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ORIGINAL RESEARCH

A High-Throughput Organoid Microinjection Platform to Study Gastrointestinal Microbiota and Luminal Physiology

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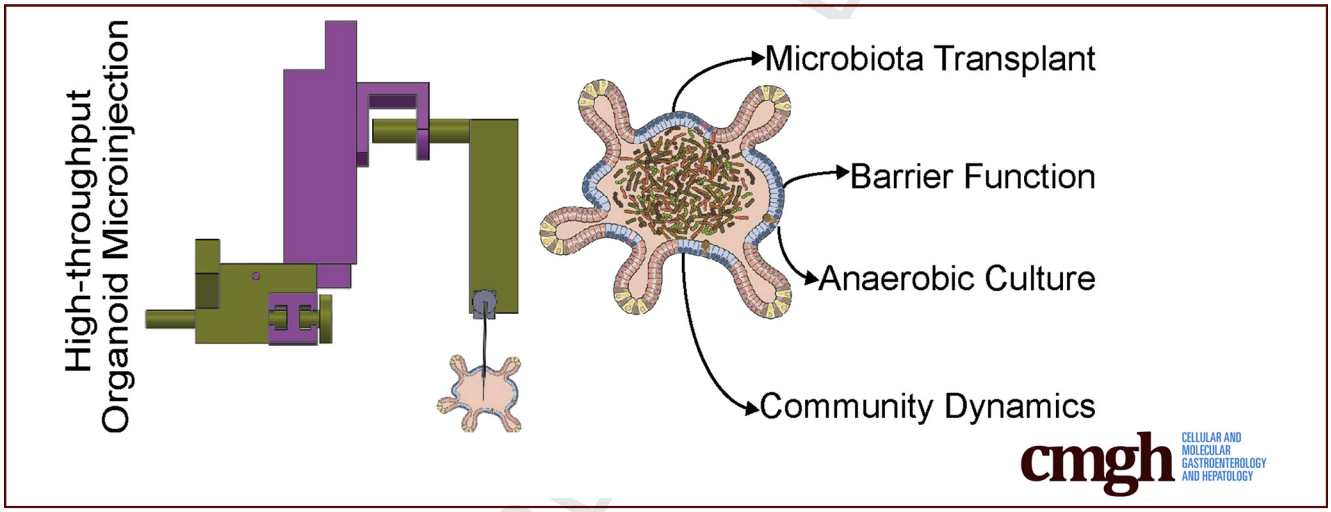

SUMMARY

A high-throughput organoid microinjection platform was developed to study gastrointestinal physiology and the microbiome. Monitoring and quantification of injected microbes and other cargos was achieved by automated imaging. Human fecal microbiota including highly oxygen-sensitive anaerobic taxa were transplanted into the organoid lumen and maintained over time in stable monocultures or microbial communities.

BACKGROUND & AIMS: The human gut microbiota is becoming increasingly recognized as a key factor in homeostasis and disease. The lack of physiologically relevant in vitro models to investigate host–microbe interactions is considered a substantial bottleneck for microbiota research. Organoids represent an attractive model system because they are derived from primary tissues and embody key properties of the native gut lumen; however, access to the organoid lumen for experimental perturbation is challenging. Here, we report the development and validation of a high-throughput organoid microinjection system for cargo delivery to the organoid lumen and high-content sampling.

METHODS: A microinjection platform was engineered using off-the-shelf and 3-dimensional printed components. Microinjection needles were modified for vertical trajectories and reproducible injection volumes. Computer vision (CVis) and microfabricated CellRaft Arrays were used to increase throughput and enable high-content sampling of mock bacterial communities. COMSOL modeling predicted a hypoxic luminal environment that was functionally validated by transplantation of fecal-derived microbial communities and monocultures of a nonsporulating anaerobe.

RESULTS: CVis identified and logged locations of organoids suitable for injection. Reproducible loads of 0.2 nL could be microinjected into the organoid lumen at approximately 90 organoids/h. CVis analyzed and confirmed retention of injected cargos in approximately 500 organoids over 18 hours and showed the requirement to normalize for organoid growth for accurate assessment of barrier function. CVis analyzed growth dynamics of a mock community of green fluorescent protein– or DsRed-expressing bacteria, which grew within the organoid lumen even in the presence of antibiotics to control media contamination. Complex microbiota communities from fecal samples survived and grew in the colonoid lumen without appreciable changes in complexity.

CONCLUSIONS: High-throughput microinjection into organoids represents a next-generation in vitro approach to investigate gastrointestinal luminal physiology and the gastrointestinal microbiota. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.05.004)*

Keywords: Organoid; Microinjection; High-Throughput; Fecal Microbiota; Anaerobic; Barrier Function; High-Content Sampling.

The human gastrointestinal tract contains a remarkably dense and diverse microbial community.[1,2] The interactions between gut microbiota and host are becoming increasingly recognized as key factors in homeostasis and disease.[3] Many studies have indicated that community imbalances, known as dysbioses, are associated with the onset and progression of diseases including diabetes,[4] obesity,[5–7] colorectal cancer,[8–10] and inflammatory bowel disease.[11] Despite tight statistical associations between dysbiosis and disease, the ability to formally test cause-and-effect relationships is severely limited by a lack of in vitro experimental models that enable controlled interrogation of host–microbe interactions.

Sequencing of the 16S ribosomal RNA (rRNA) gene is used routinely to characterize microbial communities and is a powerful tool to identify bacteria that may contribute to disease.[12] Although 16S rRNA gene sequencing provides a signature of microbial composition within a community, alone it is insufficient to define specific microbial mechanisms that impact host biology. Germ-free (gnotobiotic) animal models commonly are used to investigate host–microbe interactions in a physiologically relevant system, but germ-free animal models often are impractical for researchers to use because of the scarcity of gnotobiotic facilities and the high cost of gnotobiotic experimentation.[13] In addition, the inherent low-throughput nature of germ-free rodent studies limits the ability to decipher the individual role that each microbial species plays in health and disease.

A recent assessment of microbiota research in the United States identified the development of high-throughput tools as a key common unmet need for this field.[14] With the recognition of this problem, concerted efforts now are being made to build a "translational microbiome toolbox" to create innovative and high-throughput approaches to test detailed mechanisms of host–microbe interactions.[13] For instance, engineering *Bacteriodes thetaiotaomicron*, *Bacteriodes fragilis*, *Bacteriodes vulgatus*, *Bacteriodes ovatus*, *Bacteriodes eggerthii*, and *Bacteriodes uniformis* with 6 different fluorescent proteins enabled delineation of species within the gut of mice, and showed that the priority of gut colonization determines colonic crypt microbial occupancy.[14] Similarly, engineering-inducible promoters in *Bacteroides* has enabled the study of host–microbe interactions through measurement of commensal sialidase activity and liberation of mucosal sialic acid, a nutrient for pathogens.[15] Furthermore, new methods have been developed that permit genetic manipulation and analysis of "genetically intractable" bacteria from the intestine.[16] As these types of tools are being increasingly developed to test mechanistic questions, and studies are expanded to interrogate the thousands of different microbes that inhabit the gut, high-throughput in vitro models will be essential for these next-generation microbiome studies.

Transplanting the complex microbial communities found in the gut lumen to a physiologically relevant system in vitro is particularly challenging because most microbes comprising the human gastrointestinal (GI) microbiota are highly sensitive to oxygen exhibited limited viability or proliferative capacity in the presence of oxygen.[17] A recent study suggested that 50%–60% of the oxygen-sensitive bacterial genera in the GI microbiota can produce resilient spores and can be detected on specialized agar plates incubated in an anaerobic environment,[17] however, non-sporulating anaerobes remain difficult or impossible to cultivate in vitro and require an environment sufficiently hypoxic for survival and growth.[17] Minibioreactors without a host cellular component have been built to cultivate fecal samples in a hypoxic environment.[18] This system increased throughput by allowing up to 48 communities to be cultivated per anaerobic chamber. Although this system proved sufficient to stably culture complex communities, only 15%–25% of the initial fecal operational taxonomic units (OTUs) were observed, suggesting that other system components are required to cultivate the full complexity of fecal microbiota.

Designing culture environments that possess a host cellular component in combination with the physiologically relevant luminal environment may enable more complex communities to be cultivated while facilitating the study of host–microbial interactions. Culturing techniques have been developed that permit growth of primary intestinal epithelium on 2-dimensional (2D) surfaces,[19–21] however, generating a steep oxygen gradient over a single cell layer in monolayer cultures remains an engineering challenge owing to the requirement of a constantly intact monolayer, which is difficult to achieve in 2D cultures. Gut organoids, also known as *enteroids* or *colonoids*,[22] represent an alternative in vitro system to culture fecal-derived microbiota by the virtue of their morphologic, cellular, and physiologic properties that are unavailable in 2D culture systems.

Organoids are microscale spherical structures composed of an epithelial monolayer that surrounds a hollow lumen containing mucous and cellular debris, and serves as a

Abbreviations used in this article: CAG, _____; CFU, colony-forming unit; CRA, CellRaft Array; CVis, computer vision; DsRED, _____; EGFP, _____; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; GI, gastrointestinal; HF, hydrogen fluoride; hiPS, _____; LB, _____; L-WNR, _____; OUT, operational taxonomic unit; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; QIIME, Quantitative Insights Into Microbial Ecology; rRNA, ribosomal RNA; 3D, 3-dimensional; 2D, 2-dimensional; WT, wild-type.

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