MICROPHYSIOLOGICAL SYSTEMS

Human-gut-microbiome on a chip

A microfluidic chip incorporating oxygen gradients, a diverse human microbiota and patient-derived cells, mimics interactions between microorganisms and host tissue in the human gut.

Roberta Poceviciute and Rustem F. Ismagilov

Recent evidence that microbiota inhabiting the gut play key roles in human health and disease has fuelled research into deciphering host–microorganism interactions. In human studies, the manipulation and control of experimental variables, such as host and microbial genetics, diet, and other environmental factors, is difficult. Studying the direct interactions between human gut microbiota and the host tissue also remains challenging. Faecal samples can be obtained easily, but this is not the case for intestinal aspirates and biopsies[1]. Many effects of the gut microbiota are manifested at specific locations in the gut (such as at sites of inflammation), and probably change over time[2,3]. However, in situ measurements of both the microbiota and the host responses, at sufficiently high temporal and spatial resolution (on the order of hours and centimetres, respectively), are not currently possible in humans. Animal models, which offer advantages such as a similar gastrointestinal architecture, straightforward sample collection, and finer control over diet and genetics[4], cannot currently be interrogated via in situ measurements at the necessary temporal and spatial resolutions either, nor do they perfectly recapitulate human disease[5]. Reporting in *Nature Biomedical Engineering*, Donald Ingber and colleagues now describe an in vitro system that mimics the host–microorganism interactions in the human intestine. By taking advantage of a previously reported microphysiological intestine-on-a-chip[6], the researchers established an oxygen gradient across the endothelium–epithelium–lumen axis to co-culture human endothelial and epithelial cells together with a diverse human-derived microbiota, consisting of both aerobes and anaerobes, for at least five days[7]. The microbial diversity and extended duration of co-culture enabled by the chip could be leveraged to study host–microorganism crosstalk.

Ingber and co-authors' gut-microbiome-on-a-chip consists of two channels separated by a permeable membrane, with endothelial cells growing in the lower channel, and epithelial cells growing in the upper channel in direct contact with the microbiota (Fig. 1, left). An oxygen gradient is established by placing the chip in an anaerobic chamber and by continually flowing oxygenated mammalian-cell growth medium through the lower channel. Oxygen levels are monitored in real time across the device via built-in fluorescent oxygen sensors. The authors first co-cultured two established cell lines (human intestinal microvascular endothelial cells, and Caco2 epithelial cells) and a model anaerobe (*Bacteroides fragilis)* on the chip. Both the endothelial and epithelial cells remained viable, and counts of *B. fragilis* colony-forming units increased, thus indicating adequate oxygen supply to the mammalian cells and a sufficiently low oxygen concentration in the lumen so as to permit the growth of the bacteria. Furthermore, epithelial cells polarized, differentiated into villi,

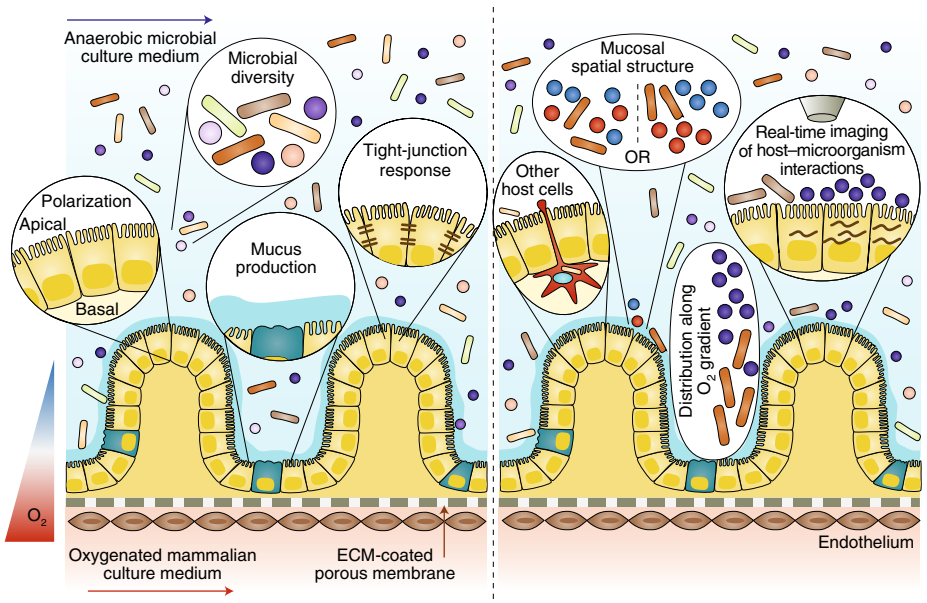

Fig. 1 | Human-gut-microbiome on a chip. Left, Current features. A porous membrane coated with extracellular matrix (ECM) separates endothelial cells (lower channel) from epithelial cells cultured in direct contact with gut microorganisms (upper channel). An oxygen gradient across the endothelium–membrane–epithelium is established by placing the device in an anaerobic chamber and by continually flowing oxygenated mammalian-cell culture medium through the lower channel. Epithelial cells on the chip differentiate to form villi, and polarize to form microvilli on the apical side. The resulting epithelium, which is covered by a mucus layer produced by goblet cells (blue) and is connected by tight junctions that respond to the presence of microorganisms, co-exists in direct contact with a diverse human microbiota. Right, Potential future developments and applications. Other host cells, such as immune cells, can be introduced into the chip to better recapitulate the complexity of the host–microorganism interface in the human gut. The spatial structure of mucosal communities can be examined to determine whether they are well-mixed or 'patchy'. Differences in the mucosal and lumenal microbiota along the direction of the oxygen gradient can be analysed. Real-time imaging can be integrated in situ, and microbiota and host cells can be actively manipulated to study the dynamics of host–microorganism and microrganism–microorganism interactions.

produced mucus and formed tight junctions (Fig. 1, left). To increase the complexity of the system, the authors inoculated the chip with complex gut microbiota from humanized mice. The resulting microbiota remained diverse, falling within the range of microbiota compositions reported by the Human Microbiome Project. Furthermore, consistent with in vivo observations, the co-culture with microbiota increased epithelial barrier function. As proof-of-concept that the chip could be made patient-specific, the authors used organoids derived from an ileal biopsy of a patient as a source of epithelial cells, and co-cultured them with fresh stool microbiota. Similar to the co-cultures using the Caco2 cell line, the primary human intestinal cells recapitulated the features of the ileum, and a diverse microbiota was sustained on the chip for at least five days.

The gut-microbiome-on-a-chip, which at present is at an early stage of development, offers opportunities for further characterization. For example, what microbial loads are in the lumen and at the mucosa in the chip, how microbial and host-cell viability change over time, which bacteria are metabolically dominant and how their gene-expression profiles evolve during culture, and how these dynamics compare to the in vivo situation. Future technology developments and applications would also benefit from characterizing the degree of spatial heterogeneity in both the microorganism and the host (Fig. 1, right). Considering that microorganisms are seeded at low numbers to prevent rapid overgrowth, it would be fitting to establish whether the chip microbiota is well-mixed or is 'patchy' — that is, forming distinct local communities on different parts of the mucosal surface or even in different chips. Because microorganism–microorganism and microorganism–host interactions are driven by diffusing molecules and are often nonlinear, one may expect counterintuitive 'patchy' effects in microbial colonization and the corresponding response of the host cells[8]. The chip would be uniquely suitable for identifying such effects.

Further developments could be introduced into the chip. As alluded by Ingber and co-authors, the complexity of the system can be further increased by incorporating immune or other human cells so as to create more realistic chips specific to a particular intestinal segment or even to a particular patient. However, increases in complexity bring about new limitations and would have to be carefully targeted to specific scientific questions that do not require mimicking the physiology of the entire host. For example, the metabolites produced by the microorganisms can be absorbed by the host, and therefore metabolite concentrations are determined by the balance of production and absorption fluxes. For a metabolite produced by microorganisms at high flux, the concentration in the lumen can be high if the absorption flux is low, or it can be low if the absorption flux is high. Therefore, tuning the chip to faithfully recapitulate the production and removal fluxes of metabolites occurring in vivo would be a formidable challenge. Also, distant organs can affect host–microorganism interactions. For example, bile acids are produced by the liver, secreted as potent antimicrobials that affect microbial composition, and then they are transformed by the microbiota in the intestine and re-absorbed[9]; transformed bile acids also interact with host receptors, which in turn further alter the bile-acid metabolism of the host[10,11]. Such complex feedback loops would be difficult to capture realistically in simple in vitro models.

The complexity of the microbiome-on-a-chip may be increased by supplementing the microbial growth medium with sterilized intestinal fluid that may contain essential microbial growth factors, a strategy that would enable the cultivation of previously uncultured microorganisms[12]. Furthermore, additional diet-derived or host-derived factors can be added to the microbial growth medium to either further enrich the microbiota or to study host–microorganism interactions. As a first step towards spatially resolved analysis, it would be informative to isolate and separately profile lumenal and mucosal microbial communities to understand the differentiation of the microbiota along the device's oxygen gradient (Fig. 1, right). In the long run, it would be exciting to develop in situ, spatially resolved tools to analyse bacterial communities and host cells in the chip, including analyses of metabolism, RNA expression, and genetic and phenotypic composition. In situ sensing and imaging technologies are probably best suited for this purpose (the real-time imaging of the oxygen gradient in the current version of the device is already a step in this direction). Ultimately, new device developments could provide real-time, dynamic and spatially resolved information of host–microorganism crosstalk.

If such new capabilities are integrated, the microbiome-on-a-chip could be used to answer fundamental questions about host–microorganism interactions that would otherwise be challenging to answer in human and animal studies. For instance, the chip could be used to identify driving forces that govern variations in microbiota composition along the mucosal–lumenal axis (Fig. 1, right). Mucosal microbial communities can be distinct from those in the lumen[13]; however, large-scale 2D imaging suggests that the degree of such variation diminishes when microbiota-accessible carbohydrates are removed from the diet[14]. Oxygen gradients may also play a role in microbial composition. Similarly, the chip could be used to study factors that shape the spatial structure of microbial communities on the mucosal surfaces (as opposed to along the mucosal–lumenal axis). Metabolic coupling, stochastic events and surface topology are all factors influencing compositional variation. For example, crypt microbiota has been reported to be distinct[15], which raises the question of whether such communities require crypt architecture to persist. The chip would offer control over the topography of the epithelial surface and, as a result, could be harnessed to answer whether surface irregularities, such as crypts, villi and folds, shape the structure and heterogeneity of mucosal microbial communities. Overall, after further development, Ingber and colleagues' gut-microbiome-on-a-chip will offer ample opportunities for the detailed study of how direct host–microorganism interactions in the gut take place, with control of external conditions and host–microorganism genotypes. ❐

Roberta Poceviciute[1] and Rustem F. Ismagilov[1,2*]

[1]Division of Chemistry & Chemical Engineering, California Institute of Technology, Pasadena, CA, USA. [2]Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA, USA.

**e-mail: rustem.admin@caltech.edu*

Published online: 5 July 2019
https://doi.org/10.1038/s41551-019-0425-0

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