Published in final edited form as:

Microbiol Spectr. 2019 March ; 7(2): . doi:10.1128/microbiolspec.BAI-0013-2019.

New Age Strategies to Reconstruct Mucosal Tissue Colonization and Growth in Cell Culture Systems

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CHAPTER SUMMARY

Over the past few decades, *in vitro* cell culture systems have greatly expanded our understanding of host-pathogen interactions. Yet, studies using these models have been limited by the fact that they lack the complexity of the human body. Therefore, recent efforts that allow tissue architecture to be mimicked during *in vitro* culture have included the development of methods and technology that incorporate tissue structure, cellular composition, and efficient long-term culture. These advances have opened the door for the study of pathogens that previously could not be cultured and for the study of pathophysiological properties of infection that could not be easily elucidated using traditional culture models. Here we discuss the latest studies using organoids and engineering technology that have been developed and applied to the study of host-pathogen interactions in mucosal tissues.

INTRODUCTION: THE STUDY OF HOST-PATHOGEN INTERACTIONS IN CULTURE

With the current understanding that microbes play a large role in shaping human physiology, there has been renewed interest in the study of host-microbe interactions. Much of our present knowledge of host-pathogen interactions has resulted from studies with animal models or cultured immortalized cell lines that attempt to reproduce specific events during host-microbe interactions (1). Underlying these strategies is the goal of attempting to reproduce the important events that occur during human disease. The closest approximation to human infection has been to perform animal infection studies using model organisms such as the mouse, to attempt to generate a system with complexity and biological functions similar to humans. Unfortunately, many important pathogens do not readily colonize or establish disease in common mammalian models and the response to infection does not always recapitulate the human response (2, 3). A particularly confounding problem is that the infection site is often inaccessible in the animal, interfering with the ability to perform manipulations in real time, or allow direct observations at the microscopic level.

As an alternative to performing infections in animals, an entire subfield of microbiology has matured over the past 30 years that involves direct interrogation of microbial interactions with cultured mammalian cells (4). This has allowed access to the infection site and improved visualization of the host-pathogen interface. This strategy also has resulted in great advances in our understanding of how mammalian cell processes are manipulated by microbes (5, 6), but there are several shortcomings to this approach. First, microbial interactions in the host are often complex, with diverse subsets of both immune and non-immune cells interfacing with the microorganism simultaneously or in rapid succession. Most cell culture systems, in contrast, only include one cell type and therefore are unable to represent these complex interactions. Secondly, microbial interactions often involve targeting epithelial or other cells that have topological constraints with defined polarity (7). Most cell culture systems involve monolayers of immortalized cells, which lack these constraints. Finally, there is the problem that cell culture systems often use immortalized cells, which can behave quite differently from primary cells in the host (8).

To overcome some of these shortcomings, a few immortalized epithelial cell lines can be grown as confluent impermeant monolayers with tight junctions on Transwell® filters, which has allowed for the generation of defined apical and basal surfaces of the monolayer (9–11). These systems also have shortcomings, as they lack other biophysical properties and three dimensional (3D) structure of the particular organ system that they are trying to represent (12). One strategy to solve this problem has been to perform primary culture of cells from model mammals, such as the mouse or rat, but again this raises the problem of species differences in trying to reproduce events that take place in the human host (13). Thus, primary cells from various human organs have been harvested and cultured *in vitro* but have to be used for very short intervals as the tissues often deteriorate in culture (14). The lack of a system that fully represents the intricacy of the human body has left an incomplete understanding of what occurs during infection with a pathogen.

Recent technological advances in propagating human tissue long-term in culture have provided exciting avenues for the study of host-pathogen interactions. Of particular excitement is the use of organoids, which are 3D structures that can be derived from a variety of tissue types and include many of the cell types relevant to their tissue (15). Since they are derived from pluripotent stem cells or adult tissue stem cells, they maintain their ability for self-renewal and self-organization into structures that mimic the given organ (15). Furthermore, engineering advances have improved the methods used for cell culture, allowing for the inclusion of additional biophysical tissue features such as flow and mechanical stresses. These exciting new developments will likely provide a path for culturing organoids in physiologically relevant conditions and could prove to be invaluable for the study of host-pathogen interactions. In this chapter, we address recent advances in *ex vivo* models for the analysis of host-microbe interactions in various mucosal tissues.

DEVELOPMENT OF INTESTINAL ORGANOIDS

In 2009, two groups developed methods for long-term culture of primary mouse intestinal cells from single Lgr5⁺ stem cells or stem cells originating from crypts that could recapitulate intestinal differentiation (16, 17). The LGR5 gene had recently been identified

as a marker for multipotent intestinal stem cells that give rise to all intestinal cell types (18). Prior to these discoveries, methods to develop long-term culture of primary cells had uniformly failed and attempts to induce cultured primary cells to mimic intestinal villi and crypt morphology were unsuccessful. Supplying stem cells with proper growth factors resulted in the formation of 3D structures, termed organoids, that maintain stem cell quality and therefore could self-renew *in vitro* for long-term culture (19). Removal of stem cell-promoting factors resulted in differentiation of the organoids to exhibit the main cell types found in the intestine including enterocytes, Paneth cells, goblet cells, and enteroendocrine cells (19). These methods were also applied to human tissue from the colon for the culture of colonoids and have since been applied to other regions of the gastrointestinal tract from both diseased and healthy tissues (19). Perhaps one of the most exciting aspects of these cells is that they can be isolated from human patients during very common standard procedures, such as colonoscopies, allowing diverse patient populations to be analyzed as well as provide important tools directed toward studying human genetic diseases.

Another method to establish long-term human intestinal cell culture involves the culture of human pluripotent stem cells (hPSCs) and the resulting 3D structures are often termed human intestinal organoids (HIOs) (20–22). Thus, to distinguish the origin of the derived tissue, the organoids previously described that are derived from intestinal Lgr5⁺ stem cells are now often termed human intestinal enteroids (HIEs). Organoids developed from hPSCs contain an epithelial layer and a mesenchymal layer and are more fetal-like in nature and therefore may be particularly useful for studies of intestinal development (21). Both enteroids and organoids maintain physiological properties of the intestine such as functional ion transport via absorption and secretion (23) and peptide transport (21). They also maintain intestinal structure as they self-assemble into stem cell-containing proliferative zones and differentiated zones (21). Altogether, the development of these human “mini-intestines” has opened the door for the study of intestinal development, pathogenesis of disease, and host-pathogen interactions.

USE OF INTESTINAL ORGANOIDS FOR THE STUDY OF HOST-PATHOGEN INTERACTIONS

Organoids As 3D Structures And 2D Monolayers

One of the primary complications of analyzing pathogen interaction with organoids is that their multicellular 3D structure results in topological constraints. There is an opportunity cost because organoids allow for new analysis strategies, in that the 3D cellular structures can mimic a mini-organ in structural topology and function. However, as the apical side of the epithelium faces the lumen of the structure, microorganisms must be microinjected into the organoid to allow contact with the relevant cells (24) (Figure 1). In this fashion, Spence and coworkers have used HIOs to analyze bacterial interactions. They have shown that the anaerobe *C. difficile* can colonize the lumen of these structures and cause damage to epithelium due to the secretion of clostridial Tcd toxins (25). Therefore, the luminal environment is of sufficiently low oxygen tension to allow survival and expression of toxin genes by an anaerobe. The Spence group also took advantage of the fact that the culture of HIOs allows researchers to study the interaction between immature stem cells and

microbiota components to determine how microbes affect host epithelial development. To this end, HIOs were microinjected with non-pathogenic *E. coli* to study the initial colonization of the microbes (26). Colonization promoted antimicrobial responses, such as the production of inflammatory cytokines as well as mucin production, under conditions in which the epithelial barrier integrity was maintained. This system could provide key tools to interrogate events linked to microbial control of intestinal epithelial cell development.

To facilitate infection of the apical side of the intestine, enteroids grown as 3D cultures can be broken up into single cells and seeded onto Transwell® filters (27) (Figure 1). In this model, the enteroids form a polarized monolayer with defined apical and basolateral surfaces. Importantly, the monolayers contain differentiated cell types found in the intestine and a mucus layer covers the apical surface. Pathogenic strains of *E. coli* adhere to both ileal and rectal derived epithelial monolayers indicating that this model is amenable to the study of host-pathogen interactions (27). Furthermore, enterohemorrhagic *Escherichia coli* (EHEC) colonizes preferentially on differentiated monolayers when compared to undifferentiated monolayers, which may be due to the presence of mucin on the differentiated monolayers, a target of EHEC during infection (28). Transmission scanning electron microscopy images of the colonoid monolayers reveals typical attaching and effacing lesions that are associated with the natural infection of the human intestine. Thus, the monolayers provide a useful tool for visualization of early events during host-pathogen interactions.

Organoids as a Model For Pathogens That Are Difficult To Culture

Studies of enteric viruses have been hindered by the lack of good models, with the downstream consequence being that there is a lack of effective intervention therapies. For example, human noroviruses do not replicate to high levels when cultured with transformed intestinal cell lines (29, 30) and do not readily infect animal models (31). To determine if enteroids can be used for culturing norovirus, human norovirus strains were isolated from stool filtrates and infected onto jejunum derived monolayers (32). Using the HIE model, human norovirus strains of the GII.4 genotype, which cause the majority of norovirus disease worldwide, replicate to high titers. Other relevant clinical strains require pre-treatment of the enteroid cultures with bile in order to replicate. Since replication can be detected, this system can be exploited to test the efficacy of viral neutralization techniques by subjecting HIEs to norovirus strains that had undergone heat inactivation or γ irradiation (32). In addition, HIOs have been found to express histo-blood group antigens which can bind human noroviruses, although replication is not as robust as seen in the HIE model (33). Differences in replication levels between the models may be due to the differentiation state of the enterocytes, however, taken together, these results demonstrate that each system holds promise for analyzing various aspects of norovirus infection.

Similar approaches using both organoids and enteroids have been taken to study rotavirus replication. Replication of clinical isolates of rotavirus in HIOs was shown to reach levels 10X that of the conventional monkey cell line MA104 (34). This study provided evidence that organoids could be used as a model for achieving rotavirus replication and studying rotavirus infection. Using HIEs as a model for rotavirus infection, this same group found

that human rotavirus can replicate in enteroids derived from all three sections of the small intestine (35). 3D enteroids were shown to swell and expand in response to rotavirus infection or treatment with rotaviral enterotoxin, likely representing fluid movement to the lumen, which occurs during a diarrheal episode. This indicates that enteroids can be used to study pathophysiological features of infection. In more recent work, rotavirus infection was shown to induce type III IFN response by HIEs, although this is ineffective at blocking viral replication in this system (36). By contrast, viral growth can be restricted when the HIEs are treated with exogenous type I IFNs. Enteroids have also been used to test the effectiveness of anti-rotavirus activity of IFN- α and ribavirin (37). The effect of these antivirals is variable among different patient strains of enteroids and is less effective when compared to immortalized cells used as model hosts. Since this result more closely relates to clinical outcomes of the antivirals, it suggests that enteroids can be used to evaluate drug efficacy possibly on a person-by-person basis.

Investigating Intestinal Tropism

Organoids can also be used to determine cell type tropism or differences between the intestinal segments, which is impossible with immortalized cell lines that lack the segment specificity and cellular diversity found in the intestine (38). Using differentiated ileum enteroids, the respiratory adenovirus HAdV-5p primarily infects intestinal goblet cells, but interestingly this cellular preference is not true for the enteric serotype HAdV-41p (39). Similarly, goblet cells are resistant to the enterovirus echovirus 11 (E11), but enteroendocrine cells are permissive for this virus (40). Rotavirus infection of HIOs shows tropism for replication in enterocytes as well as mesenchymal cells (34).

Intestinal enteroids, derived from the duodenum, jejunum, ileum, and the colon of three patients, infected with multiple pathogenic strains of enteroaggregative *Escherichia coli* (EAEC) demonstrates the remarkable tropism of the pathogen (41). There are five patterns of aggregative adherence of EAEC to these tissues, which can be seen in both a segment specific and host specific manner. Minimal diffuse adherence to the jejunum occurs across all donors and among the adherent EAEC strains. Total adherence to the duodenum, ileum, and colon varies among donors but is consistently robust in the duodenum. Adherence to the ileum occurs in a stacked-brick pattern whereas adherence to the colon is mesh-like. The pattern of adherence to the duodenum is more dependent on donor and occurs in sheet-like or microcolony patterns. Thus, enteroids provide the potential for studying host variability and intestinal tropism.

Incorporation of Immune Cells

The immune system is an essential component of the host response to infection, but organoid and enteroid cultures do not include immune cells. Therefore, there have been efforts to include immune components in enteroid cultures, especially in the context of studying host-pathogen interactions. HIOs have been co-cultured with human neutrophils prior to microinjection with the Shiga toxin producing *E. coli* O157:H7 strain or a commensal *E. coli* strain (42). In response to infection with O157:H7, neutrophils migrate to the lumen of the enteroids to a greater extent compared to enteroids harboring the commensal strain (42). To develop an enteroid-macrophage co-culture model, Transwell®

filters with human enteroid monolayers have been constructed that allow human macrophages derived from human peripheral blood monocytes to be seeded on the basolateral side (43). During apical infection with enteropathogenic *E. coli* (EPEC) or enterotoxigenic *E. coli* (ETEC), macrophages extend projections through to the apical side, decreasing the recovery of ETEC relative to culture without macrophages (43). Addition of immune components is critical to understanding the host response to infection, so continuing to develop ways to increase cellular complexity in these models will contribute to a fuller understanding of the events that occur at the interface of infection.

Use of Gastric Organoids To Study Host-Pathogen Interactions

The stomach is composed of two main regions, the corpus and the antrum, that each harbor specialized cell types. The corpus contains parietal cells that secrete acid, chief cells that aid in digestion, surface mucous pit cells, mucous neck cells, and endocrine cells, while the antrum contains few parietal or chief cells, but has mucous pit cells, endocrine cells, and basal gland cells that secrete mucus (44). Recently, methods for culturing intestinal organoids were applied to the development of organoids from the stomach, from both iPSCs and human gastric glands that contain self-renewing stem cells, making gastric organoids the first long-term primary human cell culture system for gastric tissue (45–48). The organoids from each stomach region express the distinct gastric cell types and can form typical gland and pit organization (45, 47, 48).

Gastric organoids have proved useful for studying infection by *Helicobacter pylori* (45–49), a bacterium that causes inflammation of the stomach epithelium and is known to be a major risk factor for the occurrence of peptic ulcers and gastric cancers. Interestingly, *H. pylori* colonizes in the stem and progenitor cell-containing gland region of microinjected gastric organoids and can induce epithelial cell proliferation, a tantalizing finding given the association between *H. pylori* infection and stomach adenocarcinoma (45, 47). *H. pylori* infection can also be supported by 2D monolayers of polarized epithelium derived from gastric organoids leading to upregulation of host inflammatory response genes (48). Gastric organoids will likely be valuable not only for investigating host-pathogen interactions, but also for investigating links between infection-generated tissue damage and long-term gastric disease.

ENGINEERING ADVANCES THAT HAVE IMPROVED THE INVESTIGATION OF HOST-PATHOGEN INTERACTIONS

Organ On Chip

A promising area of research for the application of host-pathogen interactions is the development of microfluidic devices, termed organs-on-a-chip, which have been reviewed extensively by Bhatia and Ingber (50). In this approach, human cells from a given organ are seeded into a chamber and subjected to continuous flow, with additional mechanical forces that mimic physiological organ conditions. These devices are exciting because they provide opportunities to increase assay complexity and provide a mimic for tissues subjected to hydrostatic forces. For instance, the devices can include multiple chambers that are separated by a porous membrane that is seeded with cells on each side, providing a tissue-

tissue interface. Alternatively, they can consist of multiple chips seeded with cells from different organs that connect together. These multi-organ models have great potential for studying how infections propagate between organs.

Lung-on-a-chip for the study of respiratory pathogens

The lungs and respiratory tract, like the digestive system, are topologically exterior mucosal tissues and are therefore subject to pathogenic and environmental insults from inhaled respiratory droplets. The lungs consist of highly structured conducting airways and alveoli. The epithelium of the conducting airway is made of ciliated, goblet, basal and other secretory cells, while the epithelium of the alveoli is made of alveolar type I and type II cells (51). Underlying smooth muscle cells, fibroblasts, and immune cells also contribute to the overall architecture of the lung (51). Due to the complexity of the lung and the various cell types that contribute to proper lung function, the lung has not been well mimicked *in vitro*. The recent development of lung-on-a-chip devices provides an opportunity to recreate various features of the lung microenvironment including the simultaneous culture of multiple cell types and cyclic breathing forces. Furthermore, development of lung organoids from the different regions of the lung has allowed for studies with defined lung cell types that are functionally more similar to *in vivo* responses than immortalized cell lines. As these models have only recently been described, they have been underutilized for the study of host-pathogen interactions but have great potential.

A lung-on-a-chip model, developed by Huh et al, includes two chambers separated by an extracellular matrix-coated porous membrane (52). The upper chamber is seeded with immortalized airway epithelial cells and interfaces with air while the opposite side of the membrane facing the lower chamber is seeded with human pulmonary microvascular endothelial cells and exposed to culture media. To mimic the pressures that occur during normal breathing, the device includes additional chambers connected to a vacuum that when activated reproduces the forces of breathing onto the epithelium. Interestingly, when TNF α is added to the upper chamber to mimic an inflammatory stimulus, the epithelium induces a change in the endothelium resulting in an increase in surface expression of ICAM-1. Neutrophils flowing through the lower endothelial chamber representing the vasculature, adhere to ICAM-1 and migrate through the endothelium and across the epithelium. This exciting system should provide an excellent model for the study of host-pathogen interactions to examine the immune response to infection.

Several laboratories have developed lung-on-a-chip models for studying airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) (53, 54). In addition, differentiated primary human airway epithelial cells have been seeded into a microfluidic model at the air-liquid interface and subjected to a pollen extract challenge (55). The basolateral flow-through can be collected through the fluidics system allowing secretion of the inflammatory marker interleukin (IL)-8 by epithelial cells to be measured in response to pollen, again providing evidence that this system provides the potential for novel analyses of the immune response and the mechanisms of disease.

Excitingly, methods have been described creating lung organoids from isolated stem cells from various regions of the lungs or from iPSCs (reviewed by Barkauskas et al (56)). While

these lung organoids are still new and being functionally explored, a few groups have used them for the study of virus infection. Lung organoids developed from pluripotent stem cells can be infected with respiratory syncytial virus (RSV), a virus that causes respiratory illness in very young children (57). After two days, infected cells begin to swell, detach from the epithelium, and are shed into the lumen of the organoid, a pathological response seen in humans during disease. Furthermore, in a different study, human lung organoids derived from adult patient tissues were demonstrated to be infected with various strains of enterovirus 71, a virus that infects the gastrointestinal and respiratory tracts (58). The virus replicates in lung organoids in a strain-dependent manner. The successes with both the lung-on-a-chip model and the lung organoids indicates that combining these two technological advances has the potential to broaden our knowledge of host-pathogen interactions in the lungs, to further evaluate the effect of drugs on tissues and infections, and to study pathogenesis of long-term infectious and non-infectious diseases in the lung.

Gut-On-A-Chip

Taking advantage of the microfluidics systems that have recently been developed to model the lung, the Ingber group created a gut-on-a-chip in which the top chamber of the microfluidics device is seeded with Caco-2 cells, an immortalized human intestinal cell line (59). This model supports peristalsis movements and flow, important physiological forces that contribute to the intestinal microenvironment and are essential for maintaining proper epithelial barrier function (60). Compared to the static Transwell® filter system that results in a flat monolayer of Caco-2 cells, the microfluidics chamber with flow results in the formation of a taller monolayer of cells that were columnar in shape, more closely mimicking *in vivo* conditions (59). Also, due to flow, the chip model supports the growth, but not overgrowth, of a microbe *Lactobacillus rhamnosus* GG which improves the barrier function of the epithelial layer as assessed by trans epithelial electrical resistance (TEER), a measurement of the resistance between each side of the chip. The Caco-2 cells under flow conditions form villus-like structures with defined proliferative and basal regions with cells that express intestinal markers (61).

To study the intestinal epithelial response during chronic inflammation, human peripheral blood mononuclear cells (PBMCs) added to the lower chamber of the gut-on-a-chip model allow recruitment of white blood cells to the underlying lamina propria to be mimicked (62). In the absence of underlying immune cells, addition of a non-pathogenic strain of *E. coli* causes no barrier dysfunction. In the presence of PBMCs, however, addition of the commensal leads to disruption of the monolayer barrier as detected by TEER measurement, suggesting that the presence of chronic inflammation promotes epithelial injury, as may occur during inflammatory bowel diseases (IBDs). Thus, the chip model could be useful for studying how the interplay between the immune system and the microbiome contributes to disease pathology during conditions of chronic inflammation.

The chip model has also been used to study the epithelial response to the enteric pathogen coxsackievirus B1 (CVB1) with loss of villus morphology detected by immunofluorescence after 24 hours post infection (63). CVB1 can infect these monolayers both apically (via inoculation in the top chamber of the microfluidic device) or basolaterally (via inoculation in

the bottom chamber). Interestingly, virions and inflammatory cytokines are released apically under both infection conditions. Future modifications to the gut-on-a-chip model such as the inclusion of immune cells, mesenchymal cells, and/or nerve cells would improve the physiological relevance to the human intestine and make it a more robust model for the study of host-pathogen interactions (64).

Combining Engineering Technologies With Enteroids

There have been recent exciting efforts to combine engineering technologies with intestinal organoids to create microenvironments that more closely represent the actual human intestine and exceed the capabilities of static 2D cell culture. The Kaplan group has developed an *in vitro* silk-based 3D scaffold model of the intestine that is cylindrical in nature and consists of a hollow lumen for the seeding of intestinal cells and a porous bulk that can support intestinal myofibroblasts (65) (Figure 2). Using immortalized intestinal cell lines to seed the lumen, this tissue model exhibits luminal mucus secretion and oxygen levels similar to *in vivo* conditions and could support *C. difficile* growth, toxin production, and epithelial damage (65, 66). Connecting the 3D scaffolds to a bioreactor perfusion system allows for the incorporation of luminal flow, induction of peristaltic-like contractions, and tunable oxygen levels (67). The addition of flow and peristalsis is essential for mimicking the microenvironment of the intestine that works to constantly clear luminal contents. Furthermore, the 3D scaffold model has since been seeded with cells from human intestinal enteroids resulting in monolayers that express a dense brush border of microvilli, markers of the four major intestinal cell types, and digestive enzyme production (68). This provides evidence that the silk-based scaffolding model can support and sustain growth and differentiation of a polarized monolayer of intestinal epithelium derived from enteroids. The design of these scaffolds could be modified to include immune cells on the basolateral side and to further mimic physiological conditions present during initial infection.

The Ingber group has improved the gut-on-a-chip with the development of the Intestine Chip, in which Caco-2 cells have been replaced with enteroids (69) (Figure 3). By seeding human intestinal duodenum enteroid fragments on the Intestine Chip and subjecting the device to continuous flow, Kasendra et al. have demonstrated that villus-like structures can be formed that extend out from a polarized epithelium (69). Further, when compared to human duodenum, cells grown in the Intestine Chip model are closer in gene expression profile to the human duodenum than to the cultured enteroids from which the chips were derived (69). They also increased the complexity of the microenvironment by seeding primary human intestinal microvascular endothelial cells in the chip model on the opposite surface from the intestinal enteroids in the lower chamber of the device. The inclusion of these cells results in enhanced confluency of the enteroid layer. This is a promising model that can now be applied to the study of host-pathogen interactions.

CONCLUSIONS

Researchers have barely skimmed the surface of the usefulness of organoids, but these promising studies demonstrate that organoids can be used for a variety of biomedical applications including infectious disease modeling, pathogenesis, and drug testing. One of

the most exciting aspects of this approach is the ability to derive organoids from different patients with distinct genetic backgrounds exposed to different microbiota populations, providing great potential for patient-specific therapies and personalized medicine. The development of organoids is a monumental step forward in terms of having a long-term culture system of non-transformed cells that have many of the properties of the cell types found in the respective tissue. Yet, many challenges remain, as organoids lack many players that affect the physiology of disease, such as immune cells, microbiota, stromal cells as well as distinct tissue forces and fluid flow. The interplay between of all these features and a pathogen is essential for a complete understanding of pathophysiology of a given disease. Therefore, the advancement of engineering technologies will further harness the great power of organoids through the development of innovative ways to incorporate human parameters into robust culture systems with the ultimate goal of improving human health.

ACKNOWLEDGEMENTS

We thank our collaborators and members of our laboratories for useful discussions. ACF was supported by NIAID T32 AI007077; JM was supported by NIAID U19 AI131126 and NIAID R21 AI12809. RRI was supported by NIAID R01 AI110684.

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Figure 1: Methods for using intestinal organoids to study host-pathogen interactions.

(a) Image of undifferentiated intestinal cysts containing stem cells. (b) Cysts can be differentiated in Matrigel and used for microinjection with bacteria. Image of differentiated enteroids (right). (c) Cysts can be broken up enzymatically with trypsin and seeded on Transwell® filters and differentiated into a polarized monolayer with an apical side (A) and a basolateral side (B). Microbes can be added to the apical side using a pipette. Image looking onto apical surface of monolayer (right). All images taken through 4X objective lens on optical microscope.

Figure 2: Methodology for development of 3D silk scaffold to model human intestines.
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Figure 3: Methodology for development of Intestine Chip using enteroids to model human intestines.

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