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# Organoid culture systems to study host–pathogen interactions

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Recent advances in host–microbe interaction studies in organoid cultures have shown great promise and have laid the foundation for much more refined future studies using these systems. Modeling of *Zika virus* (ZIKV) infection in cerebral organoids have helped us understand its association with microcephaly. Similarly, the pathogenesis of bacterial (*Helicobacter pylori*, *Clostridium difficile*) and viral (*Norovirus*, *Rotaviruses*) infections have been precisely dissected in organoid cultures. Additionally, direct associations between microbial colonization of tissues and diseases like cancer have also been deciphered. Here we discuss the most recent and striking studies on host–microbe interactions in organoid cultures, highlighting various methods which can be used for developing microbe–organoid co-culture systems.

## Addresses

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## Introduction

The “germ theory of diseases”, which hypothesizes that diseases are caused due to the action of microorganisms, was the crowning achievement of a French scientist Louis Pasteur, who in 1860s refuted the theory of spontaneous generation [1]. Ever since, various hypothesis on the microbial pathogenicity have been proposed and established [2,3]. Initially believed to primarily be assailants leading to disease states i.e. pathogenic (pathos is the Greek word for disease and genes means “born of”), scientists now recognize that host–microbe crosstalk is not always detrimental but also beneficial as in the case of gut symbionts (derived from symbiōsis, meaning “state of living together” in Greek) [4]. More so, disease states are a result of a two-way interaction that occurs between the host cells or tissues and the microorganisms. As per the

chain of infection model, host–pathogen interactions can lead to either host immunity or an aggravated immune response due to infection, depending on six factors including the susceptibility of the host, route of entry and colonization potential of the microbe (Figure 1) [5,6]. Recent years have seen a surge in interest in understanding this complex interplay between the microbes and the host organism. According to the World Health Organization (WHO), at least 12% of all human pathogens are considered as Emerging Infectious Diseases (EID) including malaria, Severe acute respiratory syndrome (SARS), *Zika virus* disease, HIV/AIDS etc., thus making it indispensable for us to understand the mechanism of action of molecular components involved in host–pathogen interaction during infection with EIDs [7].

Model organisms and animal models like fruit fly *Drosophila melanogaster*, zebrafish *Danio rerio* and mice have been instrumental in providing valuable insights into host–microbe interactions; however, their limited translation potential to humans due to uncontrollable microbial diversity and significant inter-specie variances proves to be a major disadvantage [8–11]. Recently developed humanized mice models are more relevant to human diseases, allowing better understanding of microbe interactions, but are expensive and difficult to maintain [12,13]. Ex vivo two-dimensional (2D) cell cultures of immortalized cell lines grown as monolayers and are functionally closer to the ‘real situation’ in humans, but lack the three-dimensional (3D) in vivo architectural details. In recent years, matrix or scaffold based 3D in vitro culture systems grown as spheroids or aggregates have gained widespread interest [14]. 3D Organoids or “mini organs on a dish” are adult stem cell (ASC) or pluripotent stem cell (hPSC) derived structures that can be grown from resident stem cells and present all organ specific cell types on their surface. Organoids from various tissues have been generated using both adult and pluripotent stem cells [15–18,19\*]. They recapitulate the composition, diversity and organization of cell types much better than any other existing in vitro system, therefore providing better opportunities to develop more efficacious control measures against emerging pathogens. In this review, we discuss the past, present and future of the use of 3D organoid cultures of various tissues as disease models for host–microbe interaction studies (Table 1).

## Modeling infectious diseases in organoids Intestinal organoids model gastrointestinal diseases

In 2009, in the first of its kind model system, ever expanding 3D intestinal organoids were grown from

Figure 1

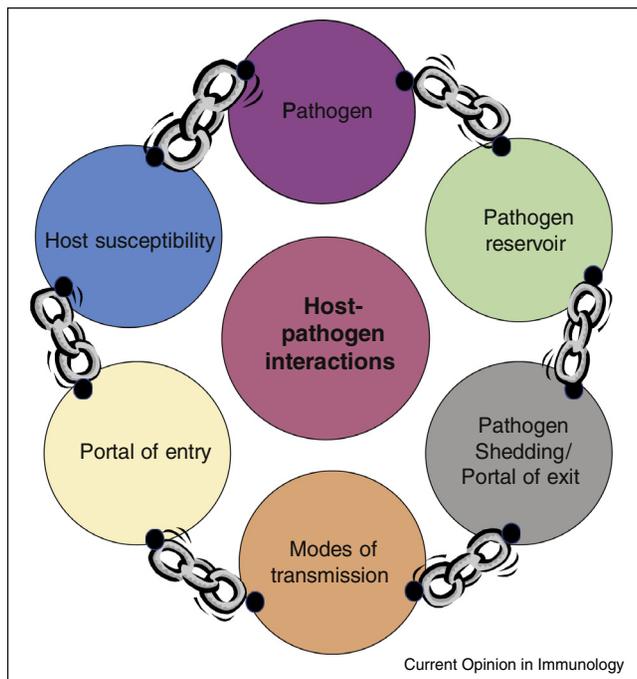


Illustration of the “chain of infection” model. Infection results from the interaction between the host, microbe and the environment. Six elements together constitute the chain of infection starting from pathogen, reservoir, portal of exit, means of transmission, portal of entry and ends with the infection of a new host. Organoids can be used to study the various links of the “chain of infection” model and help in the prevention and treatment of infectious diseases.

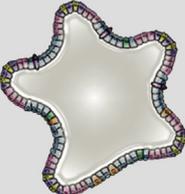
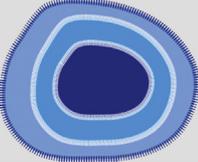
non-transformed mouse adult tissue stem cells [20,21]. Subsequently, conditions for growing organoids from adult human colon, small intestine and adenocarcinoma were also developed [22]. Intestinal organoids can be maintained in culture for long-term without procuring genetic aberrations or alterations and they retain their apico-basal polarity. Intestinal organoids are Wnt activity dependent, consisting mostly of resident proliferating stem cells which can be directed towards a differentiated cell state by withdrawal of niche factors. They have also been generated from human pluripotent stem cells or hPSCs (including embryonic stem cells — ESCs and induced pluripotent stem cells or iPSCs) [23]. In both these systems, organoids are grown in scaffolds with extracellular matrix like Matrigel<sup>®</sup> (Corning) or Basement membrane extract (BME) supplemented with a cocktail of growth factors essential for stem cells proliferation. Designer matrices or synthetic hydrogel networks with a well-defined composition have recently been tested to support organoid growth. These will further improve the reproducibility and applicability of organoid culture systems [24<sup>••</sup>].

Intestinal organoids have been used to model diseases such as colorectal carcinoma (CRC) and Cystic fibrosis

(CF) [25–27]. Another compelling application of intestinal organoids has been their use in studying the pathogenesis of various infectious diseases and in understanding host–microbe dynamics [28,29]. Organoids can be used to study the various links of the “chain of infection” model (Figure 1). For example, the epithelial cells of the intestinal organoids can be modeled as a reservoir and portal of exit for intracellular parasites like *Cryptosporidium* etc. Organoids can also be used to study the mechanism of transmission e.g. if a pathogen is airborne and can spread from an infected to an uninfected organoid. The study of portal of entry of pathogens and the role of specific cell types for e.g. modeling the penetration of intestinal epithelium by *Shigella* via the M-cells is also possible using organoid cultures. Studies using mouse small intestinal organoids with terminally differentiated secretory Paneth cells co-cultured with *Escherichia coli* or its antigens have given insights into the effects of microbial antigens on the function and changing facets of Paneth cells, identifying IFN- $\gamma$  as a potent immune component which facilitates release of antimicrobial factors into the gut lumen [30<sup>•</sup>]. *Clostridium difficile* (*C. difficile*) and *Salmonella typhi* (*S. typhi*) are the two-major bacterial intestinal pathogens causing diarrhea and gastrointestinal failures in humans. These pathogens have affinity towards the apical side of the epithelium, thus to mimic that interaction in 3D organoids, groups now use two different methods — 1) microinjection, 2) mechanical disruption of organoids and subsequent introduction of the microbe [31<sup>•</sup>,32<sup>•</sup>]. Alternatively, 3D organoids can be dissociated into single cells and grown as a monolayer with the apical side facing upwards. These monolayers can then be exposed to pathogens via their addition to the media (Figure 2). However, in this case, assessment of effects on the basolateral surface is not possible. In proof of principle studies, live *Salmonella typhimurium* was microinjected into the closed iPSC derived intestinal organoid lumen [33<sup>••</sup>,34]. Gene expression profiling and biochemical analysis of these organoids revealed massive NF- $\kappa$ B activation and upregulation of cytokine-mediated signaling. Factors like Interleukin (IL)-6, 8 and TNF $\alpha$  were also found to be secreted, consistent with previous findings in animal studies. Likewise, in a model for obligate anaerobe *C. difficile* infection (CDI), the Spence lab used pluripotent stem cell derived intestinal organoids and microinjected *C. difficile* toxin A (TcdA) and Toxin B (TcdB) purified from strain VPI 10463 into the lumen. While TcdA had previously been shown to be more potent in mice models, TcdB had a stronger effect in cell lines [35<sup>••</sup>]. Interestingly, infection in the 3D organoid model was closer to the in vivo situation. Within a few hours of infection, the distribution of tight junctional marker zonula occludens (ZO-1) was altered. Furthermore, cell–cell adhesion marker E-Cadherin and actin cytoskeletal rearrangements were seen in organoids injected with *C. difficile* toxin A but not *C. difficile* toxin B. In another study, the Worrell laboratory

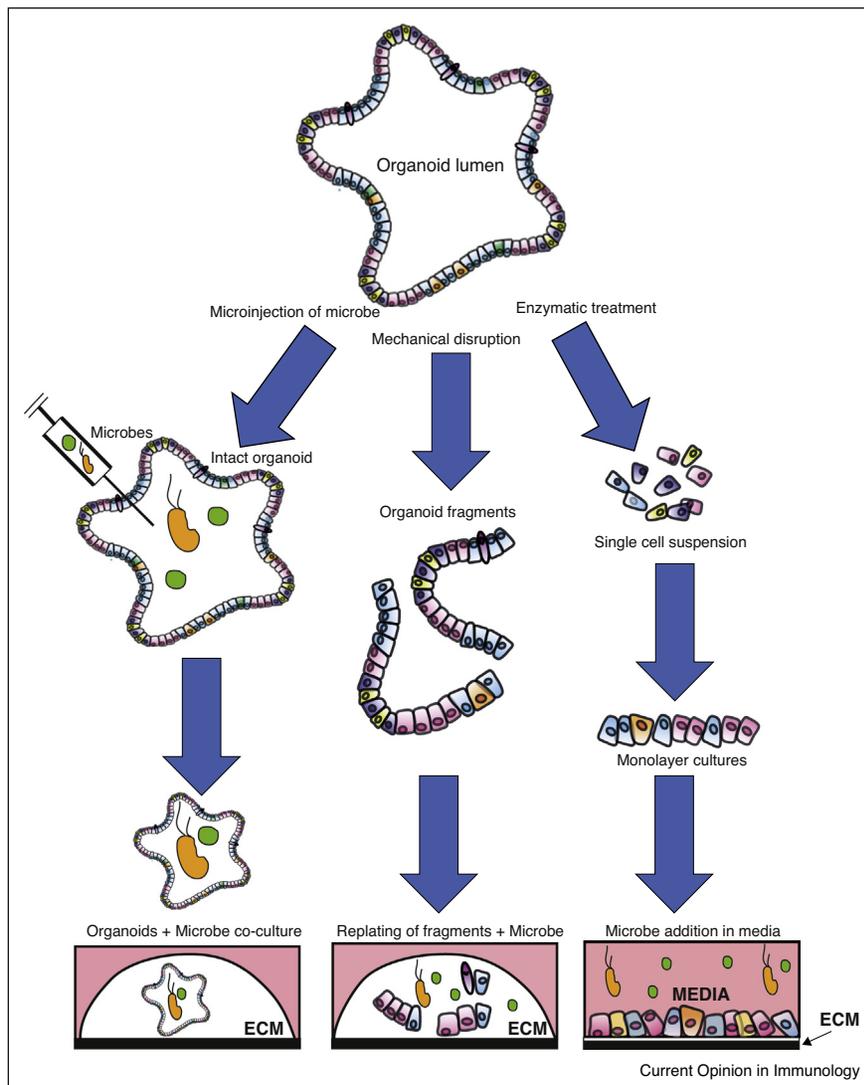
**Table 1**

**Studying host–microbe interactions in organoid cultures** Table shows organoids of different organs being used for studying microbe–host interactions. List shows microbes tested in intestinal, gastric, brain and gall bladder organoids and other organisms which can be potentially studied in the future in organoid cultures

	Intestine/colon	Stomach	Brain	Gall bladder	Liver	Lung
Organoid						
Microbe/infection modeled	<ul style="list-style-type: none"> <li>- <i>C. difficile</i></li> <li>- <i>S. typhi</i></li> <li>- Norovirus</li> <li>- Rotavirus</li> <li>- <i>Shigella</i><sup>a</sup></li> <li>- Enteric adenovirus<sup>a</sup></li> <li>- Cryptosporidium<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>- <i>H. pylori</i></li> <li>- Epstein Barr Virus<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>- Zika virus</li> <li>- Chikungunya virus<sup>a</sup></li> <li>- Japanese encephalitis virus<sup>a</sup></li> <li>- Venezuelan equine encephalitis virus<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>- <i>S. typhi</i></li> </ul>	<ul style="list-style-type: none"> <li>- <i>P. vivax</i><sup>a</sup> (Malaria)</li> <li>- Hepatitis virus A, B, C, E<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>- Rous sarcoma virus<sup>a</sup></li> <li>- Influenza virus<sup>a</sup></li> <li>- Rhinovirus<sup>a</sup></li> <li>- Coronavirus<sup>a</sup></li> </ul>
Source Reference	Human ASCs, iPSCs [28,33**,34,35**,36*,37,38**,39*,40]	Human ASCs, PSCs [32*,33**,34,35**,36*,37,3,39*,40,41,42**,43,44**]	Human iPSCs [45*,46,47**,48*,49*,50,51**,52*,53**]	Mouse ASCs [55**]	Human ASCs, PSCs <sup>a</sup> –	Human ASCs, PSCs <sup>a</sup> –

<sup>a</sup> Not yet published/potential future studies.

Figure 2



Methods of studying host-microbe interactions. Organoids can be microinjected with the microbe using a fine capillary microinjector. In this case the microbe is in direct contact with the apical side of epithelial cells. Organoids can also be sheared into smaller pieces, mixed with pathogen and re-plated with Matrigel/BME. Alternatively, 3D organoids can be dissociated into single cells by enzymatic treatment and grown as 2D monolayer cultures. Microbes are then introduced into the media.

observed a reduction in NHE3 and MUC2 protein levels in *C. difficile* infected organoids as compared to normal organoids. This could be a way the microbe creates a favorable environment for its colonization [36\*].

Viral pathogen *Human Norovirus (HuNoV)* infection leads to a self-limiting stomach flu or viral gastroenteritis and is one of the most common causes of acute gastroenteritis in the world [37]. Following close behind is *Rotavirus*, which is the second most common cause of gastric diarrhea in humans. Despite the rampant nature of both these viruses, no proper vaccine has yet been developed against them due to the lack of a good model organism or in vitro

system supporting their growth. In striking studies, Ettayebi and group modeled *HuNoV* infection in an organoid — virus co-culture system, successfully showing that the virus can infect and faithfully replicate inside the absorptive enterocytic cells of the epithelium, with only a specific *GII.3 HuNoV* strain displaying bile requirement [38\*\*]. Furthermore, a differential response of patients with histo-blood group antigen (HBGA) variability was observed towards different *HuNoV* strains, a fact which was also seen previously in cultured gastrointestinal epithelial cells (Caco-2) upon *Norovirus* infection. Similarly, researchers have shown that *Rotavirus* strain (*simian SA11*) from clinical samples can replicate in iPSC-derived

intestinal organoids [39\*,40]. Future research on gastrointestinal viruses, parasites and bacterial pathogens using organoid cultures should help identify therapeutic targets and in developing novel diagnostics and vaccines.

#### Understanding the pathophysiology of *Helicobacter pylori* infection in gastric organoids

The occurrence of bacterium *Helicobacter pylori* (*H. pylori*) is so common that at any given point, more than 50% of the world's population harbors *H. pylori* in the upper gastrointestinal tract. [41]. Acute *H. pylori* infection is associated as a major risk factor for peptic ulcers, gastric adenocarcinoma and gastritis. To study this association further, researchers microinjected *H. pylori* into the lumen of gastric organoids derived from human gastric mucosa. Schlaermann and group developed a monolayer culture from similar 3D gastric organoids [42\*\*,43]. Colonization of the bacteria led to an increase in proliferation of Lgr5<sup>+</sup> stem cells which was in turn found to be induced by bacterial virulence factor *CagA* expression [44\*\*]. Furthermore, an inflammatory response was induced and promoted by the differentiated cells of the bacteria infected organoids. It would not be far-fetched to hypothesize that such an inflammatory response could be the bridging factor connecting excessive microbial colonization of *H. pylori* in the stomach to the occurrence of gastric cancer in humans. In another study, scientists used gastric organoids to determine that a potent chemoattractant — urea which emanates from the epithelial cell wall is essential for *H. pylori* colonization in the gastric mucosa [45\*]. Gastric organoid models would thus not only be beneficial to study *H. pylori* pathogenesis but also to dissect the implications of such microbial colonizations in the organ and in understanding their role in the causation of diseases like cancer and inflammatory bowel disease (IBD) in humans.

#### Cerebral organoids as a model of ZIKV infection

A breakthrough in the field of neuroscience came with the development of brain organoids from human iPSCs by the Knoblich group in 2013 [46,47\*\*]. Around the same time, *Zika virus* (ZIKV), a mosquito-borne flavivirus came into prominence into the modern world after a public outbreak of the virus in Brazil. ZIKV was first identified in 1947 from the blood of a rhesus monkey found in Uganda and in humans in 1952. By Feb 2016 World Health Organization (WHO) had declared *Zika virus* infection as a public health emergency. The virus spreads mainly by the *Aedes aegypti* mosquito and its occurrence is strongly associated with microcephaly. However, the pathogenesis of the viral infection and how it effects the brain neurons was not fully understood until recently. Employing pluripotent stem cell (ESCs and iPSCs) derived cerebral organoids multiple recent studies have now deciphered the sequence of disease progression in *Zika virus* infection [46,47\*\*,48\*]. Multiple groups demonstrated that ZIKV infection causes disruption of cerebral organoid cortical

layers, abrogating growth and thus halting the process of neurogenesis. Researchers found that the Toll-like receptor 3 (TLR3) activation, which occurs upon ZIKV infection, leads to deregulated neurogenesis and thus decrease in the pool of functional neurons [49\*]. Gabriel and group further showed that the pattern of pathogenicity was different when two new ZIKV isolates were used instead of the highly passaged MR766 strain. The new strains infected apical proliferating progenitors, interfering with centrosomal protein assembly, which in turn led to their premature differentiation and apoptosis, giving rise to features of microcephaly [50,51\*\*,52\*]. In a drug repurposing screen of ~6000 compounds, caspase-3 activity inhibitors Emricasan and Niclosamide, a category B anthelmintic, were found to be effective in limiting ZIKV induced neural cortical progenitor death and ZIKV replication [53\*\*]. Scientists have now developed innovative cost-effective miniature spinning bioreactors to generate cerebral organoids from human iPSCs [54\*]. In light of these recent studies, the United States Centers for Disease Control Prevention (CDC) in April 2016 concluded that ZIKV infection causes microcephaly (CDC, 2016).

#### Dissecting associations between microbes and cancer — gall bladder organoids

Neefjes and colleagues recently exploited gallbladder organoids derived from *Ink4a/Arf* (*Cdkn2a*) tumor suppressor deficient mice for to draw a direct association between chronic *Salmonella enterica serovar typhimurium* infection and gallbladder carcinoma (GBC). WT *Salmonella* infection leads to colorectal adenocarcinoma formation in mice. When mouse gallbladder organoids were infected with WT *Salmonella*, they presented features of loss of polarity, like those seen in the GBC mouse model. Additionally, WT *Salmonella* pre-exposed organoids were found to have neoplastic transformations via activation of AKT and MAPK signaling and could grow in a growth-factor deficient media [55\*\*]. Organoid and microbe co-cultures would be instrumental in further dissecting the molecular basis of such associations.

#### Future of organoid — microbe studies

Given that organoid cultures of various other tissues including liver, lung, kidney and ovary have already been established (unpublished data from Clevers lab), it would be exciting to mimic other infectious diseases such as malaria (*P. vivax* and *P. falciparum*) and hepatitis (HBV) in liver organoids, *Rous sarcoma virus* (RSV) in lung organoids and Epstein Barr Virus (EBV) infection in gastric organoids. Hepatitis C (HCV) and Human Immunodeficiency virus (HIV) co-infection studies have been a subject of immense interest [56\*]. While HIV is known to enhance HCV infection, the direct alteration of the course of HIV infection and AIDS upon HCV infection remains debated. It would be interesting to study HCV–HIV co-infection in organoid cultures. While most commensal microbes (microbiome) are anaerobic and the organoid

lumen is about 5% aerobic, it would be interesting to tweak the current organoid culture systems to understand the host-microbiome interplay that exists in our body. Another important addition would be the inclusion of immune and endothelial cells to fully access how microbial fluctuations modulate immune cell responses, leading to disease states. Future research using organoid models to dissect the pathogenesis of various diseases is bound to open exciting new avenues to tread and lead us towards novel drug discoveries and improved worldwide healthcare.

### Conflict of interest

The authors declare no conflict of interest.

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