OrganoReady® Colon Organoid

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BME 410L
Spring 2025

1.

The native tissue that was modeled was human colon epithelium. The key cell types include colon epithelial cells (enterocytes), goblet cells, and stem/progenitor cells. Colon epithelial cells are main absorptive cells that line the colon and are responsible for the absorption of water and electrolytes. Goblet cells specialize in secretory cells that produce mucus. They protect and lubricate the intestinal lining. Stem/progenitor cells are located at the base of the crypts and constantly regenerate the colon's epithelium (Barker, 2014). Although OrganoReady focuses primarily on enterocytes, goblet cells, and stem cells, there are other epithelial subtypes including enteroendocrine cells, tuft cells, and Paneth-like cells. Although Paneth cells are primarily small intestine, there exist some Paneth-like cells in the human colon that are involved in antimicrobial peptide secretion. The turnover rate for the human colon epithelium is roughly 3-5 days, meaning it renews itself. The stem cells at the crypt base continuously divide and new cells migrate upwards, then differentiating into enterocytes or goblet cells. It's important to also note that epithelial cells aren't simply a wall. They actually interact actively with immune cells. These cells secrete antimicrobial peptides such as defensins and present signals to resident macrophages and dendritic cells via cytokine release. Some of these include IL-8 and IL-1ß. Not only are there immune interactions in the microbiome, but the colon epithelium also has dynamic interactions with the gut microbiota. This influences epithelial differentiation, mucus production, and immune tolerance (Sommer and Bäckhed, 2013).

The tissue architecture consists of a crypt-villus structure, a single-layered epithelium, and a basement membrane. The surface of the colon is organized into crypts of Lieberkühn, which are invaginations lined by proliferative stem cells at the base and differentiated cells towards the top. There are no villi in the colon, unlike the small intestine. A simple columnar epithelial layer containing tight junctions is what forms a selective barrier between the colon lumen and underlying tissue. Lastly, the basement membrane lies under the epithelial cells, and they provide structural support.

The main functions of the human colon epithelium are barrier protection, water and electrolyte absorption, and mucus secretion (Allaire et al., 2018). With regards to barrier protection, it prevents pathogen invasion and maintains immune homeostasis. Water is recovered from intestinal contents in order to form solid stool. The purpose of mucus secretion is to protect epithelial cells from mechanical and chemical injury.

2.

The OrganoReady Colon Organoid technology platform created by MIMETAS has notable features such as cells, biomaterials, microfluidics, sensors, and assay capabilities. The primary cells are human colon epithelial cells derived from healthy donors. They retain features of stem cells, differentiated epithelial cells, and goblet cells (Trietsch et al., 2017). The biomaterial of the technology platform is an extracellular matrix (ECM) hydrogel that is similar to Matrigel and supports 3D growth. This allows cells to maintain polarity as well as crypt-like structures. As for microfluidics, the OrganoPlate 384 platform uses phaseguides to create microfluidic channels without the use of pumps or valves. This gravity-driven flow is what supports gentle culture conditions. There are no integrated electronic sensors. The OrganoReady Colon Organoid technology platform doesn't use integrated electronic sensors. Additionally, it is compatible with optical and fluorescence assays for viability, barrier integrity, and imaging. There are also multiple assay capabilities. These include barrier function assays such as FITC-dextran permeability, drug screening with high-throughput capability, cytotoxicity testing, viability assays (live/dead staining), immunostaining and imaging, and gene expression profiling. The benefit of the high-throughput screening capability is that the 384-channel

OrganoPlate allows for parallel experiments. Drug screenings, toxicity, and permeability assays with many different conditions can be performed at the same time. This is a significant advantage for pharmaceutical preclinical testing or even personalized medicine approaches.

3.

When comparing OrganoReady Colon Organoids and native human colon tissue, one feature that is modeled closely is the crypt architecture and cellular composition. Organoids create the 3D structure with colon stem cells, differentiated epithelial cells, and goblet cells that are embedded in the ECM. Meanwhile in native human colon tissue, native colon has crypts filled with stem cells at the base with differentiated cells at the top. Mucus production by goblet cells mirrors the structure of the organoid. A feature that is not modeled closely is the lack of complex vasculature and immune cell environment. Organoids don't include immune components such as macrophages and T-cells, blood vessels, or stromal cells. In vivo, the colon tissue is extremely vascularized and interacts constantly with mucosal immune cells which are crucial for maintaining the barrier function and responding to other pathogens. Blood vessels in the colon supply are what supply the oxygen and nutrients to the basal side. This also assists with the removal of waste. Oxygen gradients, which are more hypoxic near the lumen, shape cell metabolism as well as barrier function. Organoids are purely diffusion-limited, which means they create non-physiological conditions if they become larger. If angiogenesis or vasculature is missing, this has a major effect, particularly for long-term cultures. Potential future improvements include the incorporation of co-culture systems with endothelial cells to stimulate vasculature, immune cells such as macrophages, and bacterial consortia that stimulate the microbiota. Mechanical forces are more accurately modeled by embedding organoids into a dynamic flow.

References

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