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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 semi-permeable 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

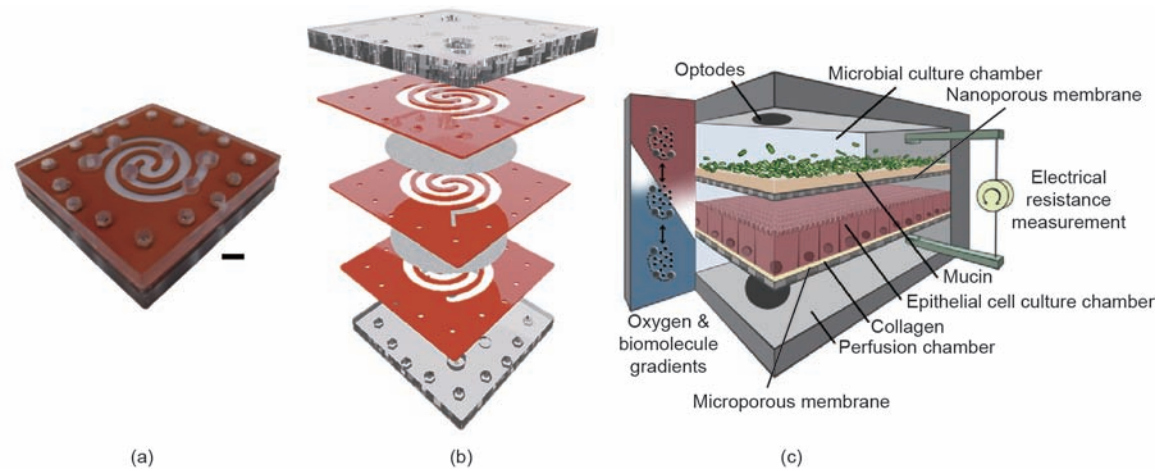

Fig. 1. The HuMiX platform [25]. (a) Image of the assembled HuMiX platform (the scale bar is equivalent to 1 cm); (b) exploded view of the HuMiX platform; (c) annotated schematic illustration of the key features in the HuMiX platform.