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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

The Next MacGyver: A Platform to Study Intestinal Organoids Using High-Throughput Computer-Driven Microinjection

The complex microbial community that lives in the colon plays an important role in diverse biological pathways and is linked to many diseases including inflammatory bowel disease, obesity, and cancer. Defining how microbial interactions influence host cellular molecular pathways is crucial for exploring the potential therapeutic benefits of manipulating microbial communities to treat intestinal disorders. In 2009, pioneering work in the gastrointestinal field led to the development of intestinal organoid cultures, either derived from pluripotent embryonic or reprogrammed somatic stem cells,[1] or from adult stem cells derived directly from intestinal tissue or biopsy specimens.[2,3] These organoid cultures are 3-dimensional (3D) structures composed of intestinal epithelium comprising multiple differentiated cell types in the appropriate ratios.[3] This epithelium is polarized into apical and basolateral surfaces, forms a hollow lumen lined by the apical surface in the center, and shows many physiological functions present in the original intestinal tissue.[3] These cultures now are being used in modeling colorectal cancer and in drug testing,[4] and provide a new platform in which microbiome–host cellular interactions can be explored.

One particular challenge that needs to be overcome to use organoid cultures to study microbial communities is the delivery of cargo to the lumen of these structures. Microinjection of organoid cultures is labor intensive, requires extensive technical expertise, and currently lacks throughput tools to assess a large number of organoids. Other options previously used for delivery of cargo to organoids include plating in 2-dimensional format,[5] which does not maintain the luminal environment present in 3D cultures, or dissociation of the organoid for exposure to the cargo followed by reassociation,[6] which provides no mechanism to control the amount of cargo retained in the 3D lumen. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Williamson et al[7] have "macGyvered" (made in an improvised or inventive way making use of whatever items are on hand) off-the-shelf and 3D printed components to design an elegant new high-throughput system combining microinjection of 3D organoid structures with automated delivery and imaging. They have combined conventional remote-controlled microinjection hardware with a fluorescent microscope and imaging system in a physiologic chamber resulting in accurate, reproducible, quantitative, and precise delivery of cargo to the 3D organoid lumen. They optimized both the fittings to ensure precise needle articulation over the organoids as well as the microinjection needle to allow a vertical approach to the organoid in a multiwell plate. Detection of fluorescein-dextran showed that delivered cargo was retained inside the lumen of the colonoids with minimal leakage from the needle puncture.

In addition to automating the mechanical components of microinjection, Williamson et al[7] also tackled the problem of time consumption and labor intensiveness of microinjection. To do this, they designed a computer vision (CVis) program that is able to automatically identify the colonoid on a cell raft array, allowing imaging, tracking, and quantification of thousands of individual organoids both before and after injection. This platform showed greater than 95% concordance to manual organoid microinjection. Their apparatus also allowed them to standardize organoid size for injection by measuring the organoid cross-sectional area and using it as a proxy for volume. Williamson et al[7] then could determine a relationship between volume and successful microinjection efficiency; in effect, standardizing individual organoids based on size.

To show the applicability of their new tools to a biological question, Williamson et al[7] explored whether their new system could be used to deliver microbial communities to the lumen of 3D colonoids and monitor the dynamics of their growth. They microinjected several strains of commensal bacteria labeled with different fluorescent tags or microbial communities isolated directly from stool into individual organoids using their system and monitored survival and growth of the organisms over time by quantifying fluorescence per organoid, colony formation assays at the individual organoid level, or using 16S amplicon sequencing. They were able to estimate delivery of microorganisms to approximately 90 organoids per hour compared with manual injection of organoids at 10–12 per hour, which substantially increases biologic and technical replicates using this method. The organoid lumen was hypoxic enough to cultivate individual or complex anaerobic microbial communities over a 4-day period. This supports and extends work with anaerobic pathogenic bacteria, which also were reported to grow in the organoid lumen in the absence of organoid damage.[8,9]

In conclusion, the establishment of an automated method to deliver microinjected commensal organisms into the lumen of colonic organoids, use of CVis to standardize microinjection delivery based on organoid volume, and recording of microbial survival and growth kinetics are significant advancements in technology necessary to further the use of organoid cultures to study intestinal biology. First, the ability to select organoids based on automated determination of size overcomes a substantial limitation in organoid experimentation by reducing variability introduced by organoid size that exists in 3D cultures. Second, by semi-automating microinjection, such that it surpasses what

an individual could accomplish on a per-hour basis, Williamson et al[7] provide a precise delivery system targeted to the organoid lumen that now has the power of technical replicates that will function to increase statistical confidence. Their work has wide implications for use in the study of both anaerobic and anaerobic organisms, which now can be rapidly and directly delivered to the 3D organoid lumen. The development of techniques to study complex microbial communities of the gastrointestinal tract has lagged behind the explosion of in vitro models of the intestine. Williamson et al[7] offer a valuable technical platform with potential to expand the use of organoid cultures systems to advance the understanding of interactions between microbial communities and the cellular host.

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Conflicts of interest
The author discloses no conflicts.

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2352-345X
https://doi.org/10.1016/j.jcmgh.2018.06.010