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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 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 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;6:123–131; https://doi.org/10.1016/j.jcmgh.2018.03.002)*

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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 (Corning Inc, Corning, NY) culture of Caco-2 cells. These 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

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, semi-naphtharhodafluor; TCRS, 2-component regulatory system; TEER, transepithelial/transendothelial electric resistance; 3D, 3-dimensional; 2D, 2-dimensional.**

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for over a year with proper techniques,[12] the 2D and microfluidic cultures, even when 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 (HuMiX) 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 ratiometry	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.

lower-than-ambient O_2 concentrations that are most relevant to GI biology. Electrochemical sensors consume O_2 and hence can produce significant measurement errors in hypoxic environments such as the intestinal lumen.[32]

Despite the advantage of optical O_2 sensors, most commercial systems use a thin probe that must be inserted into the sensing area, causing damage and disruption when applied to intestinal organoids.[33] This is particularly problematic when making multiple measurements over time because the puncture site creates major inhomogeneities, such as leakage of bacteria or virus from the lumen. A promising emerging technology is the use of dispersible bead-type O_2 microsensors,[34,35] in which small sensing beads are placed and imaged remotely (Figure 1*A*).

pH Sensing

Both electrochemical and optical mechanisms are used in biological pH sensing. For typical quantitative acidity measurements, pH electrodes that measure electric current from hydrogen ion transport are broadly available. These systems provide easy operation and a wider detection range compared with pH-sensitive fluorophores. For long-term measurement of pH inside organoids, however, their probe-based form factor is too invasive. Moreover, the electrode requires periodic recalibration owing to significant signal drift over time. pH-sensitive fluorescent molecules[36,37] have a narrower detection range that require careful consideration in choosing the right sensor molecule for an application. Fluorescein-derived molecules such as fluorescein isothiocyanate (FITC) are pH-sensitive, but they photobleach easily; molecules synthesized more recently have improved photostability, making them a better candidate for time-lapse monitoring of pH changes in cystic organoids.

Seminaphtharhodafluor (SNARF) family pH indicators have been used for cytosolic and luminal pH measurements. These dyes have dual-absorption and dual-emission properties, making them useful for ratiometric measurement of emission intensities using confocal or 2-photon microscopy. SNARF-4F was delivered into the cells of human enteroids to measure cytosolic pH[38,39] (Figure 1*B*). Change in cytosolic pH provides information on the activity of sodium-hydrogen

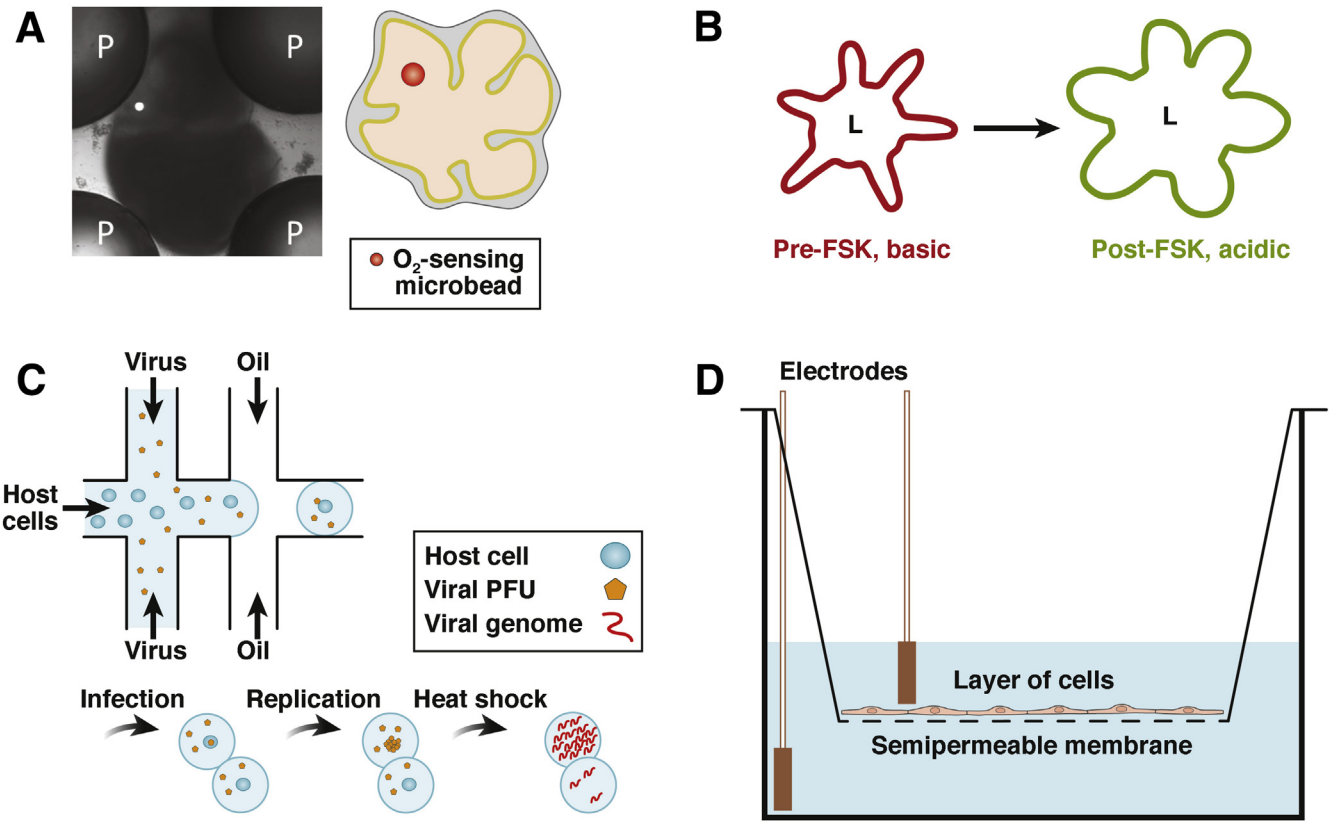

Figure 1. (*A*) *Left*: Fluorescent image of an oxygen-sensing microbead injected in a HIO overlaid on a brighfield image. The HIO is held in place by 4 PDMS pillars (P). *Right*: Schematic of oxygen-sensing microbead in the lumen of the HIO. (*B*) Schematics showing intracellular acidification induced during forskolin (FSK) swelling assay. FSK induces luminal dilation and cytosol acidification. Preloading enteroids with pH-sensitive fluorescent dye allows tracking of cytosolic pH change over time. Epithelium was alkalized before FSK treatment (red, *left*). During FSK treatment, cytosolic pH gradually decreased (yellow) and became acidic (green, right). L, lumen of the intestinal organoid. Schematics were redrawn based on the confocal images from Kovbasnjuk et al.[38] (*C*) Schematics of isolating viral infectivity in single-cell level and amplifying viral genome using a microfluidic droplet generator as part of the microfluidic in-drop RT-PCR procedure.[49] PFU, plaque-forming unit. Adapted from Tao et al[49] with permission of the Royal Society of Chemistry. (*D*) Schematics of TEER measurement set-up in the Transwell system.[52] Adapted from Srinivasan et al[52] with permission from SAGE Publications.

exchanger 3 (NHE3) channels because they transport intracellular H^+ from cytosol to the extracellular environment and uptake extracellular Na^+. With administration of forskolin, an NHE3 inhibitor, intracellular pH decreased to 6.25.[38] In the stomach, the luminal environment can get highly acidic from the hydrochloric acid secreted by parietal cells.[40] The SNARF-5F dye was used to observe a related process in which weak acidification was triggered in human gastric organoid by histamine treatment.[14]

Use of the more photostable pH-sensor dyes can be challenging, particularly for luminal pH measurements. Because the lumen is enclosed by cell layers and an extracellular matrix, optical interaction can cause single-wavelength, intensity-based sensing to be highly unreliable. Even fluorescence ratiometry can be problematic because optical artifacts can have complex absorption spectra that show different scattering and absorption behavior at different wavelengths. Thus, when the sensors are used in environments that differ substantially from the calibration conditions, the measurement result will not be as reliable.

Metabolism

The metabolic function, such as mitochondrial respiration and glycolysis, of cells and tissue can be quantified in vitro by the combined monitoring of O_2 concentration and pH. For example, the Seahorse XF Assay from Agilent Technologies (Santa Clara, CA) measures the O_2 consumption rate and the extracellular acidification rate in the culture media in the presence of different substrates, stimulators, and inhibitors.[41,42] Most commonly, this machine is used with cells cultured in 2D formats in the bottom of a specially shaped microwell. A sensor probe is lowered to within 200 μm of the cultured cells, reducing the culture media volume to just a few microliters to allow cells to rapidly (within minutes) deplete oxygen or acidify the media. The sensor proximity also allows measurements to be pericellular. Although the system is very useful, there are challenges for use with GI organoids such as the mismatch between organoid size (diameters up to millimeters) and chamber gap size (200 μm) when the sensor probes are lowered. During the measurement cycle, unwanted mechanical stress and damage may be applied to the organoids as the probe comes near the bottom. The system provides only the overall metabolic status of all cells within a well and may miss spatial variations in metabolism that are expected in GI organoids.

Although the Seahorse XF assay requires specialized instruments specific for the assay, other commercial metabolism assays using plate readers are available as well. Alamar Blue Invitrogen (Carlsbad, CA) is a well-established colorimetric/fluorimetric cell viability assay; a nonfluorescent blue molecule is reduced to become a fluorescent red molecule upon entering cells. Some studies adopt this assay to measure the relative metabolism rate; potentially, calibration may enable quantitative analysis of aerobic metabolism.[43]

Combining multiple fluorimetric assay products from Luxcel Biosciences (Little Island, Cork, Ireland) provides similar metabolism analysis to the Seahorse XF assay. For example, the MitoXpress Xtra Oxygen Consumption Assay and the pH-Xtra Glycolysis Assay (Luxcel Biosceinces, Little Island, Cork, Ireland) generates the O_2 consumption rate and the extracellular acidification rate, respectively.[44] Calibration of these products and measurement of dissolved O_2 and pH are available by measuring the intensity or lifetime of the sensors. Instead of using a plate reader, adopting advanced optical microscopy such as phosphorescence lifetime imaging microscopy could enhance spatial resolution.

Droplet-Based PCR

In studies involving GI organoids, quantitative RT-PCR has been a useful tool for cellular messenger RNA amplification and detection.[13,45] For studying viral infection and recombination, however, the usual RT-PCR technique can provide incorrect readouts and decrease the sensitivity of the technique. Norovirus in particular, an RNA virus causing acute gastroenteritis with fast transmission between hosts, has a high mutation/recombination rate.[46] Artificial RNA recombination (chimera formation) during conventional RT-PCR can interfere with the identification of rare recombinants occurring from the host–pathogen interaction.

Droplet-based RT-PCR for norovirus[47–49] can circumvent the chimera formation by encapsulating each RNA strand in picoliter droplets and enable high-throughput analysis. In addition, the droplet can isolate the host–virus interaction to a small number of host cells and virus by co-encapsulating them in a single droplet. After incubation, cells are lysed by heat shock, reagents are added to the droplet, and in-drop RT-PCR is performed[49] (Figure 1*C*). Together with recent advances in in vitro culturing of human norovirus in B cells[50] and human intestinal enteroids,[51] microfluidic PCR may be useful for elucidating in vivo infection dynamics of human norovirus, and predicting viral evolution for the prevention and treatment of the disease.

Barrier Function

TEER is a common quantitative method to measure the integrity of tight junctions in monolayers of epithelial and endothelial cells. TEER measurements are a strong measure of the integrity of a cell monolayer's ability to act as a barrier and therefore has been used in conjunction with studies on the transport of drugs, chemicals, dyes, and general membrane leakage. Benefits of TEER measurements are that it is in real time, is nondestructive, often noninvasive, and allows cell cultures to be re-used for additional studies.[52]

In general, TEER measurements are performed by growing a monolayer of cells on a semipermeable membrane with electrodes placed on each side of the membrane while in solution and then applying an AC signal to the electrodes. The current and voltage across the cell monolayer then is measured from this signal and Ohm's law is used to calculate the electrical resistance of the system. Ideally, this resistance is composed of both the transcellular resistance and the paracellular resistance in parallel. However, along with these resistances, there are nonideal factors that need to be considered such as the resistance of the media, electrode-to-media interfacial resistance, and the

support membrane resistance.[53] It is most common to perform TEER measurements using a Transwell insert with a semipermeable membrane with a monolayer of cells grown on it along with chopstick TEER electrodes (Figure 1*D*). These 2 electrodes are composed of silver and silver/silver chloride and the typical frequency of the AC electrical signal is 12.5 Hz with a current of 10 μA.

In recent years there has been great progress in growing intestinal cell monolayers. This ability to design an experimental system to model intestinal epithelial cells as a confluent polarized monolayer has been an important milestone in understanding intestinal pathology. One major difficulty of studying primary intestinal epithelial cells in Transwells with monolayer culture and TEER measurements is that it requires a large amount of viable cells.[20] This lack of cells has been overcome by generating intestinal cells from stem cell–derived intestinal organoid cultures. Adult stem cell–derived organoid cultures are made from dissociated primary crypts/stem cells whereas pluripotent stem cell–derived organoid cultures are made from embryonic stem cells or induced pluripotent stem cells. Both of these cultures use media containing canonical Wnt ligand, R-spondin, and Noggin to support intestinal epithelial stem cell growth. Once enough intestinal cells from these 3D spheroid/organoid cultures are generated, 2D intestinal epithelial monolayers can be created on Transwell membranes for assays. Before such intestinal epithelial cells were capable of being reproducibly grown in 2D cultures on Transwells, it was common to use immortalized cell lines such as Caco-2 or T84, which can show microvilli formation. Monolayers of these cell lines typically provide TEER values ($>$300 Ωcm^2). This is higher compared with TEER of human small intestinal tissue ($\sim$40 Ωcm^2) measured from freshly obtained surgical samples using an Ussing chamber to immobilize the tissue.[17]

Studies by Moon et al[20] and VanDussen et al[21] developed 2D monolayers of intestinal epithelial cells derived from spheroids (enteroids) on semipermeable Transwell membranes. These platforms showed TEER values of approximately 395 Ωcm^2 and approximately 400 Ωcm^2 for rectal and ileal monolayers, respectively.[21] A similar study created monolayers of intestinal epithelial cells from intestinal organoids but on a natural extracellular matrix based on porcine small intestinal scaffold instead of the standard Transwell semipermeable membranes.[17] The values of these intestinal epithelial monolayers were approximately 40 Ωcm^2, which is closely matched to the native human small intestine. Fibroblast co-cultures did not alter TEER readings, but did provide a more heterogeneous monolayer with prismatic cells and luminal cystic structures in the epithelium as shown by hematoxylin and eosin (H&E) staining. To further investigate the barrier function of the 2 different monolayer scenarios, FITC-dextran (FITC-Dex) permeability studies were performed. The FITC-Dex permeability studies showed that the fibroblasts created a more robust and consistent membrane with a permeability of approximately 1% compared with approximately 4% for the monolayer without fibroblasts, a difference that was not discernable by TEER measurements.

There also has been research on the co-culture of intestinal epithelial monolayers with human monocyte–derived macrophages to investigate the importance of the interaction of the intestinal epithelium with the mucosal immune system. Here, it was found that the presence of monocyte-derived macrophages with intestinal epithelial cells derived from differentiated enteroids increased TEER and barrier function from approximately 800 Ωcm^2 to approximately 1000 Ωcm^2,[18] suggesting a potential role of the macrophages in enhancing maturation of the intestinal epithelium and thickening the physical barrier.

TEER measurements and dye flux assays, such as the FITC-DEX assay described earlier, frequently are performed together to provide a thorough characterization of the barrier function of cell monolayers. Similar to TEER measurements, the traditional experimental configuration for dye flux assays uses a monolayer of cells grown on the semipermeable membrane of a Transwell dish. After a confluent monolayer of cells is grown on the Transwell membrane, a dye of a specific concentration and molecular size is placed in the liquid media in the top chamber of the Transwell dish. Then the solution present in the bottom chamber is sampled at various time intervals for the concentration of the dye.[54] This approach can be used to either quantitatively to calculate the permeability of the cell monolayer for the specific dye, or qualitatively to compare cell monolayer treatments/scenarios.[55] By using FITC-Dex with a molecular weight of 150 kilodaltons, 1 study showed good barrier function of commercially available primary human intestinal epithelial cells from various donors, induced pluripotent stem cell–derived intestinal cells, as well as Caco-2 cells, which are commonly used for human intestinal models.[16]

As 3D cultures of organoids (eg, intestinal and lung) become more common,[23,56] there is a growing need to convert cell monolayer-based permeability and TEER assays into a format that is directly compatible with organoids. Although there are no published works on direct TEER measurement of organoids, there have been permeability/leakage studies of human intestinal organoids (HIOs).[23,24] In this work, the barrier function of the HIOs was evaluated by microinjecting FITC-Dex with an average molecular mass of 4 kilodaltons into the lumen of the HIOs. The HIOs retained 58.8% of their fluorescent intensity over 18 hours in control cultures whereas exposure to ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, a calcium chelator that disrupts tight junctions, caused a 70% loss of fluorescence intensity in just 6 hours. An additional variation of this test was performed in which 4 kilodaltons were added to the surrounding media of the HIOs to investigate barrier leakage in the basal-to-apical direction (opposite of the microinjection method). The 2 complementary experiments show that HIOs can have good paracellular epithelial barriers.

Bioengineered Bacteria for Proinflammatory Small-Molecule Sensing

During gut inflammation, increased reactive oxygen species oxidize thiosulphate ($S_2O_3^{2-}$) to produce tetrathionate ($S_4O_6^{2-}$). Thus, $S_4O_6^{2-}$ is of interest as a potential

marker of GI tract disease if there were convenient ways to sense this molecule. Compared with other bacteria, pathogenic *Salmonella* are able to uniquely thrive under conditions of high $S_4O_6^{2-}$ by using $S_4O_6^{2-}$ as a respiratory electron acceptor to fuel their outgrowth.[57] These *Salmonella* possess a 2-component regulatory system (TCRS) that specifically recognize $S_4O_6^{2-}$ to up-regulate genes required for $S_4O_6^{2-}$ utilization.[58] By inserting this $S_4O_6^{2-}$-sensing TCRS system into *Escherichia coli*, and engineering the system to turn on a lambda-derived cI/Cro memory circuit,[59] Riglar et al[60] developed a commensal bacteria that can retain a memory of exposure to $S_4O_6^{2-}$ in the gut.

In a different approach, Daeffler et al[61] inserted $S_2O_3^{2-}$- and $S_4O_6^{2-}$-sensing TCRSs from marine *Shewanella* species to *E coli*. To avoid cross-repression of $S_4O_6^{2-}$ TCRS in *Salmonella typhimurium* by O_2 and nitrate, they identified TCRS homologous to that of *S typhimurium* using bioinformatics. With a fluorescent reporter gene inserted, both bacterial sensors expressed fluorescence in response to the respective target species in vitro. Although the response time for these sensors is approximately an hour,[61] the approaches used in this work, fluorescent-sensing modality and use of bioinformatics for homolog TCRS identification, may allow the sensors to be incorporated into organoid systems for real-time responses in the future.

Another interesting small molecule is nitric oxide. Under inflammatory conditions, NO is produced in large amounts that can reach micromolar levels. Interestingly, NO has many beneficial effects at the picomolar to nanomolar range, such as increasing tissue perfusion, vasodilation, and protecting tissue against acute inflammation.[62,63] High concentrations of NO, however, cause injury both directly and indirectly through byproducts such as peroxynitrate, produced by a reaction with superoxide anion.[64] Because high levels of NO are a proinflammatory signature of inflammatory bowel disease and necrotizing enterocolitis,[65–67] living bacterial sensors have been engineered to detect NO in the micromolar range.[68] These sensor bacteria also may be useful for in vivo and GI organoid NO sensing.

Microperfusion and Integrated Biosensors for Real-Time Organoid Monitoring

Perfusion can benefit organoid cultures through application of fluid mechanical stress, supply of nutrients, removal of waste, or dynamic application of different culture media or drugs. In one example, Jin et al[69] loaded mouse intestinal organoids in a microfluidic device to create an array of organoids to facilitate organoid volume change measurements. The investigators re-created secretory diarrhea by adding cholera toxin to the media, which induced swelling of the organoids. This microfluidic array potentially could be used to perform multiplex drug screening. Although there are increasing numbers of microfluidic intestine culture systems, most are used for 2D cultures and there are still few examples of microperfusion of intact, 3D organoids. It already has been shown that stimulating the apical side of the intestinal epithelium in 2D fluidic cultures induced intact and differentiated intestinal epithelium with in vivo recapitulating microstructural changes.[8–10]

Hill et al[33] analyzed cell signaling molecules such as interleukin 6, interleukin 8, vascular endothelial growth factor, and β-defensin that have been secreted to the basal side of the bacteria-colonized HIOs using enzyme-linked immunosorbent assays. Also, in the same work, the investigators measured bacterial translocation across HIOs by microinjecting *E coli* in the lumen and counting the number of bacteria in the external media over time.[33] These time-lapse microscale media analytics still are limited to external culture media. Adding a microperfusion component to 3D intestinal organoids will allow access to the media in the luminal compartment as well. The increasing interest and need for microperfusion coupled with a lack of solutions make this an important area of opportunity.

Dynamic environments created by microperfusion and real-time monitoring go hand-in-hand. An important consideration for future development include decreasing the cost and the development of perfusion systems and biosensors that can go directly inside the incubator where temperature and humidity is much better controlled than on a microscope stage. One recent example is a miniaturized microscope constructed from an inexpensive webcam that can be placed inside an incubator to provide real-time images of microscale tissue dynamics.[70]

Conclusions

The dynamic environment in the GI tract is associated with various physiologic responses within the human body. Advances in stem cell–derived GI organoid cultures enabled human-specific studies, complementing animal models and conventional in vitro models. The development of engineered biosensors appropriate for cystic organoids still is lagging, and spatiotemporal changes in organoids and their microenvironment are difficult to monitor. Here, we reviewed sensors that had been incorporated into GI organoid studies, targeting pericellular O_2, cytosolic and luminal pH, metabolism, and transepithelial barrier function. In addition, we discussed emerging biosensing techniques that may add value to understanding dynamic host–microbiota–pathogen interactions in GI organoids. Scaling down and developing new form factors of existing biosensors are crucial. Because the size of GI organoids is typically in the range of a few hundred microns to a few millimeters, adaptation of existing micro/nanoscale technologies, for example, microfluidics, to GI organoid cultures will create synergistic effects. For broad use of these capabilities, cost reduction, miniaturization, and engineering sensors that can be used inside cell culture incubators also will be important to consider.

References

1. Greiner T, Bäckhed F. Effects of the gut microbiota on obesity and glucose homeostasis. Trends Endocrinol Metab 2011;22:117–123.
2. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. J Psychiatr Res 2015;63:1–9.

3. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. Nature 2016;535:85–93.
4. Gray J, Oehrle K, Worthen G, Alenghat T, Whitsett J, Deshmukh H. Intestinal commensal bacteria mediate lung mucosal immunity and promote resistance of newborn mice to infection. Sci Transl Med 2017;9:eaaf9412.
5. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, Lampis A, Eason K, Huntingford I, Burke R, Rata M, Koh D-M, Tunariu N, Collins D, Hulkki-Wilson S, Ragulan C, Spiteri I, Moorcraft SY, Chau I, Rao S, Watkins D, Fotiadis N, Bali M, Darvish-Damavandi M, Lote H, Eltahir Z, Smyth EC, Begum R, Clarke PA, Hahne JC, Dowsett M, de Bono J, Workman P, Sadanandam A, Fassan M, Sansom OJ, Eccles S, Starling N, Braconi C, Sottoriva A, Robinson SP, Cunningham D, Valeri N. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science 2018;359:920–926.
6. Kernéis S, Bogdanova A, Kraehenbuhl JP, Pringault E. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 1997;277:949–952.
7. Hubatsch I, Ragnarsson EGE, Artursson P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. Nat Protoc 2007;2:2111–2119.
8. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 2012;12:2165–2174.
9. Kim HJ, Li H, Collins JJ, Ingber DE. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. Proc Natl Acad Sci U S A 2016;113:E7–E15.
10. Shah P, Fritz JV, Glaab E, Desai MS, Greenhalgh K, Frachet A, Niegowska M, Estes M, Jäger C, Seguin-Devaux C, Zenhausern F, Wilmes P. A microfluidics-based in vitro model of the gastrointestinal human–microbe interface. Nat Commun 2016;7:11535.
11. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.
12. Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, Sugihara H, Fujimoto K, Weissman IL, Capecchi MR, Kuo CJ. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med 2009;15:701–706.
13. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011;470:105–109.
14. McCracken KW, Aihara E, Martin B, Crawford CM, Broda T, Treguier J, Zhang X, Shannon JM, Montrose MH, Wells JM. Wnt/β-catenin promotes gastric fundus specification in mice and humans. Nature 2017;541:182–187.
15. Uchida H, Machida M, Miura T, Kawasaki T, Okazaki T, Sasaki K, Sakamoto S, Ohuchi N, Kasahara M, Umezawa A, Akutsu H. A xenogeneic-free system generating functional human gut organoids from pluripotent stem cells. JCI Insight 2017;2:e86492.
16. Kauffman AL, Gyurdieva AV, Mabus JR, Ferguson C, Yan Z, Hornby PJ. Alternative functional in vitro models of human intestinal epithelia. Front Pharmacol 2013;4:79.
17. Schweinlin M, Wilhelm S, Schwedhelm I, Hansmann J, Rietscher R, Jurowich C, Walles H, Metzger M. Development of an advanced primary human in vitro model of the small intestine. Tissue Eng Part C Methods 2016;22:873–883.
18. Noel G, Baetz NW, Staab JF, Donowitz M, Kovbasnjuk O, Pasetti MF, Zachos NC. A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. Sci Rep 2017;7:45270.
19. Yissachar N, Zhou Y, Ung L, Lai NY, Mohan JF, Ehrlicher A, Weitz DA, Kasper DL, Chiu IM, Mathis D, Benoist C. An intestinal organ culture system uncovers a role for the nervous system in microbe-immune crosstalk. Cell 2017;168:1135–1148.e12.
20. Moon C, VanDussen KL, Miyoshi H, Stappenbeck TS. Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. Mucosal Immunol 2014;7:818–828.
21. VanDussen KL, Marinshaw JM, Shaikh N, Miyoshi H, Moon C, Tarr PI, Ciorba MA, Stappenbeck TS. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 2015;64:911–920.
22. Finkbeiner SR, Hill DR, Altheim CH, Dedhia PH, Taylor MJ, Tsai Y-H, Chin AM, Mahe MM, Watson CL, Freeman JJ, Nattiv R, Thomson M, Klein OD, Shroyer NF, Helmrath MA, Teitelbaum DH, Dempsey PJ, Spence JR. Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo. Stem Cell Reports 2015;4:1140–1155.
23. Leslie JL, Huang S, Opp JS, Nagy MS, Kobayashi M, Young VB, Spence JR. Persistence and toxin production by Clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. Infect Immun 2015;83:138–145.
24. Hill DR, Huang S, Tsai Y-H, Spence JR, Young VB. Real-time measurement of epithelial barrier permeability in human intestinal organoids. J Vis Exp 2017;130:e56960.
25. Espey MG. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. Free Radic Biol Med 2013;55:130–140.
26. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H, Thom SR, Bushman FD, Vinogradov SA, Wu GD. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. Gastroenterology 2014;147:1055–1063.e8.
27. Taylor CT, Colgan SP. Hypoxia and gastrointestinal disease. J Mol Med 2007;85:1295–1300.
28. Zeitouni NE, Chotikatum S, von Köckritz-Blickwede M, Naim HY. The impact of hypoxia on intestinal epithelial cell functions: consequences for invasion by bacterial pathogens. Mol Cell Pediatr 2016;3:14.

29. Sheridan WG, Lowndes RH, Young HL. Intraoperative tissue oximetry in the human gastrointestinal tract. Am J Surg 1990;159:314–319.
30. Chen Y, Lin Y, Davis KM, Wang Q, Rnjak-Kovacina J, Li C, Isberg RR, Kumamoto CA, Mecsas J, Kaplan DL. Robust bioengineered 3D functional human intestinal epithelium. Sci Rep 2015;5:13708.
31. Clark LC Jr. Monitor and control of blood and tissue oxygen tensions. Trans Am Soc Artif Intern Organs 1956; 2:41–48.
32. Chiu JH-C, Kim G-A, Daniels R, Takayama S. Electrochemical sensors for organs-on-a-chip. In: Murphy SV, Anthony A, eds. Regenerative medicine technology. Gene and cell therapy. Boca Raton, FL: CRC Press, 2016:45–64.
33. Hill DR, Huang S, Nagy MS, Yadagiri VK, Fields C, Mukherjee D, Bons B, Dedhia PH, Chin AM, Tsai Y-H, Thodla S, Schmidt TM, Walk S, Young VB, Spence JR. Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. Elife 2017;6:e29132.
34. Wang L, Acosta MA, Leach JB, Carrier RL. Spatially monitoring oxygen level in 3D microfabricated cell culture systems using optical oxygen sensing beads. Lab Chip 2013;13:1586–1592.
35. Lesher-Perez SC, Kim G-A, Kuo C, Leung BM, Mong S, Kojima T, Moraes C, Thouless M, Luker G, Takayama S. Dispersible oxygen microsensors map oxygen gradients in three-dimensional cell cultures. Biomater Sci 2017; 5:2106–2113.
36. Han J, Burgess K. Fluorescent indicators for intracellular pH. Chem Rev 2010;110:2709–2728.
37. Wencel D, Abel T, McDonagh C. Optical chemical pH sensors. Anal Chem 2014;86:15–29.
38. Kovbasnjuk O, Zachos NC, In J, Foulke-Abel J, Ettayebi K, Hyser JM, Broughman JR, Zeng X-L, Middendorp S, de Jonge HR, Estes MK, Donowitz M. Human enteroids: preclinical models of non-inflammatory diarrhea. Stem Cell Res Ther 2013; 4(Suppl 1):S3.
39. Zachos NC, Kovbasnjuk O, Foulke-Abel J, In J, Blutt SE, de Jonge HR, Estes MK, Donowitz M. Human enteroids/colonoids and intestinal organoids functionally recapitulate normal intestinal physiology and pathophysiology. J Biol Chem 2016;291:3759–3766.
40. Yao X, Forte JG. Cell biology of acid secretion by the parietal cell. Annu Rev Physiol 2003;65:103–131.
41. Bas T, Augenlicht LH. Real time analysis of metabolic profile in ex vivo mouse intestinal crypt organoid cultures. J Vis Exp 2014;93:e52026.
42. Fan Y-Y, Davidson LA, Callaway ES, Wright GA, Safe S, Chapkin RS. A bioassay to measure energy metabolism in mouse colonic crypts, organoids, and sorted stem cells. Am J Physiol Gastrointest Liver Physiol 2015;309:G1–G9.
43. Labuz JM, Moraes C, Mertz DR, Leung BM, Takayama S. Building an experimental model of the human body with non-physiological parameters. Technology 2017; 5:42–59.
44. Correia C, Koshkin A, Duarte P, Hu D, Teixeira A, Domian I, Serra M, Alves PM. Distinct carbon sources affect structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Sci Rep 2017;7:8590.
45. Jung P, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, Auer H, Gallardo M, Blasco MA, Sancho E, Clevers H, Batlle E. Isolation and in vitro expansion of human colonic stem cells. Nat Med 2011; 17:1225–1227.
46. Bull RA, Tanaka MM, White PA. Norovirus recombination. J Gen Virol 2007;88:3347–3359.
47. Tao Y, Rotem A, Zhang H, Cockrell SK, Koehler SA, Chang CB, Ung LW, Cantalupo PG, Ren Y, Lin JS, Feldman AB, Wobus CE, Pipas JM, Weitz DA. Artifact-free quantification and sequencing of rare recombinant viruses by using drop-based microfluidics. Chembiochem 2015;16:2167–2171.
48. Zhang H, Cockrell SK, Kolawole AO, Rotem A, Serohijos AWR, Chang CB, Tao Y, Mehoke TS, Han Y, Lin JS, Giacobbi NS, Feldman AB, Shakhnovich E, Weitz DA, Wobus CE, Pipas JM. Isolation and analysis of rare norovirus recombinants from coinfected mice using drop-based microfluidics. J Virol 2015;89:7722–7734.
49. Tao Y, Rotem A, Zhang H, Chang CB, Basu A, Kolawole AO, Koehler SA, Ren Y, Lin JS, Pipas JM, Feldman AB, Wobus CE, Weitz DA. Rapid, targeted and culture-free viral infectivity assay in drop-based microfluidics. Lab Chip 2015;15:3934–3940.
50. Jones MK, Grau KR, Costantini V, Kolawole AO, de Graaf M, Freiden P, Graves CL, Koopmans M, Wallet SM, Tibbetts SA, Schultz-Cherry S, Wobus CE, Vinjé J, Karst SM. Human norovirus culture in B cells. Nat Protoc 2015;10:1939–1947.
51. Ettayebi K, Crawford SE, Murakami K, Broughman JR, Karandikar U, Tenge VR, Neill FH, Blutt SE, Zeng X-L, Qu L, Kou B, Opekun AR, Burrin D, Graham DY, Ramani S, Atmar RL, Estes MK. Replication of human noroviruses in stem cell-derived human enteroids. Science 2016;353:1387–1393.
52. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. TEER measurement techniques for in vitro barrier model systems. J Lab Autom 2015;20:107–126.
53. Elbrecht DH, Long CJ, Hickman JJ. Transepithelial/endothelial electrical resistance (TEER) theory and applications for microfluidic body-on-a-chip devices. J Rare Dis Res Treat 2016;1:46–52. Available from: http://www.rarediseasesjournal.com/articles/transepithelialendothelial-electrical-resistance-teer-theory-and-applications-for-microfluidic-bodyonachip-devices-raredis-1-1026.php. Accessed: July 20, 2017.
54. Yuan SY, Rigor RR. Methods for measuring permeability. In: Yuan SY, Rigor RR, eds. Regulation of endothelial barrier function. San Rafael, CA: Morgan and Claypool Life Sciences, 2011:21–38.
55. Siflinger-Birnboim A, Del Vecchio PJ, Cooper JA, Blumenstock FA, Shepard JM, Malik AB. Molecular sieving characteristics of the cultured endothelial monolayer. J Cell Physiol 1987;132:111–117.
56. Aurora M, Spence JR. hPSC-derived lung and intestinal organoids as models of human fetal tissue. Dev Biol 2016;420:230–238.

57. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsolis RM, Roth JR, Bäumler AJ. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature 2010;467:426–429.
58. Hensel M, Hinsley AP, Nikolaus T, Sawers G, Berks BC. The genetic basis of tetrathionate respiration in Salmonella typhimurium. Mol Microbiol 1999;32:275–287.
59. Kotula JW, Kerns SJ, Shaket LA, Siraj L, Collins JJ, Way JC, Silver PA. Programmable bacteria detect and record an environmental signal in the mammalian gut. Proc Natl Acad Sci U S A 2014;111:4838–4843.
60. Riglar DT, Giessen TW, Baym M, Kerns SJ, Niederhuber MJ, Bronson RT, Kotula JW, Gerber GK, Way JC, Silver PA. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. Nat Biotechnol 2017;35:653–658.
61. Daeffler KN-M, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, Britton RA, Tabor JJ. Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. Mol Syst Biol 2017;13:923.
62. Radomski MW, Moncada S. Regulation of vascular homeostasis by nitric oxide. Thromb Haemost 1993; 70:36–41.
63. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med 2003;9:1498–1505.
64. Beckman JS. Ischaemic injury mediator. Nature 1990; 345:27–28.
65. Kubes P, McCafferty D-M. Nitric oxide and intestinal inflammation. Am J Med 2000;109:150–158.
66. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology 2004;113:427–437.
67. Chokshi NK, Guner YS, Hunter CJ, Upperman JS, Grishin A, Ford HR. The role of nitric oxide in intestinal epithelial injury and restitution in neonatal necrotizing enterocolitis. Semin Perinatol 2008;32:92–99.
68. Archer EJ, Robinson AB, Süel GM. Engineered E. coli that detect and respond to gut inflammation through nitric oxide sensing. ACS Synth Biol 2012;1:451–457.
69. Jin B-J, Battula S, Zachos N, Kovbasnjuk O, Fawlke-Abel J, In J, Donowitz M, Verkman AS. Microfluidics platform for measurement of volume changes in immobilized intestinal enteroids. Biomicrofluidics 2014;8:24106.
70. Zhang YS, Ribas J, Nadhman A, Aleman J, Selimović Š, Lesher-Perez SC, Wang T, Manoharan V, Shin S-R, Damilano A, Annabi N, Dokmeci MR, Takayama S, Khademhosseini A. A cost-effective fluorescence mini-microscope for biomedical applications. Lab Chip 2015; 15:3661–3669.

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Ge-Ah Kim and Shuichi Takayama were responsible for preparing the outline, major drafting of the manuscript, and editing; and Nicholas J. Ginga was responsible for drafting and editing the section on barrier function.

Conflicts of interest
The authors disclose no conflicts.

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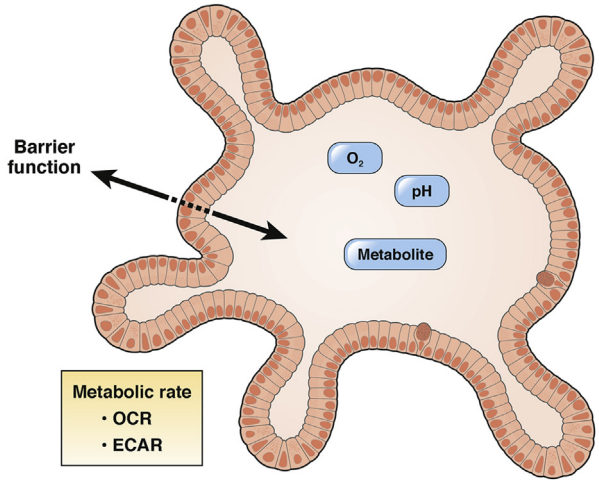

Supplemental Graphical Summary.