with LGG growing under anaerobic conditions.
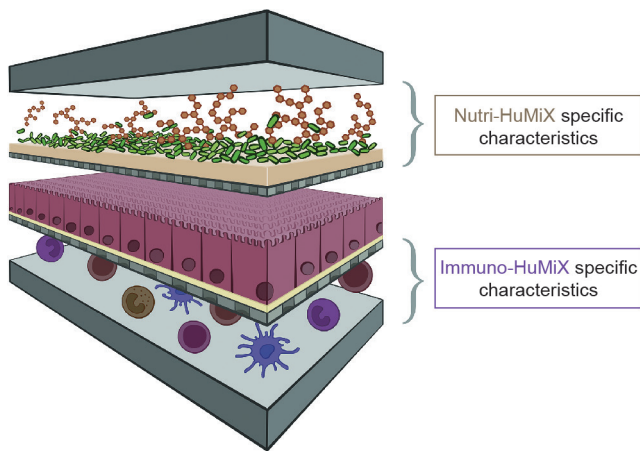
Fig. 4. Schematic illustration of the nutri-HuMiX and the immuno-HuMiX platforms. Medium rich in fibers is perfused through the bacterial chamber in the nutri-HuMiX model. Primary immune cells are inoculated into the perfusion chamber in the immuno-HuMiX model.

currently in the process of expanding this study to include different lines of primary colorectal cancer cells—T20 to represent stage II colorectal cancer and T18 to represent stage IV colorectal cancer—into the nutri-HuMiX platform, with the specific aim of examining the effects of different combinations of prebiotics, such as fiber, on the cancer cells. The knowledge and information garnered from such studies will likely have pronounced impacts in the food, biopharmaceutical, and medical fields, further highlighting the need and potential for *in vitro* experiments in platforms such as HuMiX.

3. Concluding remarks and future challenges

There is an abundance of untapped information within the gut microbiome and its effect on the human host. This information pertains to the numerous processes and interactions that shape human physiology. Over the past decade, significant advances in science and engineering have allowed researchers to gain unprecedented insights into the microbial ecology of the GIT and its role in shaping human health and disease. Breakthroughs in biomaterials, microengineering, and cell culture have resulted in the creation of *in vitro* platforms, such as microfluidics-based devices, that provide closer approximations to the environmental conditions present in the gut.

Platforms such as HuMiX and other organ-on-a-chip devices [30–32] have many potential application areas. For example, the pharmaceutical industry is under considerable pressure, both ethically and economically, to find alternative ways to expedite the drug development process and to do so in a safer, more cost-effective, and ethically responsible manner. The high failure rates associated with the development of new drugs may in part be due to the use of non-representative animal models and to the lack of consideration for the microbiome in relation to drug metabolism. Other industries facing similar challenges are the food and nutrition industries, where the efficacies of certain dietary components have yet to be mechanistically proven. There is also considerable potential to use the microbiome as a biomarker for disease, or to derive modulators that could specifically repair dysbiosis within an individual in order to potentially cure disease [20]. However, in order to capture the specific dynamics at play in a healthy or diseased gut, it is necessary to understand the interactions that drive the gut ecosystem one way or another. As such, platforms such as HuMiX, which permit studies on the effects of individual bacteria and contingents on host physiology, are cru-

cial to further our fundamental knowledge and understanding. Although developed primarily to study the host-microbe interaction in the human gut, the unique architecture of HuMiX—its ability to co-culture individual cell lines or communities in such close proximity, yet completely separately from one another—also makes it an excellent tool to study fundamental processes underlying the microecology of the human gut. For example, the ecology of stable communities established within HuMiX could be experimentally perturbed, leading to a disequilibria that is analogous to dysbiosis. In this way, the transition states could be studied in detail, leading to, for example, the development of early biomarkers for dysbiosis-linked diseases.

The use of microfabrication techniques and microfluidics represents one of the most promising means to recreate the complex features of the gastrointestinal human-microbe interface. These methods allow for the precise reconstruction of the structure, mechanics, and chemical delivery that occur at the cellular level. However, there are a number of technical and industrial challenges that still need to be solved in order to better recreate the physical and chemical environment within the gut. Foremost amongst these are better biomaterials. Most current devices are fabricated from polymers such as PDMS, due to their biological compatibility and rapid prototyping potential. However, PDMS has poor chemical resistance to organic solvents and absorbs small hydrophobic molecules, such as drugs and fluorescent dyes, thereby limiting its potential use for rigorous experiments; hence, chemical modifications are required, or other substrate materials need to be developed. Further more, from a manufacturing perspective, it is difficult to reliably reproduce devices made of PDMS on the large scale that is required for clinical validation studies, which require scalable, high-throughput systems. Additional engineering challenges include: the development and integration of sensors that can measure and detect the optical, electrical, mechanical, and chemical signals from the cells; and the integration of microfluidic valves and pumps to more accurately recreate the peristaltic motion and linked physiological conditions within the gut. Advances are also required in microbiological assays and in the inclusion of additional cell types, which may, for example, be derived from human-induced pluripotent stem cells in order to further advance our understanding of the host-microbe interactions in both a healthy and a diseased gut.

By combining the disciplines of microfluidics, microbiology, and cell biology, a number of questions pertaining to the host-microbe interactions in the GIT have been answered. However, we are just at the start of an exciting journey and further advances are required to better replicate the *in vivo* conditions *in vitro*. These advances are required to leverage the full potential of the HuMiX platform and other such platforms in order to provide much-needed comprehensive insights into host-microbe molecular interactions.

Compliance with ethics guidelines

Authors Frederic Zenhausern and Paul Wilmes have corresponding patent applications, which are currently pending PCT Pub. Nos. WO2013EP055712, WO2013EP065718, and WO201344253; US Provisional App. No. 62/166940; and PCT App. NO. PTC/EP2016/062024.

Marc Mac Giolla Eain, Joanna Baginska, Kacy Greenhalgh, Joëlle V. Fritz, Frederic Zenhausern, and Paul Wilmes declare that they have no conflict of interest or financial conflicts to disclose.

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