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REVIEW

Integration of Sensors in Gastrointestinal Organoid Culture for Biological Analysis

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SUMMARY

Bioengineered sensors characterize properties of gastrointestinal organoid epithelium, mesenchyme, and bacteria in model systems. These sensors and emerging technologies also can measure biochemicals in fluids within and proximal to the organoid.

The gastrointestinal (GI) tract regulates physiologic responses in complex ways beyond facilitating nutrient entry into the circulatory system. Because of the anatomic location of the GI tract, studying in vivo physiology of the human gut, including host cell interaction with the microbiota, is limited. GI organoids derived from human stem cells are gaining interest as they recapitulate in vivo cellular phenotypes and functions. An underdeveloped capability that would further enhance the utility of these miniature models of the GI tract is to use sensors to quantitatively characterize the organoid systems with high spatiotemporal resolution. In this review, we first discuss tools to capture changes in the fluid milieu of organoid cultures both in the organoid exterior as well as the luminal side of the organoids. The subsequent section describes approaches to characterize barrier functions across the epithelial layer of the GI organoids directly or after transferring the epithelial cells to a 2-dimensional Q7 **culture format in Transwells or compartmentalized microchannel devices. The final section introduces recently developed bioengineered bacterial sensors that sense intestinal inflammation-related small molecules in the lumen** Q8 **using lambda cI/Cro genetic elements or fluorescence as readouts. Considering the small size and cystic shape of GI organoids, sensors used in conventional macroscopic intestinal models are often not suitable, particularly for time-lapse monitoring. Unmet needs for GI organoid analysis provides many opportunities for the development of noninvasive and miniaturized biosensors.** ***(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.03.002)***

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Q9 Q10 Q11 The gastrointestinal (GI) tract is a dynamic environment that digests food, absorbs nutrients, and mediates interactions between the host and its microbiota to help maintain health.[1–4] On the other hand, it is also the target site for a variety of pathogenic bacteria, viruses, and parasites. Given inherent challenges associated with in vivo human studies, a simplified, human-relevant, GI model system would significantly accelerate mechanistic understanding, GI-focused drug development, and precision medicine efforts.[5] Fortunately, GI organoids recently have been developed that preserve key human cell physiology while allowing easier manipulation. Although the biological understanding and manipulation of these GI organoids is progressing rapidly, biosensing methods and techniques still are lagging, giving rise to a variety of challenges and opportunities. This review provides an overview of bioengineered sensor or biosensors, which are sensors engineered specifically for GI organoid measurements. The biosensors may be relatively simple adaptations of existing sensors or implementation of new technological advances.

Before the development of human stem cell–derived GI organoids, the most widely used in vitro GI models were 2-dimensional (2D) cultures of intestinal cell lines. A versatile example is the Transwell culture of Caco-2 cells. These Q12 workhorse systems are useful in having separated and basolateral compartments that allow exposure of just the apical side to bacteria,[6,7] as well as measurement of transepithelial electrical resistance (TEER). More recently, multilayer microfluidic devices with compartmentalized chambers enabled exposure of cells to dynamic mechanostimulation by fluid flow and stretch,[8,9] as well as prolonged exposure to bacteria.[9,10] Although useful, easy to image, and with the benefit of decades of sensor developments for 2D cultures, these culture formats have suffered from a lack of physiological cellular composition and maturation. That is, although human stem cell–derived GI organoid cultures[11–15] can be maintained and matured for over a year with proper techniques,[12] the 2D and microfluidic cultures, even when

Abbreviations used in this paper: **FITC, fluorescein isothiocyanate; FITC-Dex, fluorescein isothiocyanate-dextran; GI, gastrointestinal; HIO, human intestinal organoid; NO, nitric oxide; RT-PCR, reverse-transcription polymerase chain reaction; SNARF, _____; TCRS, 2-component regulatory system; TEER, transepithelial/transendothelial electric resistance; 3D, 3-dimensional; 2D, 2-dimensional.**

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seeded with the same organoid-derived cells, typically only last weeks.[8,9,16–19] It has been noted that, currently, there are significant efforts to adapt the highly physiological 3-dimensional (3D) GI organoid cultures into more convenient 2D culture formats[17,18,20,21] to take advantage of existing biosensing methods (Table 1). In this review, we take the view that it also is important to develop novel sensing methods or use existing biosensors in new ways to allow versatile analysis of 3D GI organoids.

Many methods used to analyze cellular responses in 3D GI organoid models have been adapted from tissue histology and include staining, immunofluorescence, immunoblotting, use of reverse-transcription polymerase chain reaction (RT-PCR), next-generation sequencing,[22] enzyme-linked immunosorbent assay, and other fluorescent probe-based assays. Commonly, these tools require fixation of the sample, allowing only a snapshot of the organoid at a certain time point. For sensing inside the lumen of cystic organoids, thin and long pin probe-type sensors have been adapted from the microchemistry and microbioreactor field. These are useful but challenging for long-term monitoring because of their invasiveness. Methods also have been adopted from the field of cell microinjection, in which dyes or sensor particles are injected into the organoid lumen and analyzed optically.[23,24] These methods are highly applicable but suffer from the need for specialized equipment and technique that reduce throughput. These technological challenges, coupled with rapid biological advances and significant biomedical needs, provide opportunities for productive collaborations between biosensor developers, biologists, and industry.

Capturing Real-Time Chemical Microenvironments

Oxygen Sensing

Cells in the GI tract are exposed to widely varying oxygen (O_2) environments ranging from normoxic (80 mm Hg O_2) to anaerobic (<0.01 mm Hg O_2). In the intestines, these different oxygen environments are arranged radially. The intestinal mucosa is well perfused and oxygenated by networks of blood capillaries; however, toward the center of the lumen, the environment is almost devoid of O_2, allowing obligate anaerobes to survive and be a part of the diverse gut microbiota.[25] When and how do these steep gradients develop? One explanation is that the gut lumen of a newborn becomes populated by aerobes and facultative anaerobes that deplete O_2 to create a suitable environment for obligate anaerobes.[26] Importantly, decreases in the steep radial O_2 distribution is an indicator of bacterial infection and chronic gut inflammation.[27,28] Despite the importance of O_2, accurate spatiotemporal tracking of the in vivo O_2 microenvironment is challenging because of its anatomic location.

In vitro culture systems also can create hypoxic environments through external gas environment control or owing to cellular oxygen consumption. A microfluidics-based human-microbial cross-talk device[10] consisting of multilayered modular flow compartments, created a forced transepithelial O_2 gradient mimicking that of the human colon.[29] Measured O_2 levels using an O_2-sensitive patch and accompanying optical fibers were 38.7 mm Hg O_2 (5.43% O_2) in the basal perfusion microchamber, and less than 5.70 mm Hg O_2 (0.88% O_2) in the luminal microbial microchamber. This hypoxia in the luminal compartment was created by purging dissolved O_2 from the luminal fluid with N_2, which allowed co-culture of Caco-2 with both facultative and obligate commensal anaerobes.[10] Chen et al[30] created intestine-like tissue constructs by co-culturing Caco-2, HT29-MTX cells, and human intestinal myofibroblasts on tubular silk scaffolds. This open-tube structure enabled a probe-type optical O_2 sensor to access the lumen without damaging the epithelium. O_2 levels along the longitudinal axis were effectively anoxic (<0.1%), even without bacterial colonization. These "unexpectedly" anoxic conditions in the absence of bacteria[26] may be explained by low gas transport through thick scaffold material coupled to O_2 consumption by multiple layers of intestinal cells.

Compared with the traditional Clark-type[31] of electrochemical sensors, optical O_2 sensors are more reliable at

Table 1. Bioengineered Sensors Used in In Vitro GI Models

Location	Target	Sensing methods	In vitro model	Reference
Luminal	O_2 concentration	Optical sensor patch	Nonorganoid colonic	10
		Optical sensor probe	Nonorganoid	30
			HIO	33
	pH	Fluorescent probe injection	HGO	14
Transepithelial	TEER	Ag/AgCl electrode	Adult stem cell/iPSC-derived monolayer	16
			Organoid-derived monolayer	17,18,20,21
	Permeability	FITC-Dex	Adult stem cell/iPSC-derived monolayer	16
			Organoid-derived monolayer	17
			HIO	23
	Ion transport (cytosolic pH)	Fluorescence intensity rationmetry	HIE	38,39
Overall	Metabolism (OCR, ECAR)	Seahorse XF assay	Mouse intestinal organoid	41,42

ECAR, extracellular acidification rate; HGO, human gastric organoid; HIE, human intestinal enteroid; iPSC, induced pluripotent stem cell; OCR, O_2 consumption rate.