

Intestinal organoid as an *in vitro* model in studying host-microbial interactions

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BACKGROUND: Organoid is an *in vitro* three-dimensional organ-bud that shows realistic microanatomy and physiological relevance. The progress in generating organoids that faithfully recapitulate human *in vivo* tissue composition has extended organoid applications from being just a basic research tool to a translational platform with a wide range of uses. Study of host-microbial interactions relies on model systems to mimic the *in vivo* infection. Researchers have developed various experimental models *in vitro* and *in vivo* to examine the dynamic host-microbial interactions. For some infectious pathogens, model systems are lacking whereas some of the used systems are far from optimal.

OBJECTIVE: In the present work, we will review the brief history and recent findings using organoids for studying host-microbial interactions.

METHODS: A systematic literature search was performed using the PubMed search engine. We also shared our data and research contribution to the field.

RESULTS: we summarize the brief history of 3D organoids. We discuss the feasibility of using organoids in studying host-microbial interactions, focusing on the development of intestinal organoids and gastric organoids. We highlight the advantage and challenges of the new experimental models. Further, we discuss the future direction in using organoids in studying host-microbial interactions and its potential application in biomedical studies.

CONCLUSION: In combination with genetic, transcriptome and proteomic profiling, both murine- and human-derived organoids have revealed crucial aspects of development, homeostasis and diseases. Specifically, human organoids from susceptible host will be used to test their responses to pathogens, probiotics, and drugs. Organoid system is an exciting tool for studying infectious disease, microbiome, and therapy.

Keywords bacteria, colonoids, enteroids, gastric organoids, host-microbial interactions, *H. pylori*, inflammation, intestinal organoids, microbiome, organoids, tight junctions, *Salmonella*, stem-cell differentiation, ZO-1

Introduction

Researchers have employed various *in vitro* and *in vivo* experimental models to investigate interactions taking place between microbes (e.g. bacteria, parasites, virus) and their host (e.g. humans, animals). These models consist of cell cultures deriving from human or animal cells (Dingli and Nowak, 2006), animals that can be inoculated with pathogens orally or parenterally (Fang et al., 2013), and organoids modeling host-microbial interactions (Fatehullah et al., 2016). The ultimate aim of these models is to create an environment *in vitro* that can imitate the real circumstances of

the human to elucidate physiological mechanisms of host responses in health and diseases.

In this review, we will summarize the brief history of 3D organoids, focusing on the development of intestinal organoids and gastric organoids. We will discuss the feasibility of using organoids in studying the effects and mechanisms of host-microbial interactions. Also, we will highlight the advantage and challenges of the newly developed experimental models. Further, we will discuss the future direction in using organoids as an *in vitro* model to study host-microbial interactions and its potential application in biomedical studies.

History of organoids

Cell culture was first invented at the beginning of the

twentieth century, and was used to study frog embryo nerve fiber outgrowth (Harrison, 1907). Using of dispersed cell cultures rapidly increased in the second half of the twentieth century (Hilleman 1990). Organ culture is a development from cell culture methods. It retains histological structure and the architecture characteristic of the tissue. However, compared with other types of tissue culture, organ culture has several shortcomings, including difficulty in quantification of tissues or cells, limitation in the amounts of cultured samples, the requirement of skillful manipulation of the samples, and the challenge of reproducibility.

The 3D culture system has made it possible to recapitulate partially the complexity of mammalian organogenesis *in vitro*. The newly developed organoid system acts as a bridge between *in vivo* and *in vitro* systems. They can be derived from pluripotent stem cells (PSCs), both from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), as well as from adult stem cells (AdSCs) (Kristin et al., 2016).

ESCs are derived from the inner cell mass (ICM) of the preimplantation blastocyst. Under appropriate *in vitro* culture conditions, ESCs proliferate indefinitely without differentiation, a property hereinafter referred to as “self-renewal,” and at the same time retain the developing potential to generate cells of all three primary germ layers (Huang et al., 2015a).

Over the past three decades, researchers have developed methods to derive ESCs from the epiblast and expand them continuously *in vitro*. More recently, induced pluripotent stem cells (iPSCs) can be generated from almost any mature cell type in our bodies. These breakthroughs have allowed the differentiation of various PSC populations into somatic cell derivatives (Huch and Koo, 2015). Culturing human derivatives (hESCs/hiPSCs/hAdSCs) in three-dimension (3D) has opened up new horizons for the exploration of development and regenerative medicine approaches.

In the late 1980s, organoids derived from adult stem cells experiments performed on skin proved that epidermal stem cells were expandable and could generate vast amounts of epithelium *in vitro*, supported by lethally irradiated 3T3 cells (Barrandon and Green, 1987). Until recently, the AdSC maintenance and tissue repair have been instrumental for the development of primary AdSCs cultures. AdSCs have gained much attention also for their intrinsic abilities to self-renew and differentiate into the cell types present in adult tissues while retaining genomic stability. Thus, organoids have all been derived *in vitro* from AdSCs, including mammary gland, bone, stomach, small intestine, colon and liver. A recent review from *Nature Cell Biology* has excellently summarized the organoids from different tissues (Fatehullah et al., 2016). Here, we will focus on the intestinal organoids derived from adult stem/progenitor cells. We follow the definition from (Fatehullah et al., 2016) an organoid is an *in vitro* 3D cellular cluster derived exclusively from primary tissue, ESCs or iPSCs, capable of self-renewal and self-organization, and exhibiting similar organ functionality as the tissue of origin. Most of the documented organoid cultures contain functional

tissue units that lack the mesenchymal, stromal, immune and neural cells that intersperse the tissue *in vivo*. These organoids rely on artificial extracellular matrices to facilitate self-organization into structures that resemble native tissue architecture.

In 2009, the development of intestinal organoid culture was an outstanding technological advance for the stem cell field (Ootani et al., 2009; Sato et al., 2009). Establishing intestinal organoids is challenging because it requires tissue-specific modifications that reflect the individual niche and lineage commitment factors for the resident stem cell populations and their progeny. Ootani et al. (2009) has reported a complex culture system from minced whole intestinal tissue embedded in a 3D collagen structure with support of stromal cells. Sato et al. (2009) has established a relatively simple organoid culture system, using Matrigel as an extracellular matrix (ECM) substitute, supplemented with growth factors constituting key endogenous niche signals. The system was used to create 3D structures with distinct crypt-like and villus-like domains bordering a central lumen containing dead cells extruded from the constantly renewing epithelial layer (Sato et al., 2009). Furthermore, these organoids from small intestine could be expanded for over a year *in vitro* (Huch and Koo, 2015). In the organoids, the domains bordering a central lumen containing dead cells extruded from the constantly renewing epithelial layer. This new method takes advantage of our knowledge of endogenous intestinal stem cell niche components to deliver a well-defined, stable culture system capable of sustaining the long-term growth of near-physiological epithelia from purified Lgr5⁺ stem cells or isolated crypts.

Two years later, mouse- and human-derived colonic stem cells could also be expanded in culture with a minor modification to the culture medium composition (Jung et al., 2011; Sato et al., 2011a). Human colonic organoids could be expanded for at least 1 month. From 1 month onward, the colonic organoids changed their morphology from budding structures into cystic structures (Sato et al., 2011a). Coinciding with the morphologic conversion, proliferation progressively decreased. Occasionally, cystic organoids regained their proliferative potential. However, all organoids eventually arrested growth within 3 months. Microarray analysis revealed that the human small intestinal and colonic organoids possess comparable molecular signatures of intestinal crypts, including the expression of intestinal stem cell genes. Alk receptor and p38 signaling negatively regulate long-term maintenance of human intestinal epithelial cells (Sato et al., 2011a).

Miyoshi and Stappenbeck in 2013 published a very nice paper on how to perform expansion and genetic modification of organoids (Miyoshi and Stappenbeck, 2013). Based on their protocol, isolation of epithelial cell units from mice takes up to 2 h and stem cell-enriched gastrointestinal organoids are obtained within 3 days. Genetically modified organoids with lentiviruses can be obtained in 2 weeks. As shown in Fig. 1,

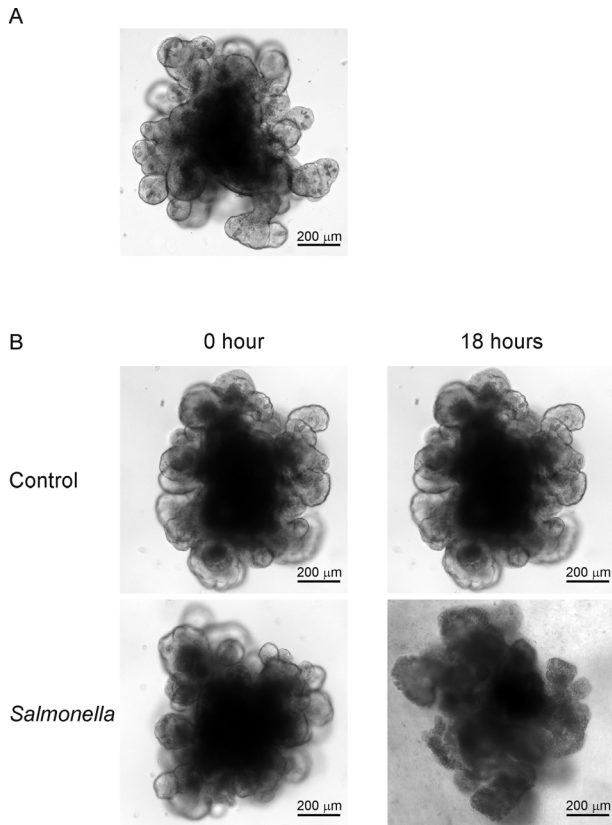


Figure 1 Normal and *Salmonella*-infected crypt-derived intestinal organoids. (A) Normal crypt-derived mouse intestinal organoids in day 7 after isolation. (B) *Salmonella*-infected crypt-derived intestinal organoids. Scale bars: 200 μm .

the organoids derived from mouse small intestinal stem cells show organ-buds 7 days after isolation from crypts and recapitulated the *in vivo* tissue architecture.

Despite the similarities with the murine organoid system, the human organoids require specific molecules to enhance and sustain their growth over time. Compared to the culture of mouse organoids, the other challenge in human organoid culture is to obtain fresh samples and establish feasible protocol. In 2015, Mahe et al. reported the detailed protocol and procedure to establish human epithelial enteroids and colonoids using whole tissue and biopsy (Mahe et al., 2015). In this methodological paper, the authors emphasize the crypt collection from whole tissue and biopsies. They recapitulate the culture modalities that are critical for the successful growth and maintenance of human epithelial organoids (enteroids) (Mahe et al., 2013) and colonoids. Commonly, termed “enteroids” when derived from small intestine and “colonoids” when derived from colon (Mahe et al., 2015).

The intestinal epithelium of mammals consists of absorptive enterocytes and of three secretory cell types, paneth, goblet, and enteroendocrine cells, which are continuously replenished from stem cells that reside in niches in the lower parts of the crypts (Crosnier et al., 2006). Organoids contain the full complement of stem, progenitor and differentiated cell

types (Sato et al., 2009; Fatehullah et al., 2016). Marker-specific antibodies for mucin 2 (goblet cells), lysozyme (Paneth cells), and chromogranin A (enteroendocrine cells) are used to validate the presence of intestinal secretory cell lineages in organoids. The enteroids/colonoids continuously produce all cell types found normally within the intestinal epithelium. Researchers who focus on specialized cell types, such as goblet cells or Paneth cells, find the organoid as a very useful *in vitro* model for insights into GI development, tissue homeostasis, and diseases (Dedhia et al., 2016). In human intestinal organoids treated with Notch inhibition dibenzazepine (DBZ, 10 mol/L), the intestinal organoids ceased their proliferation and most cells converted into goblet cells within 3 days (Sato et al., 2011a). It is reported that Mitogen-activated Protein Kinase (MAPK) signaling controls goblet/Paneth cell fate decisions in the intestine (Heuberger et al., 2014). Ablation of the tyrosine phosphatase Shp2 in the intestinal epithelium reduced MAPK signaling and led to a reduction of goblet cells while promoting Paneth cell development. Inhibition of MAPK signaling in mouse intestinal organoids changed the relative abundance of T cell factor 4 isoforms, which promoted Wnt/ β -catenin activity. The data show that Shp2-mediated MAPK signaling controls the choice between goblet and Paneth cell fates by regulating Wnt/ β -catenin activity.

Goblet and Paneth cells represent two secretory cell types in the intestinal epithelium. It is believed that the intestinal organoids derived from human iPSCs are more similar in structure to mouse organoids because of the presence of Paneth-like cells and the villus-like protrusions (Forbester et al., 2015). The organoids provide a new tool to study the development and functions of these cell types that are critical for the host-microbe interactions in health and diseases.

The organoids subsequently adapted for generating human organoids and also from animal models with different genetic modifications have been used for various basic and clinical research, including host-microbial interactions.

Feasibility and application of organoids in studying host-microbial interactions

Study of host-microbial interactions relies on model systems to mimic the *in vivo* infection. For some infectious pathogens, model systems are lacking whereas some of the used systems are far from optimal. Recent reports have shown the feasibility to use organoids as an *in vitro* model to study host-pathogen interactions (Zhang et al., 2014) (Bartfeld and Clevers, 2015). These studies have used organoids to model infections with bacteria, such as *Salmonella* (Forbester et al., 2014; Zhang et al., 2014; Forbester et al., 2015; Wilson et al., 2015), *Helicobacter pylori* (McCracken et al., 2014; Bartfeld et al., 2015; Huang et al., 2015b; Schumacher et al., 2015; Sigal et al., 2015; Schlaermann et al., 2016), *Bacteroides*

thetaitoamicon (Engevik et al., 2013), *Clostridium difficile* (Leslie et al., 2015), viruses (e.g. rotavirus (Finkbeiner et al., 2012; Finkbeiner et al., 2012; Yin et al., 2015), Cytomegalovirus (D'Aiuto et al., 2012; Penkert and Kalejta, 2013), Zika virus (Garcez et al., 2016) or Hepatitis C Virus (Yoshida et al., 2011; Roelandt et al., 2012; Schwartz et al., 2012; Wu et al., 2012; Shlomai et al., 2014)) and parasites, such as *Plasmodium falciparum* (Ng et al., 2015) or *Toxoplasma gondii* (Klotz et al., 2012).

Intestinal organoids in host-pathogenic bacterial interactions

Salmonella Typhimurium is a primary enteric pathogen infecting both humans and animals. Infection begins with the ingestion of contaminated food or water so that *Salmonella* reach the intestinal epithelium and trigger gastrointestinal disease. In 2014, we reported using an intestinal organoid culture system to study pathophysiology of bacterial-epithelial interactions post *S. Typhimurium* infection (Zhang et al., 2014). Using crypt-derived mouse intestinal organoids, we were able to visualize the invasiveness of *S. Typhimurium* and the morphologic changes of the organoids (Fig. 1B). *S. Typhimurium* entered the epithelial cells of the organoids and this resulted in disruption of the tight junctions (Fig. 2). We showed that distribution of ZO-1, a tight junction protein, was weak and disconnected in the organoids infected with *Salmonella*. Using the organoids, we also established methods for western blot, PCR, and immunofluorescence to demonstrate the changes of stem cell markers (Lgr5 and Bmi1). We found that Lgr5 and Bmi1 were significantly decreased by *Salmonella* infection. We also cultured GFP-labeled Lgr5 organoids to study the pathogen regulation of stem cells (Zhang et al., 2014).

The NF- κ B pathway in intestine is activated by *Salmonella*

infection *in vitro* and *in vivo*. We examined the changes of NF- κ B pathway in the organoids infected with *Salmonella*. *Salmonella*-infected organoids had a significantly decreased total I κ B α and increased phospho-I κ B α . The phospho-NF- κ B p65 was also increased in the *Salmonella*-infected organoids. By confocal microscopy, we found that NF- κ B p65 was translocated into the nucleus in organoids-infected with *Salmonella*. As the downstream targets of NF- κ B activation, inflammatory cytokines (e.g. IL-2, IL-4, IL-6, and TNF- α) were significantly increased in the infected organoids compared to the organoids without any infection. Moreover, we found that the ELISA was sensitive enough to detect IL-6 protein in the culture medium 1 h post *Salmonella* infection. IL-6 protein was significantly enhanced in the culture medium post 1 h, 2-, and 4- hours post infection (Zhang et al., 2014). For the first time, we have created an *in vitro* model system that recapitulated a number of observations from *in vivo* studies of the *Salmonella*-infected intestine: bacterial invasion, altered tight junctions, inflammatory responses, and decreased stem cells during host-bacterial interactions. We have demonstrated that the *Salmonella*-infected organoid culture system is a new and feasible experimental tool for studying host-bacterial interactions (Zhang et al., 2014). Our study has demonstrated the complexity of the host response to bacterial infection, even in the absence of immune cells.

Using intestinal organoids (iHOs) derived from human induced pluripotent stem cells (hiPSCs), Forbester et al. (2014, 2015) established microinjection of *S. Typhimurium* into the lumen of iHOs. They reported that 1448 genes significantly upregulated in iHOs infected with *S. Typhimurium* and 577 genes significantly downregulated compared to controls, by RNA sequencing. Genes encoding proinflammatory cytokines, including CCL20, IL1B, and IL23A, were significantly upregulated. Utilizing a *S. Typhimurium* mutant strains that lacked the *invA* component of the SPI-1 type III

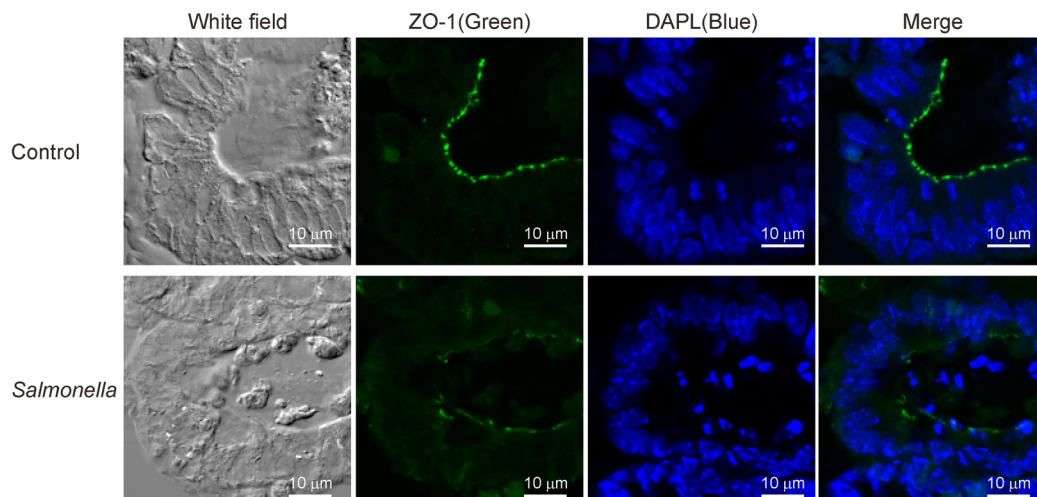


Figure 2 Distribution of tight junction protein ZO-1 in organoids. *Salmonella*-induced disruption of tight junctions in the mouse intestinal organoids. Please note the disorganized structure of ZO-1 (green staining) in organoids infected with *Salmonella*. Scale bars: 10 μ m. Images for ZO-1 protein represent three separate experiments.

secretion system, they have demonstrated that this system could be utilized to functionally assess the pathogenesis of defined mutants (Forbester et al., 2014, 2015). It is believed that the intestinal organoids derived from human iPSCs are more small intestine like in structure because of the presence of Paneth-like cells and the villus-like protrusions (Forbester et al., 2015).

Microinjection of organoids with bacteria can mimic bacterial infection in a relatively well-controlled environment, allowing for direct examination of pathogen interactions with epithelial cells in the absence of confounding variables introduced by immune cells or the commensal microbiota. Wilson et al. reported that Paneth cells in organoids from both wild-type mice and *Mmp7*^{-/-} mice produced granules containing pro- α -defensins. Organoids form a sealed lumen that contains concentrations of α -defensins capable of restricting growth of *S. Typhimurium* for at least 20 h postinfection (Wilson et al., 2015). In human intestinal organoids (Leslie et al., 2015), toxin production by *Clostridium difficile* result in disruption of epithelial paracellular barrier function.

Organoids also provide a novel and powerful *ex vivo* model for studying commensal bacteria, probiotics, and microbiome studies. For example, inoculation of *Bacterioides thetaiotaomicron*, a Bacteroidetes member, in wild-type and NHE3^{-/-} terminal ileum organoids displayed increased fut2 and fucosylation. These data suggest that *B. thetaiotaomicron* alone is sufficient for the increased fucosylation seen *in vivo* (Engvik et al., 2013).

Probiotic *Lactobacillus rhamnosus* GG (LGG) has been reported to be therapeutically effective against acute secretory diarrhea by rotavirus infection; however, the underlying mechanisms remain to be completely elucidated. Intestinal organoids derived from small intestinal crypts treated with LGG showed increased Toll-like receptor 3 (TLR3) mRNA levels, by quantitative real-time polymerase chain reaction (Aoki-Yoshida et al., 2016).

A recent study has examined the transcriptional response of organoids upon exposure to short-chain fatty acids (SCFAs) and products generated by two abundant microbiota constituents, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* (Arnold et al., 2016). *A. muciniphila* metabolites affect various transcription factors and genes involved in cellular lipid metabolism and growth, supporting previous *in vivo* findings. Contrastingly, *F. prausnitzii* products exerted only weak effects on host transcription. In addition, *A. muciniphila* and its metabolite propionate modulated expression of *Fiaf*, *Gpr43*, histone deacetylases, and peroxisome proliferator-activated receptor gamma, important regulators of transcription factor regulation, cell cycle control, lipolysis, and satiety. *A. muciniphila* induces stronger effects on the host than *F. prausnitzii*. This study thus illustrates that specific bacteria and their metabolites differentially modulate epithelial transcription in mouse organoids.

VanDussen et al. (2015) reported a culture system for

human gastrointestinal epithelial cells from multiple regions of the gastrointestinal tract. Key advantages of this system include use of endoscopic biopsy tissue as starting material and the rapid expansion of the spheroids, which allows for line establishment from an individual patient within a time frame that is commensurate with patient care (~2–3 weeks). The mucus layer is a critical component of the physical barrier separating the host from the luminal environment, thus providing protection from pathogens. The spheroid-derived intestinal epithelial cells produced a mucus layer that could be mechanically dissociated. The adherence phenotypes of diarrheagenic *E. coli* was also test in this culture system. It has great potential for use in patient-specific assays (VanDussen et al., 2015).

Gastric organoids/enteroids in host-bacterial interactions

Gastric organoids have evolved to a new state-of-the-art *in vitro* tool for *Helicobacter pylori* research. *H. pylori* is a gastric pathogen that colonizes approximately 50% of the world's population. Infection with *H. pylori* causes chronic inflammation, peptic ulcers and ultimately leads to gastric cancer (Wroblewski et al., 2010; Salama et al., 2013). Bartfeld et al. generated human gastric organoids (hGO) from surgical samples of gastric corpus. hGO maintained many characteristics of their respective tissues based on their histology, expression of markers, and euploidy. They performed microinjection of GFP-expressing *H. pylori* into the lumen of gastric organoids. Plating of bacteria from organoids 2 h after injection verified that the bacteria are alive inside the organoids. Electron microscopy showed that bacteria were engaged in very intimate contact with the epithelial cells. The primary response of the hGO to *H. pylori* showed robust NF- κ B pathway, like IL-8, by microarray (Bartfeld et al., 2015).

McCracken et al. reported that bacteria were tightly associated with the hGO epithelium. The major physiological changes include an increase in proliferation due to oncogenic *H. pylori* protein CagA and increased β -catenin signaling (McCracken et al., 2014). Schumacher and colleagues has examined the epithelial response to infection with *H. pylori*, using both mouse and human gastric enteroids. Enteroids are epithelial organoids. *H. pylori* infection of gastric organoids induced Shh expression, triggering Shh expression via CagA dependent activation of NF- κ B (Schumacher et al., 2015). Sigal et al. (2015) described a direct colonization of Lgr5⁺ stem cells by *H. pylori*. Gland-associated *H. pylori* induce increased Lgr5-lineage tracing at the level of individual glands. Antral glands from infected mice formed organoids with a significantly higher capacity and larger sizes (Sigal et al., 2015). These data indicate bacterial ability to alter the stem cells and important implications for gastrointestinal stem cell biology and *H. pylori*-induced gastric pathology.

The studies in gastric enteroids allow researcher to identify new mechanisms which was not found in the animal models.

Using spent media from both gastric enteroids and polarized cell lines, Huang et al. (2015b) demonstrated that *H. pylori* used the chemoreceptor TlpB to sense urea emanating. It suggests that *H. pylori* concurrently senses and modulates its environment while colonizing its gastric niche. Surprisingly, the authors noted that TlpB is sensitive to low levels of urea and that physiological levels of urea in the stomach would inactivate the receptor. Ultimately, they found that *H. pylori*'s ability to break down urea via its urease facilitates its ability to sense host urea. Gastric enteroids were particularly useful as the absence of immune cells in this model enabled the authors to determine that NF- κ B activation resulted directly from *H. pylori* infection rather than recruitment of other cell types, a finding that would be more difficult to make in an animal model. This group has continued to use enteroids to demonstrate that the host receptor CD44 played a functional role in the epithelial cell proliferation triggered by the *H. pylori* Cag pathogenicity island (Bertaux-Skeirik et al., 2015).

Organoids/enteroids for host-virus interactions

Organoids have been used for host-virus interactions, including rotavirus (Finkbeiner et al., 2012; Yin et al., 2015), Cytomegalovirus (DAiuto et al., 2012; Penkert and Kalejta, 2013), Zika virus (Garcez et al., 2016) or Hepatitis C Virus (Yoshida et al., 2011; Roelandt et al., 2012; Schwartz et al., 2012; Wu et al., 2012; Shlomai et al., 2014). Organoids/enteroids potentially can be used to determine ways to correct the diarrhea-induced ion transport abnormalities via drug therapy (VanDussen et al., 2015).

Spence et al. (2011) has reported directed differentiation of stem cell lines into intestine-like tissue called “induced human intestinal organoids” (iHIOs). iHIOs is used as a new model to cultivate and study enteric viruses (Finkbeiner et al., 2012). iHIOs support replication of rotavirus, on the basis of detection of nonstructural viral proteins by immunofluorescence, increased levels of viral RNA, and production of infectious progeny virus. iHIOs also support replication of 12/13 clinical rotavirus isolates directly from stool samples. Interestingly, rotavirus infection is not only detected in the epithelial cells, but also in the mesenchymal cell population of the iHIOs. Thus, iHIOs offer a new model to study rotaviruses and other gastrointestinal viruses (Finkbeiner et al., 2012).

Garcez et al. (2016) used human iPS-derived human neural stem cells (NSCs) infection with Zika virus. They demonstrated that Zika virus was detected in NSCs after 24 h. Zika Virus targets human brain cells by reducing their viability and growth and causing cell death in neurospheres and brain organoids.

Researchers have made advancement by using human enteroids for rotavirus and norovirus research (Foulke-Abel et al., 2014; Saxena et al., 2015; Ettayebi et al., 2016; In et al., 2016). Particularly, norovirus culture is an important

advancement made by using enteroids (Ettayebi et al., 2016). Bile is required for strain-dependent human norovirus replication. Lack of appropriate histoblood group antigen expression in intestinal cells restricts virus replication, and infectivity is abrogated by inactivation (e.g., irradiation, heating) and serum neutralization. Multiple human norovirus strains are cultured in enterocytes in stem cell-derived, nontransformed human intestinal enteroid monolayer cultures. This culture system permits human host-pathogen studies of previously noncultivable pathogens, and allows the assessment of strategies to prevent and treat norovirus infections.

Organoids for studying host-parasite interaction

Studying human protozoan parasites and their interaction with the host remain severely limited, because of non-existent or inappropriate animal models and challenge to culture parasites *in vitro* due to strict human-host specificity or physiology. Using organoids is a strategy to address many of these experimental bottlenecks (Klotz et al., 2012). Studies on *Plasmodium falciparum* (Ng et al., 2015) and *Toxoplasma gondii* (Klotz et al., 2012) in organoids allow us to address questions of cell and developmental biology, immunology, and pharmacology in unprecedented ways.

Advantage and challenges of using organoid system in studying host-microbial interactions

The first advantages of using organoid systems in studying host-microbial interactions is that adult stem cells from many murine and human tissues can be grown *in vitro* and self-organize into organoids that resemble the *in vivo* counterpart. Using the traditional *in vitro* models to investigate interactions between microbes and intestinal epithelial cells, many studies fail to recreate the differentiated tissue components and structure observed in the normal intestine. One approach to creating differentiated cells is through a suspension culture technology using a rotating wall vessel bioreactor that allows cells to remain in suspension with bubble free aeration (Unsworth and Lelkes, 1998). However, this system may lack normal stem cell niches, which are responsible for the renewal of normal intestinal tissues.

Stem cells of the gastrointestinal tract, pancreas, liver and other columnar epithelia are known to resist cloning in their elemental states. A recent study has reported cloning and propagation of highly clonogenic, ‘ground state’ stem cells of the human intestine and colon (Wang et al., 2015). Interestingly, derived stem-cell pedigrees sustain limited copy number and sequence variation despite extensive serial passaging and display exquisitely precise, cell-autonomous commitment to epithelial differentiation consistent with their origins along the intestinal tract. Using clonally derived colonic epithelium, toxins A or B of the *Clostridium difficile*

recapitulate the salient features of pseudomembranous colitis (Wang et al., 2015). These stem cells may have certain advantages for use in host-microbial interactions.

The second advantage of organoids is that researcher can work on the tissue-specific or site-specific host interaction with a particular pathogen. Organoids can be generated from different organs or specific sites of the gut, including the small intestine and colon (Sato et al., 2011a, 2011b; Yui et al., 2012). Organoids represent more closely the intestinal epithelium than often-used colon cancer cell lines (e.g., CaCo2 or HCT116). Thirdly, studies in organoids allow researchers to have findings that would be more difficult to make in an animal model. Small biopsy specimens taken from adult donors can be expanded without any apparent limit or genetic harm, the technology may serve to generate transplantable epithelium for regenerative purposes (Sato et al., 2011b). Schwank and colleagues have demonstrated the possibility to use the CRISPR/Cas9 system to edit the genome with and correct the mutation on the CFTR gene causing a cystic fibrosis (Schwank et al., 2013). Overall, intestinal organoids in host-microbe interactions allow us to address questions of cell and developmental biology, microbiology, immunology, and pharmacology in unprecedented ways.

Ideally, the cell-cell interactions are needed in host-microbial interactions. However, the intestinal organoids still have their disadvantages/limits. First, organoids lack several components of the intestine *in vivo*, such as the enteric nervous system and the vascular, lymphatic and immune systems. Organoids cocultured with immune cells are needed for more comprehensive studies. Second, studying tissue patterning and organ morphogenesis has still been hindered by the lack of optimal culture condition for laboratory usage. Last, organoids relied on animal-derived matrices, which can be highly variable and are poorly defined, a problem that also makes them unsuitable for clinical application. A recent study has reported now designed modular synthetic hydrogen networks to support the formation of intestinal organoids from mouse and human intestinal stem cells (Gjorevski et al., 2016). A highly accurate, reproducible culture model could help to overcome current limitations that hinder the technology's transition from bench to bedside.

Conclusion and future direction

Organoids are one of the most accessible and physiologically relevant models to study the dynamics of host-microbial interaction in a controlled environment. The progress in generating organoids that recapitulate the human *in vivo* tissue composition has extended organoid applications from being just a basic research tool to a translational platform with a wide range of uses. In combination with genetic, transcriptome and proteomic profiling, both murine- and human-derived organoids have revealed crucial aspects of development, homeostasis and diseases. The commercial

development of more standardized, validated organoid culture media, and affordable materials will be valuable in ensuring that the organoid system becomes accessible to a wide range of academic and clinical researchers, to further maximize its potential. Specifically, human organoids from susceptible host will be used to test their responses to pathogens, probiotics, and drugs. The physiological relevance of the system makes organoids one of the most exciting and promising technologies for studying human development, infectious disease, microbiome, and therapy.

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Compliance with ethics guidelines

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