

EDITORIAL

Using Microfluidics to Model Mucus



Much of our understanding of molecular mechanisms of diseases has relied historically on work performed in cancer cell lines. Although cancer-derived cell lines offer many advantages such as being easy to grow, relatively inexpensive, and amenable to experimental manipulation, the degree to which cell lines faithfully recapitulate biological processes that occur in vivo is unclear. The development of intestinal organoids or enteroids from primary adult epithelium by Sato et al¹ in 2009 provided an innovative and valuable in vitro tool for scientists. Intestinal organoids mimic in vivo tissue more closely than traditional cell lines. The presence and maintenance of all intestinal cell types including enterocytes, tuft cells, goblet cells, enteroendocrine cells, stem cells, Paneth cells, and so forth in intestinal organoid culture offers a distinct advantage over cell lines that lack cell type diversity. Moreover, intestinal organoids can be derived from limited amounts of tissue, such as biopsy specimens, and expanded exponentially, making them a useful tool for studying disease mechanisms. Importantly, intestinal organoids recapitulate in vivo physiology with limited genetic alterations after long-term culturing.

For the past decade, scientists have been tailoring intestinal organoid culture systems and techniques to fit specific experimental needs. Intestinal organoids originally were grown and manipulated in Matrigel (Corning, Bedford, MA) in a 3-dimensional format. However, 2-dimensional monolayers of intestinal organoids recently were generated to address questions that require access to the lumen or the apical membrane. In addition to these formats of organoid cultures, other techniques have begun to be implemented to better mimic in vivo conditions. One new piece of technology is the organ-on-a-chip system, which features a multichannel 3-dimensional microfluidic cell culture chip. The organ-on-a-chip platform simulates the mechanics and physiological conditions present in vivo.

An important step in achieving more physiologically relevant in vivo-like conditions in the colon is the generation of a mucus bilayer in vitro. In vivo, the colonic epithelium is lined by a thick mucus bilayer produced by goblet cells that protect epithelial cells from bacterial contact and potential pathogens. The importance of the colonic mucus layer is shown by the fact that disruption of the layer results in colitis. Previously, the generation of a physiologically functional mucus bilayer in vitro had not been realized. Sontheimer-Phelps et al² have reported successful in vitro generation of a mucus bilayer using human organoid-derived colonic epithelial cells cultured using an organ-on-a-chip microfluidic device. They show that dynamic flow conditions result in a polarized, confluent colonic epithelial layer that maintained barrier function in vitro. In addition, organoid-derived

colonic epithelial cells cultured using organ-on-a-chip resulted in spontaneous goblet cell differentiation as determined by the presence of mucin (MUC2)-positive goblet cells, Trefoil factor 3 immunostaining, and gene expression of goblet cell markers.

A unique aspect of the culture system described by the Ingber laboratory is the generation of an inner and outer mucus layer in vitro. The colonic mucus layer in vivo is characterized by a dense inner mucus layer that is impenetrable to bacteria and an outer mucus layer that is looser and populated by commensal bacteria.^{3,4} By using fluorescent microbeads and transmission electron microscopy, Sontheimer-Phelps et al² showed the delineation of the inner and outer mucus layer formed by organoid-derived colonic epithelial cells cultured using organ-on-a-chip. They further showed that organ-on-a-chip colonic epithelium treated with prostaglandin E₂, which is increased in patients with ulcerative colitis, increased mucus volume through hydration via the sodium-potassium-chloride cotransporter, NKCC1, ion channel.

In summary, Sontheimer-Phelps et al² reported a robust in vitro culture system that provides a model to investigate the physiology of colonic mucus that can be applied to study the mechanism of various gastrointestinal disorders. In the future, this tool will be important to dissect the mechanism of mucus secretion and formation of the mucus bilayer. The role of the microbiome in functional maturation of the mucus bilayer under homeostatic and pathophysiological conditions also remains to be addressed. Johansson et al⁵ previously reported the necessity of bacterial colonization in vivo in mice to form a mucus bilayer. In contrast, Sontheimer-Phelps et al² have reported the establishment of an inner mucus layer sans bacterial interaction in their colon-on-a-chip in vitro model. Further investigations will likely shed light on these paradoxes and establish organ-on-a-chip microfluidic platforms as models for studying mucus production. Ultimately, this study provides new directions for organoid platforms and provides a new sophisticated means to generate more physiologically relevant models.

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Conflicts of interest

The author discloses no conflicts.

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