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MUCOSAL BIOENGINEERING: GUT IN A DISH

Ivaylo I. Ivanov

Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

Abstract

Studying the interactions between commensal microbes and host intestinal tissue networks is challenging due to the complexity and inaccessibility of the system. A recent study reports a novel organ culture system that will enhance our ability to dissect these interactions.

Organization of cells into tissue networks is crucial for all biological processes. These networks are complex and allow not only modification and regulation of functions of individual cells or systems, but also of synchronized tissue responses. For example, in the mammalian intestine interactions between multiple cell types, including immune, neuronal, enteroendocrine, epithelial, and muscle cells, are required to generate, regulate and coordinate appropriate responses to the numerous physiological and non-physiological stimuli present in the digestive tract. Characterization of the behavior and function of immune cells in peripheral tissues is an exciting research frontier in biology. However, the paucity of *ex vivo* experimental systems to study tissue responses is a particular challenge. This is especially true for the intestine, which in addition to tissue complexity, contains a unique luminal microenvironment that harbors a large community of mostly unculturable commensal microbes. Recent advances, such as “gut-on-a-chip”, intestinal organoids and 2D transwell culture of primary epithelial cells, have invigorated studies in this area by allowing *ex vivo* modeling of epithelial cell development, intestinal flow, or interactions between defined components, including intestinal microbes and epithelial cells [1–4]. The next step is modeling tissue microarchitecture in order to study cell recruitment, migration and interaction between multiple cellular components and gut microbes. A new study now reports an exciting intestinal organ culture system and demonstrates how it can empower such experimentation.

One of the main challenges in developing *ex vivo* intestinal systems is the rapid deterioration of tissue architecture. *In vivo*, intestinal epithelial cells (IECs) are concomitantly exposed to vastly different environments on their luminal and basolateral sides, which is virtually impossible to replicate *ex vivo*. Therefore, primary IECs are especially prone to cell death when taken outside the body. In a new study in *Cell*, Yissachar et al., reasoned that

Correspondence: ii2137@cumc.columbia.edu.

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maintaining the environmental differential, including intestinal flow, is crucial for maintaining epithelial cell viability, and could improve tissue viability *ex vivo* [5]. They designed a sealed silicon tissue culture system unit that generates two independent flow environments outside and inside an extracted intestinal segment. This allows for 1) maintenance of physiological environmental differences and 2) introduction of controlled treatments to either the tissue or luminal sides. Amazingly, this system allows for maintenance of large intestinal segments (3–4 cm) *ex vivo* for up to 30 hours without evidence of deterioration. Intestinal tissue microarchitecture is preserved and this includes tissue histology, epithelial cell barrier function, immune cell subsets, intestinal smooth muscles and the enteric nervous system (ENS). The intestinal segments even exhibit spontaneous peristalsis [5].

The ability to maintain whole intestinal segments *ex vivo* for an extended period of time can be used to dissect cell migration and functional responses to different treatments. Importantly, this includes responses of desired combinations of microbes, which can be introduced directly into the intestinal lumen. As a proof-of-concept the authors used several immunomodulatory commensals, including segmented filamentous bacteria (SFB). In contrast to other commensals, SFB interact directly with and modify the function of IECs and this is important for their ability to induce mucosal Th17 cells [6, 7]. Introduction of SFB from feces of SFB-monocolonized mice into the luminal side of the *ex vivo* cultures induced a number of transcriptional profile changes in IECs that were also induced by the bacteria *in vivo*. Although restricted to a subset of the *in vivo* genes, this demonstrates that the *ex vivo* system is functional and can be used to study physiological tissue responses. In addition, this experiment demonstrates that the system can be used to study early responses to unculturable microbes.

To directly demonstrate that the system can be used to uncover new physiological mechanisms, Yissachar et al., introduced commensal microbes known to induce ROR γ^+ regulatory T cells (Treg). ROR γ^+ Tregs are a recently identified Treg subset that is present exclusively in the gut and appears to have important roles in intestinal homeostasis [8, 9]. The intestinal signals controlling presence of this subset are not known. Somewhat unexpectedly, colonization of colonic explants with ROR γ^+ Treg inducing microbes led to changes in genes associated with neurobiological processes. In particular, a set of genes involved in nociceptor neuronal function was suppressed, including the substance P neuropeptide precursor protein Tac1. Moreover, this was a property of multiple ROR γ^+ Treg inducing microbes and suggested that these microbes may engage ENS. The authors further explored this by showing that ROR γ^+ Treg inducing microbes activate neurons, including nociceptor neurons, in primary cultures [5]. Neuronal activation required live bacteria and was mediated by filtered bacterial supernatants, suggesting that gut neurons detect secreted products from metabolically active bacteria. To examine whether neuronal activation and Treg induction are causally connected, the authors went back to animal models and demonstrated that Tac1-KO mice have increased levels of ROR γ^+ Tregs in the gut. Conversely, nociceptor neuronal stimulation with dietary capsaicin suppressed ROR γ^+ Treg levels, but not total Treg levels, *in vivo*. Thus the *ex vivo* intestinal organ system can identify new complex physiological tissue interactions.

Modeling intestinal tissue responses *ex vivo* will be invaluable in investigating biological processes that require intersystem cellular interactions (Figure 1). The system developed by Yissachar et al. represents the first organ culture system for the gut. As any first iteration, the system has limitations. The gut explants can currently only be cultured for no more than 40 hours without losing tissue integrity, which limits readouts to early events and interactions. The culture is also adapted to intestines of young mice (under 14 days of age) and may need to be optimized for specific interactions in adult tissues. Although currently limited to mice, with the advances in humanized mouse models, the system could allow for examination of the behavior of human cells and tissues in a whole-organ context. Despite these limitations, this Gut-in-a-Dish system provides unprecedented opportunities to study cell-cell communication, migration and function in a controlled environment and upon external perturbations. Immune cell movement can be imaged directly *ex vivo* and multiplexing allows simultaneous culture of intestinal segments from different animals or under different treatments. Preserved tissue architecture also allows for investigation of physiological interactions between immune cells and stromal, neuronal, epithelial and other tissue networks. The organ culture system developed by Yissachar et al., brings new dimension to gut biology. Together with previous tissue engineering approaches, e.g. epithelial organoids, mucosal immunologists now seem well equipped to dissect the complex networks controlling immune responses in this fascinating organ (Figure 1).

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Figure 1. Modeling the gut

Replicating the critical functions of intestinal epithelial cells (IECs) is crucial for *ex vivo* models of intestinal biology. Cell line based models such as transwell assays allow for generation of polarized epithelia and study of epithelial cell responses *in vitro*. Further advances in this technology, e.g. Gut-on-a-Chip, allow for modeling of luminal flow and peristalsis. They also provide opportunity to better study basic interactions between epithelial cells, luminal microbiota and basal immune cells. Primary cell based models are aimed at utilizing primary IECs. This allows not only investigation of IEC development and function, but also modeling of intestinal epithelial responses from individual patients. Organ based models will allow investigation of the complex tissue interactions between microbiota, epithelial, immune, neural, and other cells in a physiological context.