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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.

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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

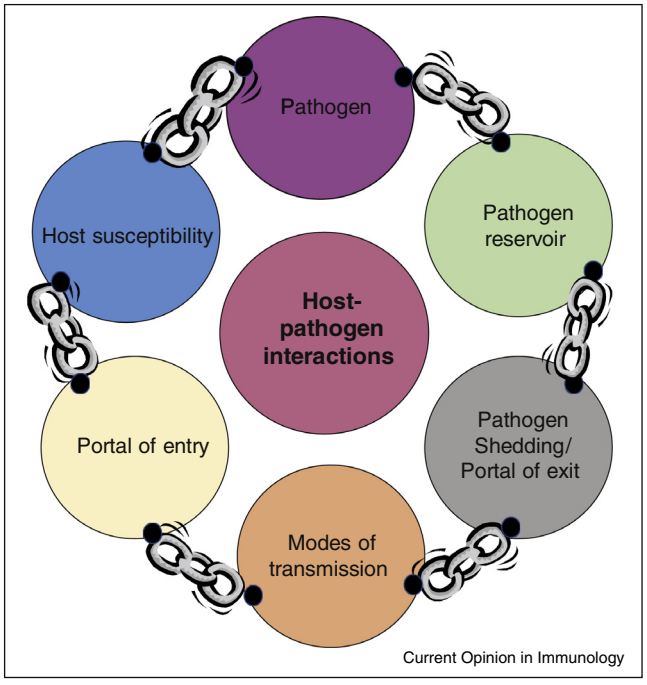

Illustration of the "chain of infection" model. Infection results from the interaction between the host, microbe and the environment. Six elements together constitute the chain of infection starting from pathogen, reservoir, portal of exit, means of transmission, portal of entry and ends with the infection 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 withdrawl of niche factors. They have also been generated from human pluripotent stem cells or hPSCs (including embryonal 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® (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••].

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-γ as a potent immune component which facilitates release of antimicrobial factors into the gut lumen [30•]. *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•,32•]. 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••,34]. Gene expression profiling and biochemical analysis of these organoids revealed massive NF-κB activation and upregulation of cytokine-mediated signaling. Factors like Interleukin (IL)-6, 8 and TNFα 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••]. 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