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MINIREVIEW

Modeling Host-Pathogen Interactions in the Context of the Microenvironment: Three-Dimensional Cell Culture Comes of Age

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ABSTRACT Tissues and organs provide the structural and biochemical landscapes upon which microbial pathogens and commensals function to regulate health and disease. While flat two-dimensional (2-D) monolayers composed of a single cell type have provided important insight into understanding host-pathogen interactions and infectious disease mechanisms, these reductionist models lack many essential features present in the native host microenvironment that are known to regulate infection, including three-dimensional (3-D) architecture, multicellular complexity, commensal microbiota, gas exchange and nutrient gradients, and physiologically relevant biomechanical forces (e.g., fluid shear, stretch, compression). A major challenge in tissue engineering for infectious disease research is recreating this dynamic 3-D microenvironment (biological, chemical, and physical/mechanical) to more accurately model the initiation and progression of host-pathogen interactions in the laboratory. Here we review selected 3-D models of human intestinal mucosa, which represent a major portal of entry for infectious pathogens and an important niche for commensal microbiota. We highlight seminal studies that have used these models to interrogate host-pathogen interactions and infectious disease mechanisms, and we present this literature in the appropriate historical context. Models discussed include 3-D organotypic cultures engineered in the rotating wall vessel (RWV) bioreactor, extracellular matrix (ECM)-embedded/organoid models, and organ-on-a-chip (OAC) models. Collectively, these technologies provide a more physiologically relevant and predictive framework for investigating infectious disease mechanisms and antimicrobial therapies at the intersection of the host, microbe, and their local microenvironments.

KEYWORDS 3-D, 3D, RWV, rotating wall vessel, gut-on-a-chip, host-microbe interaction, host-pathogen interactions, mechanotransduction, organ-on-a-chip, organoid

Mucosal surfaces lining the gastrointestinal, respiratory and urogenital tracts continuously interface with the external environment and serve as a barrier against pathogens, commensals, chemicals, drugs, and toxins. These tissues possess a complex architecture with multiple cell types organized into three-dimensional (3-D) structures that facilitate tissue-specific functions. The biological, chemical, and biomechanical characteristics that define microenvironmental niches along these surfaces provide the

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structure and context in which infection takes place. Pathogens have adapted to detect specific host structures, polarity, and changes in local environmental stimuli (pH, temperature, oxygen, nutrients, hormones, physical forces, etc.) to know where and when to activate specific virulence programs during different infection stages (1–7). A major challenge in tissue engineering for infectious disease research is recreating *in vivo* spatiotemporal properties of dynamic 3-D microenvironments to more accurately model host-pathogen interactions in the laboratory.

Historically, infectious disease has been commonly studied *in vitro* by assessing the interaction of a single microbe with a single host cell type, with the latter grown as flat 2-D monolayers. This reductionist approach has enabled important discoveries and advanced our understanding of mechanisms that underlie infection and disease. However, the study of disease in isolation or out of context can change the native behavior of both host and microbe, thus creating a barrier for researchers to correlate *in vitro* and *in vivo* responses. In this data-rich period where multiple -omics technologies are being synergistically applied for unparalleled insight into host-pathogen interactions, it is critical to consider the context in which these investigations are performed. Reconstructing host microenvironments is key, including 3-D tissue architecture, multicellular complexity, microbiota composition/localization, oxygen tension, transport processes, and biomechanical forces (e.g., fluid shear, stretch, compression) (1, 8–11). Within this context, *in vitro* models are positioned along a continuum between 2-D and 3-D, with flat monolayers of a single cell type representing the most basic system and more complex models located further down the spectrum that recreate multiple aspects of the native tissue microenvironment (Fig. 1). Since tissues and organs function in a 3-D context, consideration of proper structure is essential for development of models that better mimic *in vivo* responses. Since no current *in vitro* model fully accomplishes this task, multidisciplinary teams of biologists, engineers, physicists, mathematicians, and clinicians are creatively working together to develop next-generation 3-D models with enhanced predictive capabilities to open new avenues for clinical translation.

Present-day 3-D culture techniques result from a series of progressive advances in tissue engineering over the past century to better mimic the native structure and microenvironment of normal and diseased tissues (reviewed in reference 12). Indeed, long ago the cancer research community recognized that appropriate modeling of the 3-D microenvironment is important for mimicking disease, leading to development and application of 3-D organoid models developed within or on top of extracellular matrix (ECM) (12–16). The bidirectional exchange of biological and physical signals between cells and their microenvironment regulates cell structure/function and is largely manifested by tensile connections between ECM, cell surface receptors (e.g., integrins), and the cytoskeleton to transduce signals to and from the nucleus (17–31). This structural network is also engaged by certain invasive pathogens (e.g., *Salmonella*, *Shigella*, *Listeria*, rotavirus, and influenza virus) that hijack and remodel host cell architecture to facilitate their internalization, intracellular trafficking, and/or dissemination (9, 32–34). Similarly, we and others have demonstrated that bacteria also respond to biomechanical forces like fluid shear, which can regulate virulence, gene expression, and/or stress responses (1–5, 35–47). Indeed, the discovery of biomechanical forces as environmental regulators of microbial pathogenesis was made by our team almost 2 decades ago with the finding that fluid shear forces globally reprogram *Salmonella* gene expression, stress responses, and virulence (35). Fluid shear also plays a central role in regulating a number of host responses, including differentiation (48–50).

Although 3-D models have long been applied for cancer research (12–16), they remained largely unincorporated by the infectious disease community until the late 1990s and early 2000s. As expected for many new ideas in an established field, the use of 3-D models to study host-pathogen interactions was initially met with skepticism. The first reports of 3-D models to study viral infections were by Long et al. in 1998 (rhinovirus) and bacterial infections by Nickerson et al. in 2001 (*Salmonella enterica* serovar Typhimurium) (11, 51). Recently, infectious disease researchers have broadly

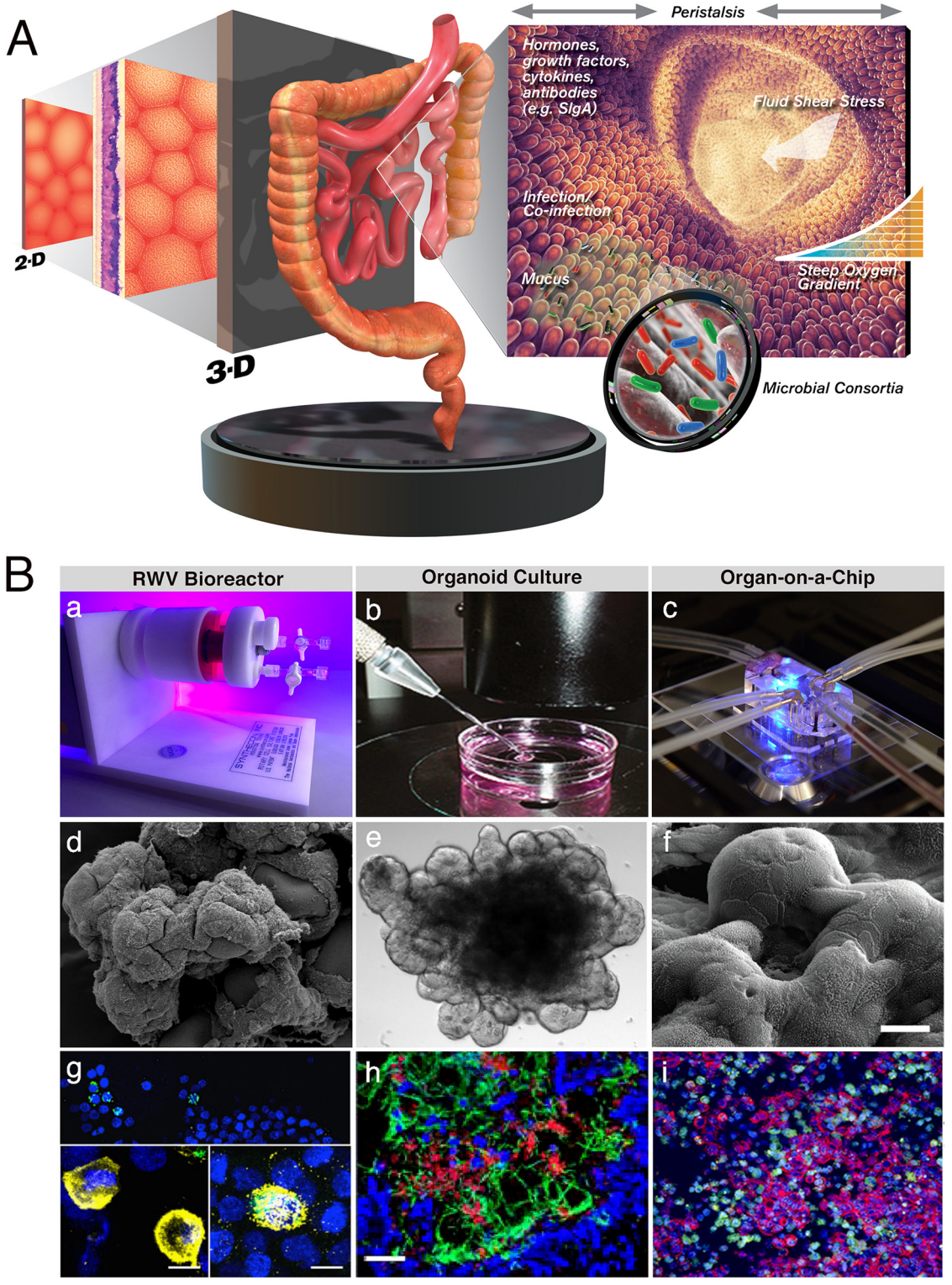

FIG 1 Recreating the complex intestinal microenvironment to study host-pathogen interactions. (A) *In vitro* model advancement from 2-D to 3-D by incorporation of physiological factors to better mimic the *in vivo* environment. (Intestinal lumen, cell, intestine, and intestinal microbe images are republished from references 398 to 401, respectively, with permission of the publisher.) (B) Three-dimensional approaches routinely used to develop advanced intestinal models: (a) RWV bioreactor, (b) (republished from reference 307 with permission of the publisher), and (c) OAC (republished from reference 344 with permission of the publisher). (d) Scanning electron micrograph (SEM) showing an RWV colon model. (Republished from *PLoS One* [152].) (e) Light micrograph of an enteroid model. (Republished from *Physiological Reports* [240].) (f) SEM of a gut-on-a-chip model (republished from reference 341 with permission of the publisher). (g) Oxygen-dependent host cell colocalization of *S.* Typhimurium in an RWV 3-D coculture model of intestinal epithelium and macrophages. Following aerobic culture of bacteria, no macrophages were found, but following microaero-

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embraced 3-D models for studying pathogenesis mechanisms, for biomarker discovery, and for drug candidate screening. In this review, we highlight key microenvironmental factors to consider when selecting *in vitro* 3-D intestinal models to study host-pathogen interactions. We focus on three key technologies for model development, (i) the rotating wall vessel (RWV) bioreactor, (ii) ECM-embedded/organoid models, and (iii) gut-on-a-chip models, and propose a vision for future model advancements. We also provide proper historical context for use of 3-D cell cultures in studying host-pathogen interactions, which is finally gaining a critical mass of scientists who understand and appreciate the value of studying disease in the proper context of tissue form and function.

MICROENVIRONMENTAL CUES IN HOST-MICROBE INTERACTIONS

Mucosal tissue function and homeostasis are meticulously controlled by complex bidirectional interactions between cells and their microenvironment (15, 20, 25, 27–29, 52–55). The microenvironment includes 3-D tissue architecture, multiple cell types, ECM, innate immunity mediators, indigenous microbiota, and physical forces. These factors are regulatory signals for mucosal pathogens and may be beneficial or detrimental for infection (1–5, 8, 35–45, 47, 56–64). Below we address key cellular, biochemical, and biophysical cues that dictate infection outcome and are important considerations when modeling host-enteric pathogen interactions.

Cellular factors. Intestinal mucosal epithelium contains an array of specialized epithelial and immune cells that work in synergy to protect against infection by (i) serving as a barrier against luminal toxins, commensals, and pathogens, (ii) sampling microbial antigens, and (iii) recruiting innate and adaptive immune effectors (65). The intestine contains multiple epithelial cell types, including enterocytes (absorptive functions), enteroendocrine cells (hormone secretion), Paneth cells (antimicrobial production), goblet cells (mucin production), M cells (luminal antigen sampling/induction of mucosal immunity), Tuft cells (Th2 immunity), and cup cells (unknown function) (66, 67). The intestine also contains immune cells for innate and adaptive responses to pathogen attack, including macrophages, dendritic cells, and T and B cells, including those organized in lymphoid structures termed Peyer's patches, sites of induction of mucosal immunity. As the body's largest immune organ, the composition, organization, and function of the intestine vary by region and consist of integrated cross-communication networks of different cell types and effectors critical for protection against pathogens (described in references 59, 65, and 68 to 72).

Epithelial cell polarity establishes barrier function, regulates uptake/transport of nutrients, and maintains epithelial architecture (65, 73–75). In the intestine, apical surfaces face the lumen and regions between villi/folds, lateral surfaces face adjacent cells, and basal surfaces face the basement membrane and lamina propria. Along Peyer's patches and isolated lymphoid follicles, the basal side of the follicle-associated epithelium overlies a subepithelial dome region containing a mixture of immune cells (76). The distinct biochemical composition (e.g., protein and lipid) of apical and basolateral surfaces facilitates their specific functions (75). Given that many pathogens have evolved to recognize surface-specific molecules for attachment and/or to disrupt barrier integrity to enable their uptake and dissemination (6, 74, 77–79), appropriately modeling polarity *in vitro* is critical, as pathogens infect host cells differently depending on whether they are polarized or nonpolarized (80–83). Maintaining barrier integrity requires proper expression and localization of tight and adherens junctions. Adherens junctions are mediated by E-cadherin and catenin interactions, while tight junctions are

FIG 1 Legend (Continued)
bic culture, macrophages were present and either were empty (left inset) or contained internalized bacteria (right inset). Macrophages (CD45; yellow), *Salmonella* (green; white when overlaid with CD45), and nuclei (4′,6-diamidino-2-phenylindole [DAPI]; blue) are visible. Scale bar = 10 μm. (Republished from *npj Microgravity* [171].) (h) iHIOs injected with *E. coli* O157:H7. Nuclei (blue), neutrophils (CD11b; red), and *E. coli* (green) are visible. Scale bar = 100 μm. (Republished from *PLoS One* [260].) (i) CVB-infected gut-on-a-chip. CVB (green), F-actin (red), and nuclei (blue) are visible. (Republished from *PLoS One* [343].)

composed of transmembrane proteins (e.g., claudins and occludins) and cytoplasmic plaque proteins (e.g., zonula occludens). While generally protective, junctional complexes are also exploited by pathogens to facilitate invasion (74) and some enteric viruses utilize receptors localized to these junctions (77, 78).

Another major cellular component encountered by enteric pathogens is the diverse microbial community—termed microbiota (referring to microorganisms) or microbiome (referring to microbial genomes). The intestinal tract contains prokaryotes, viruses, archaea, and eukaryotes, some of which protect the host against pathogen colonization by a variety of mechanisms, including epithelial cell turnover, mucin synthesis, and triggering bacterial sensors on host cells (84–86). Reciprocal interactions between host and microbiota contribute to tissue function and homeostasis and determine microbiota composition, thereby playing an important role in infection and disease (87). For example, members of the intestinal microbiota regulate production of antimicrobial peptides by Paneth cells (88) and shape immune responses by regulating numbers, subsets, and/or functions of T, B, and myeloid cells (65). Microbiota-induced changes in immunity also determine intestinal microbiota composition (85, 89).

The intestinal microbiota is comprised of $\sim 10^{14}$ bacteria ($>$1,000 species), with *Firmicutes* and *Bacteroidetes* most abundant (90–93). Interpersonal variation in intestinal microbiome occurs, with each individual carrying a subset of the total known microbiome (94). Temporal and spatial variation occurs throughout the intestinal tract (95, 96). Increasing data suggest a relationship between an imbalanced intestinal microbiome and various diseases, including obesity, inflammatory bowel disorders, and cancer (97). The importance of the gut microbiota to health is highlighted by successful clinical application of fecal microbiota transplants from healthy individuals to patients with recurrent, antibiotic-resistant *Clostridioides difficile* infections (98–100).

Biochemical cues. Mucosal tissues contain an array of small molecules, including innate defense mediators that target pathogens and regulate downstream host defenses. Intestinal mucus harbors compounds from the innate and adaptive systems that protect against microbial insult, including digestive enzymes (e.g., lysozyme), lactoferrin, antimicrobial peptides, complement, and antibodies (e.g., secretory immunoglobulin A [sIgA]) (65). In addition, cells of the innate defense system respond to pathogen-associated molecular patterns (PAMPs) using pathogen recognition receptors (PRRs). Depending on the pathogen, PRR-mediated signal transduction results in different cellular outcomes (e.g., cell proliferation, apoptosis, antimicrobial peptide production, autophagy, and cytokine secretion). Cytokine production leads to recruitment of innate and adaptive immune effectors to the infection site, representing a bridge between these two arms of immunity (65, 101).

Mucins are complex mixtures of high-molecular-weight, glycosylated macromolecules that bind and remove pathogens and their products (7, 101). Enteric pathogens sense and respond to cues within mucus and overcome this barrier to reach underlying epithelium (7). Normal intestinal mucus consists of two layers: an outer layer colonized by microbes and a sterile inner layer (102–104). The composition and thickness of these mucin layers vary throughout intestinal regions to accommodate their different functions and microbial burdens. Within the small intestine, the inner and outer mucosal layers are thinner to facilitate nutrient absorption, with thicker regions found toward the ileum, where microbial burden is heavier (7). In the colon, both layers are thicker to accommodate the burden of several trillion commensals (7). The presence of sIgA and other mucin antimicrobials also serves to reduce bacterial colonization (105).

The ECM is another key contributor to tissue homeostasis. The ECM has historically been neglected as a signaling entity, but seminal discoveries have revealed the central role of ECM in regulating tissue architecture/function (20, 53). The ECM is a three-dimensional noncellular scaffold comprised of proteins (e.g., collagens, elastins, laminins, and fibronectins), proteoglycans, and water. Two main types of ECM include (i) interstitial connective tissue matrix, which serves as a cellular scaffold, and (ii) basement membrane matrix, which separates epithelium from interstitium (106, 107). ECM com-

ponents also serve as ligands for cell receptors like integrins, which transduce physical forces into biological responses (mechanotransduction). Additionally, immune responses are mediated through interactions with the ECM (107, 108). Furthermore, the ECM controls availability/release of growth factors and other signaling molecules (hormones and cytokines) (107). The complexity, composition, and structure of ECM are highly dynamic and specific (as are the biochemical gradients it contains) and depend on tissue type, developmental stage, and health/disease state (107).

Biophysical forces. The role of physical forces in cell and tissue development/function is as important as those of genes and biochemical signals (28, 109). Physical forces regulate cell proliferation, differentiation, and homeostasis (110, 111). Forces experienced by intestinal cells include fluid shear, pressure (112), and contractile peristalsis of muscles (113). Hydrodynamic calculations suggest that fluid shear forces on the exposed epithelial brush border microvilli are ~200 times greater than those between microvilli (<0.01 dynes/cm^2) (114).

The cytoskeleton and its linkage with the ECM play an essential role in enabling cells to sense and respond to biophysical forces. While the governing role of the ECM as a dynamic signaling entity that regulates tissue form/function is now appreciated, it was initially considered a purely static scaffold. However, tissue-specific architecture and function are regulated by the biophysical properties of the ECM (20, 115, 116), which exerts physical influences transduced by cell surface receptors through the cytoskeleton to the nucleus to ultimately alter cellular and molecular properties. These structural networks are critical for regulating cell shape/architecture and have been modeled using the principle of tensegrity, which refers to structures that are stabilized under continuous tension by balancing opposing tension and compression forces (27–29, 31). The integration of biophysical forces across cells and tissues using this structural network regulates a wide range of biological processes (e.g., cell proliferation, apoptosis, differentiation, adhesion, migration, gene expression, and architecture) (8, 20, 21, 23, 25, 27, 29–31, 55, 117). Accordingly, ECM composition and stiffness are critical regulators of cellular responses (118, 119). These properties are continuously remodeled through the process of "dynamic reciprocity" (17, 20, 53, 117), theorized by Bissell in 1982 to explain how signaling between the ECM and nucleus regulates tissue function. This laid the foundation for modern 3-D cell culture approaches used today (20, 107, 120). Not surprisingly, pathogen-ECM interactions play an important role in mediating infection (121–126). In addition to impacting the host, physical forces also globally alter bacterial gene expression, stress responses, and virulence in unexpected ways to contribute to infection (5, 36–40, 47, 62–64).

MODELING THE MICROENVIRONMENT: 3-D MODELS FOR INFECTIOUS DISEASE

Several cell culture systems exist for the development and application of 3-D models of human tissues for infectious disease research, including the RWV bioreactor, ECM-embedded scaffolds (e.g., ECM extracts, purified ECM, or synthetic/semisynthetic hydrogels), and organ-on-a-chip (OAC) models. The choice of system to use depends on several factors, including the experimental question being addressed, technical complexity, and cost and expertise for model development. Different cell types in the native tissue (including immune cells) can be cocultured in these models to further enhance physiological relevance. Additionally, a single epithelial cell type can spontaneously differentiate into multiple epithelial cell types normally found in the parental tissue and undergo self-assembly into tissue-like structures using all of these 3-D technologies. To date, most *in vitro* infection studies have been performed using cell lines; however, there is a push to develop models using either primary or stem cells to better mimic the native tissue. To explore the integration of different environmental signals in regulating infection, a hierarchical series of increasingly complex 3-D model systems comprised of different cells types can be developed and applied in parallel under differing experimental conditions (e.g., different oxygen tensions and physical forces).

RWV-derived 3-D models. The RWV bioreactor is an optimized form of suspension culture that facilitates formation of self-organizing 3-D tissue-like aggregates by allow-

ing cells the spatial freedom to colocalize and self-assemble based on natural affinities within a low-fluid-shear environment (Fig. 1B, panel a) (8, 127). Fluid shear influences cell proliferation, differentiation, morphology, and function (30, 114, 128–140). Models developed within the dynamic RWV environment experience excellent mass transfer of nutrients/wastes and exhibit enhanced structure, differentiation, function, and multicellular complexity relative to 2-D monolayers (11, 80, 141–154). Along these lines, observations from the 1970s showed that flotation of collagen gels led to a more permissive environment for cellular differentiation (12, 155, 156). Moreover, the low-fluid-shear environment in the RWV is also physiologically relevant to that encountered by pathogens in low-shear regions of the infected host, including the intestine (38, 114, 129–131). Accordingly, the RWV is also used to culture pathogens to study the role of fluid shear and mechanotransduction in regulating microbial pathogenesis and host-pathogen/commensal interactions (1, 35–41, 45–47, 62, 64, 145, 157–167).

The RWV is among the most extensively used approaches to develop 3-D models to study host-pathogen interactions. It was the first technology used to develop 3-D models for infection studies with bacterial (*Salmonella*) and viral (rhinovirus) pathogens (11, 51). A range of RWV-derived 3-D models have been developed using cell lines, stem cells, and/or primary cells, including small and large intestine (11, 80, 141, 143, 145, 146, 152, 168–177), lung (144, 147, 178–182), liver (148, 153, 174, 183, 184), bladder (8, 185–187), reproductive tissue (149–151, 188–190), heart (191–193), prostate (142, 186, 194), pancreas (195, 196), nervous tissue (182, 197–199), blood-brain barrier (200), skin (201), eye (202), bone, joint, or disc (203–207), and tonsil (208), among others. These studies demonstrated that RWV-derived models exhibit enhanced *in vivo*-like characteristics, including spontaneous differentiation into multiple cell types that self-organize into 3-D structures (Fig. 1B, panel d), polarization, appropriate expression/localization of adherens/tight junctional complexes, metabolic product secretion, gene expression, cytokine production, responses to antimicrobials and microbial products, support of commensals, and/or susceptibility to infection (8, 11, 80, 141–153, 168–194, 197–208). In addition, RWV models have been advanced to incorporate immune cells to study their role in host-microbe interactions (171, 175, 177, 180).

Models are typically initiated by harvesting monolayers, combining cells with porous ECM-coated microcarrier scaffolds, and loaded into the RWV. Scaffold and ECM porosity allows the basal side of cells to experience autocrine/paracrine communications, aiding cellular differentiation/responses in a manner reflecting *in vivo* tissues. This differs from monolayers where cells proliferate on impermeable surfaces, thus hindering proper communications across apical and basolateral surfaces. Additionally, models may be developed scaffold free or using nonmicrocarrier scaffolds (e.g., decellularized tissues) for transplantation (179, 181, 209). Once developed, distribution of 3-D models into multiwell plates lends to their experimental tractability for infection assays, as their structural/functional integrity remains intact following seeding. Alternatively, pathogens or compounds can be directly added to the RWV to study interactions under physiological fluid shear. One key advantage of RWV culture is the production of large numbers of cells ($\sim 10^7$ to 10^8 per culture). Below we discuss RWV-derived 3-D models of human intestinal mucosa.

RWV-derived intestinal models. We began using the RWV to engineer 3-D models of human intestine for infection studies in the late 1990s after realizing that available models for studying bacterial pathogenesis lacked multiple aspects of the *in vivo* microenvironment (11). RWV-derived 3-D models have enabled the study of host-microbe interactions relevant to different regions of the intestinal tract, including the small intestine (11, 169) and colon (80, 143, 145, 146, 152, 171, 172, 175, 177). Imaging of these models revealed enhanced 3-D architecture relative to monolayers, including the presence of extensive 3-D folds and microvilli, that more closely resembled what is observed *in vivo* (Fig. 1B, panel d). These 3-D models are essentially "inside-out" such that the apical/luminal side faces the media and the basal side faces the scaffold, allowing for straightforward introduction of pathogens, toxins, and antimicrobials at

the apical surface, as commonly occurs *in vivo*. Collectively, these models have shown physiologically relevant expression and localization of key cellular components, including junctional proteins (e.g., ZO-1, occludin, symplekin, E-cadherin, β-catenin, and desmosomes), secretion of basal lamina components (e.g., collagen types II, III, and IV, laminin, vimentin, and fibronectin), brush border formation with villin, and/or mucus secretion (11, 80, 143, 145, 146, 152, 169, 171, 172, 175, 177). Spontaneous cellular differentiation into multiple lineages found in the intestinal epithelium is also observed, including enterocytes, M cells, goblet cells, and/or Paneth cells (enteroendocrine cells were not evaluated) (11, 80, 146, 152, 171, 175). The presence of multiple cell types within a model (e.g., epithelial and immune cells) enables study of their combined effects on infection, and in particular, pathogen colocalization patterns with different cell types. An example is described below in which an advanced 3-D RWV coculture model that combined human colonic epithelium with phagocytic macrophages was used to study infection by different *Salmonella* pathovars (171). Primary human lymphocytes have also been incorporated in a 3-D coculture model of intestinal epithelium to study *Salmonella* infection (175).

RWV-derived intestinal models have contributed to the study of a variety of pathogens, such as *S.* Typhimurium (including multidrug resistant ST313), *Salmonella enterica* serovar Typhi, enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), *Cryptosporidium parvum*, and human enteroviruses, including coxsackievirus B (CVB) and poliovirus (11, 80, 143, 145, 146, 152, 171, 172, 175). Studies with *S.* Typhimurium using 3-D models of small and large intestine displayed marked differences from monolayers in colonization, tissue morphology, apoptosis, and prostaglandin and cytokine expression (11, 80, 152). The responses of these 3-D intestinal models to *S.* Typhimurium challenge were highly predictive of *in vivo* responses in humans/animals (11, 80, 152), including rapid repair of the small intestine (initial site of *Salmonella* pathogenesis) and significant damage to the colon (primary site of pathogenesis) (210). These models were also the first *in vitro* systems to challenge the widely accepted paradigm established using monolayers that the *Salmonella* pathogenicity island 1 (SPI-1) type 3 secretion system (T3SS) is required for invasion of intestinal epithelium (80, 152). Historically, studies with monolayers contradicted *in vivo* observations wherein successful animal infections were possible with T3SS SPI-1 mutants (211, 212), and clinical isolates of *Salmonella* lacking SPI-1 function were isolated from foodborne disease outbreaks in patients experiencing gastroenteritis (213). Using a 3-D intestinal model comprised solely of epithelial cells, Radtke et al. demonstrated that SPI-1 mutants and a *Salmonella* mutant lacking all known T3SSs (SPI-1, SPI-2, and the flagellar system) still exhibited high levels of invasion relative to the wild type (although approximately 0.5 to 1 log lower) (152). As expected, in monolayers these mutants exhibited little to no invasion ($<$10 CFU), a finding which does not reflect *in vivo* observations (152). Thus, for the first time, an *in vitro* intestinal epithelial model was able to parallel *in vivo* results by supporting *Salmonella* invasion independently of SPI-1. These findings demonstrate the enhanced capability of RWV models to predict *in vivo*-like pathogenic mechanisms.

Host-pathogen-commensal and host-commensal interactions have also been investigated using RWV 3-D intestinal models (172, 177). Commensal microbes naturally enhance intestinal mucosal barrier function against pathogen colonization through complex mechanisms not yet fully characterized (214). Naturally occurring probiotic strains of bacteria are being exploited as a strategy against pathogens to combat ongoing problems of antibiotic resistance. Treatment of a 3-D intestinal model with *Lactobacillus reuteri* or its antimicrobial metabolite, reuterin, before or after challenge with *S.* Typhimurium reduced adhesion, invasion, and intracellular survival of this pathogen compared to findings for untreated cells (172). This was the first study to report the effect of reuterin on the enteric infection process for any mammalian cell type. A 3-D intestinal coculture model containing immune cells was used to profile responses to both free secretory IgA (sIgA) and sIgA complexed with a commensal strain of *E. coli* (177). Application of free sIgA to the model induced upregulation of

MUC2, interleukin 8 (IL-8), and polymeric immunoglobulin receptor (pIgR) secretion. When sIgA was complexed with *E. coli* and applied to the model, these responses were downregulated relative to models treated with free sIgA.

Barrila et al. reported advancement of a 3-D HT-29 colon model to include phagocytic macrophages, thereby improving its physiological relevance to study aspects of the innate immune response to infection (171). Characterization of this coculture model revealed macrophages integrated between and underneath epithelial cells while preserving epithelial tight junctions and the presence of multiple epithelial cell types, including enterocytes, M cells, and goblet cells (171). Macrophage phagocytosis was confirmed by evaluating their ability to engulf inert, bacterium-sized beads. The contribution of macrophages to *Salmonella* infection was assessed using *S. enterica* pathovars with differing host tropisms and disease phenotypes, including the well-studied sequence type 19 (ST19) *S.* Typhimurium strain SL1344, which causes disease in a wide range of hosts, the multidrug-resistant ST313 *S.* Typhimurium strain D23580, and the human-specific *S.* Typhi strain Ty2. Although classified as *S.* Typhimurium, ST313 strains display genome degradation similar to that of human-adapted *S.* Typhi and are associated with devastating epidemics of blood-borne infections in sub-Saharan Africa (215). Bacteria were cultured aerobically or microaerobically prior to infection to simulate oxygen environments encountered before and during intestinal infection. Colonization of all strains was reduced in the coculture model containing macrophages relative to the epithelial model, indicating antimicrobial function of macrophages. Although ST313 strains are considered highly invasive due to the systemic infection they cause, D23580 was not highly invasive in the 3-D models but instead exhibited enhanced survival/replication, thus providing clues as to what drives this organism's pathogenicity. Pathovar- and oxygen-specific differences in host cell colocalization patterns were also observed (Fig. 1B, panel g), indicating the ability of these advanced models to distinguish between closely related *Salmonella* serovars, thus providing a unique advantage over models composed of a single cell type (171).

RWV-derived intestinal models are also valuable for investigating host-pathogen interactions for which conventional cultivation strategies are unable to adequately model *in vivo* complexity. Recently, a 3-D colonic model was applied to study human CVB (146), a pathogen for which *in vitro* and *in vivo* models may not fully model the enteral infection route in humans (146, 216–219). Comparisons between polarized 2-D and 3-D cells revealed that the 3-D model displayed an enhanced number of viral particles secreted into the media at early stages of the viral life cycle, which did not coincide with increased host cell destruction relative to monolayers (146). These data suggest that 3-D models exhibit an enhancement in nonlytic release of viral particles, which might result from morphological changes (e.g., enhanced brush border formation) in 3-D cells. Similarly, another 3-D colonic model was used to study *Cryptosporidium parvum*, a parasite for which there is a lack of physiologically relevant *in vitro* and *in vivo* models (143). Following *C. parvum* infection, morphological changes were observed that were consistent with those from colonic biopsy specimens of infected patients (143). These studies further emphasize the critical importance of model complexity and physiological relevance as determinants in enabling host-pathogen interactions.

In summary, 3-D RWV intestinal models are powerful tractable research tools that advance the study of host-pathogen interactions. These models can be modularly altered to incorporate different cell types (including patient-derived cells), ECM, commensal microbiota, physical forces, etc., akin to *in vivo* scenarios, increasing their relevance. Their tissue-like architecture, differentiation and polarization, enhanced expression/localization of junctional proteins, and mucin production are necessary components of an effective barrier to invading pathogens.

Limitations and future directions of RWV-derived 3-D models. Although many key structural/functional characteristics of parental tissues have been successfully recapitulated using RWV models, several limitations remain. The full extent of 3-D

architecture, multicellular complexity, and array of physical forces of *in vivo* tissues has not yet been attained. Ongoing studies are further enhancing these features, plus incorporating patient-specific immune cells and fecal microbiota, and achieving vascularization and innervation. Models can be costly due to high medium consumption required for culturing large numbers of cells; however, researchers can scale down. Although bead porosity facilitates apical/basal cytokine secretion and there is excellent access to the apical side of the models, there is currently an inability to sample the basal side. This also prevents measurement of transepithelial electrical resistance (TEER), which measures electrical resistance across a monolayer as a proxy for assessing barrier integrity (220). The technique involves using two electrodes, one in contact with cells on a semipermeable membrane (e.g., apical side) and the other in a different chamber containing culture medium (e.g., basal side). With most RWV models grown on tiny ($\sim$175 μm) microcarrier beads, these measurements are not currently possible with off-the-shelf technology. This challenge will likely be surmounted with custom electrode design to accommodate current RWV models or the use of alternative scaffolds. Currently, immunofluorescence imaging of cytoskeletal and tight junctional markers represents an alternative method to evaluate model integrity. As these models grow in size and complexity, introduction of vasculature and nerve cells will be important. Finally, current models are not easily amenable to chronic infection due to lack of perfusion once removed from the RWV; however, inclusion of automated waste removal and nutrient delivery during infection will facilitate this approach.

3-D organoid models. The term organoid ("organ-like") has been used to describe a variety of 3-D models that resemble *in vivo* tissues. Historically, this included models engineered with different technologies using cell lines, stem cells, primary cells, or tissue explants either embedded in or cultured on top of ECM scaffolds that allow cells to self-assemble into 3-D structures (8, 12, 143, 145, 146, 169, 171, 221–229). Advances in stem cell biology led to a recent terminology shift to more specifically define organoids as 3-D models derived from stem cells, progenitor cells, or primary explants (222, 230–238). Here we focus on 3-D models cultured within a 3-D ECM that fit this definition. It is important to emphasize that current models are based on decades of work by pioneering cell biologists that laid the foundation for the current organoid field (reviewed in reference 12), representing an advancement and merging of old and new technologies to enable novel discoveries (12, 228, 239). Models cultivated using thick ECMs have deep roots in tissue engineering and cancer biology, in which they were applied to develop advanced models enabling the study of a variety of biological mechanisms, particularly with regard to understanding the interrelationship between tissue structure and function (12). This effort resulted in a critical mass of scientists who now recognize the importance of 3-D models for infection and are bringing elegant advances to the field but may not be fully aware of their historical context.

A range of different organoid models have been established, including small and large intestine (229, 230, 232, 234, 240–268), lung (269–274), stomach (275–282), breast (55, 283, 284), brain (285–287), liver (222, 288, 289), pancreas (222, 290, 291), gallbladder (292), eye (293), kidney (294), prostate (222, 295, 296) and reproductive tract (297, 298), among others. Relative to monolayers, these models more closely mimic endogenous tissues, including organization and spontaneous differentiation of multiple cell types into physiologically relevant 3-D structures (Fig. 1B, panel e), expression and localization of tight junctions, mucus production, polarity, gene expression, cell viability and proliferation, cytokine production, responses to antimicrobials, support of commensals, and susceptibility to infection (12, 55, 222, 226, 228–235, 237, 238, 240–266, 269–319).

To develop 3-D organoid models, stem cells or tissue explants containing stem cells are used. Biopsy specimens may be treated with a dissociation agent and/or mechanically disrupted prior to embedding into ECM. Stem cells isolated from biopsy specimens can be predifferentiated into progenitor cells and further differentiated into ECM-embedded organoids. Differentiation into committed cell types is enabled by stepwise supplementation and/or removal of signaling factors during culture (249, 251,

252, 254, 264, 275, 278, 303, 320–322). Purified ECM components and mixtures can be used, including Matrigel, a laminin-rich ECM isolated from chondrosarcomas (323, 324). Synthetic hydrogels help circumvent challenges associated with Matrigel, including batch-to-batch variation and potential carcinogenic issues connected with tumor-derived matrices (229).

3-D intestinal organoids. Sato et al. (249) and Ootani et al. (253) independently reported conditions enabling long-term *in vitro* culture of mouse intestinal crypts containing Lgr5$^+$ stem cells (as well as purified Lgr5$^+$ stem cells that generate villus/crypt-like structures [249]). These approaches used either Matrigel (249) or collagen (253) in combination with supplementation of Wnt agonist R-spondin 1. Sato et al. also included epidermal growth factor to enable crypt growth and noggin to facilitate passaging (249). These models displayed a polarized, multicellular epithelium (enterocytes, goblet cells, Paneth cells, and enteroendocrine cells) organized into a central lumen lined by villus/crypt-like structures (249, 253). Murine intestinal organoids developed from single Lgr5$^+$ stem cells also developed into these multicellular structures (249). Subsequently, additional factors were included to enable human colonoid culture (264).

The NIH Intestinal Stem Cell Consortium defined a standardized nomenclature to reflect model sources, approaches, and *in vitro* structures (325). Structures directly isolated include epithelial sheets, crypts, and organoids (crypts and surrounding mesenchymal elements) (325). Various structures produced *in vitro* from small intestine include enterospheres (rounded epithelial cyst-like structures), enteroids (formation of budding crypts from enterospheres), and induced intestinal organoids (multicellular clusters from induced embryonal or pluripotent stem cells, e.g., induced human intestinal organoids [iHIOs]) (325). Analogous colonic structures include colonospheres, colonoids, and colonic organoids (325). It is common to see terms used interchangeably, and the nomenclature will likely evolve as the field expands.

Model infection can be accomplished by (i) addition of pathogen directly to the media (basal side), (ii) microinjection into the lumen (Fig. 1B, panel b), (iii) shearing of models into fragments followed by pathogen addition, and (iv) complete disruption of 3-D models into flat monolayers followed by pathogen addition (230, 237). Consideration of the normal infection route is critical. Direct addition to the media is easiest; however, for pathogens that infect via the apical/luminal side, this represents a nonphysiological route of infection. Microinjection is technically challenging but preferable for pathogens that normally infect from the lumen. Due to challenges associated with microinjection, there is a growing tendency to mechanically dissociate organoids into smaller pieces or completely dissociate them into monolayers on Transwell inserts or plastic (237, 261, 281, 313, 314). This approach has been successful for a number of studies, including cultivation of norovirus (314), a major advance in the field. Use of Transwell inserts also facilitates TEER analysis and easier cytokine sampling from the apical side of the model.

When dissociating 3-D models prior to infection, it is important to note that this disconnects their form and function, similar to disrupting primary tissue into monolayers, and may render them less predictive for some (not all) phenotypes. In this approach, Transwell inserts are preferable over plastic, as the former display improved physiological relevance over conventional monolayers (326). Additional profiling should confirm the extent to which the dissociated model may have dedifferentiated and additional culture time may be required to reestablish polarity/barrier function. Key findings should be validated using intact organoids and microinjection to avoid artifacts. Additionally, since ECM-pathogen interactions are important for infection dynamics (61), infection surfaces should not contain ECM components not typically found in that location *in vivo* (e.g., lumen) if the pathogen would not normally encounter it.

A variety of pathogens have been studied using 3-D enteroid/colonoid/organoid models, including *Salmonella*, *C. difficile*, EHEC, EPEC, enterotoxigenic *E. coli* (ETEC), norovirus, rotavirus, enteroviruses, *Toxoplasma gondii*, and coronaviruses (230, 231,

233–235, 238, 240–245, 257–263, 266, 268, 307–319, 327). The first infection using iHIOs was performed using human rotavirus, which lacks robust *in vitro* culture systems (315). Both laboratory and clinical rotaviruses replicated in iHIOs and were detected in epithelial and mesenchymal cells (315). Crypt-derived enteroids also supported rotavirus replication and were used to assess antiviral efficacy against patient isolates (244, 266). Ettayebi et al. made a significant advance by the successful *in vitro* culture of human norovirus (HuNoV), known for its lack of a reproducible culture system (314). The authors initially cultured 3-D intestinal organoids and then dissociated them into monolayers on plastic or Transwell inserts (314). Successful viral replication was observed and only enterocytes were infected with HuNoVs, regardless of the strain or the intestinal region from which the model was derived. Additional viral models, including those using enteroviruses (e.g., CVB, echovirus 11, and enterovirus 71) have identified the cell-type-specific nature of these infections and the virus-specific nature of innate immune signaling in response to infection (327).

Enteroid models were also used to study *S.* Typhimurium and *E. coli*. Zhang et al. (240) and Wilson et al. (243) used crypt-derived enteroids to study *Salmonella* infection. *S.* Typhimurium successfully colonized the model (240, 243), and infection responses aligned well with *in vivo* observations, including disruption of tight junctions, inflammatory responses, and decreased stem cell numbers (240). Forbester et al. infected iHIOs with *S.* Typhimurium and observed physiological transcriptomic and cytokine profiles (257). Injection of *E. coli* O157:H7 into iHIOs containing neutrophils led to loss of actin, epithelial integrity disruption, induction of inflammatory cytokines, and neutrophil recruitment (Fig. 1B, panel h) (260). In contrast, commensal *E. coli* was retained within the lumen, with no loss of model integrity. Infection of colonoid-derived Transwell models identified MUC2 and protocadherin-24 as early EHEC infection targets (261). Colonoids were initially cultured in 3-D, followed by dissociation onto Transwells. Model differentiation correlated with expression of differentiation markers, increased TEER, and microvilli (261). EHEC preferentially colonized the differentiated model relative to an undifferentiated control, reducing colonic mucus and inducing damage to microvilli. A similar approach was applied to study EPEC and ETEC infections in coculture models containing macrophages (313). Inclusion of macrophages in the bottom chamber of the enteroid-derived Transwell model enhanced barrier function, increased epithelial height, and altered cytokine responses relative to the control. EPEC increased total macrophage numbers and induced projections that extended into the epithelium, while ETEC induced macrophage extensions across the epithelium to the apical surface. The presence of macrophages in the coculture model enhanced barrier function and correlated with decreased numbers of ETEC organisms relative to the model lacking immune cells.

iHIOs were also used to study *C. difficile* infection (CDI) (258, 259, 262, 263). CDI patients secrete acidic mucus consisting primarily of MUC1, with decreased MUC2 and altered oligosaccharide composition relative to that in healthy patients (259). Injection of the pathogen alone into iHIOs decreased MUC2, while whole CDI stool supernatant was required to induce patient-like oligosaccharide composition changes (259). iHIOs were also used to investigate nontoxigenic and toxigenic strains of *C. difficile* and purified toxins TcdA and TcdB (262). Injection of the toxigenic isolate or purified TcdA led to a loss of barrier function, while iHIOs injected with the nontoxigenic strain remained intact. Separately, colonoids helped identify Frizzled proteins as receptors for the TcdB toxin (263).

In summary, 3-D organoid models are advancing mechanistic understanding of host-microbe interactions due to their enhanced 3-D architecture and presence of $Lgr5^+$ stem cells together with multiple cell types and other functional properties. In addition, patient organoid "biobanks" have been established and are facilitating fundamental research and clinical applications (230, 231, 328, 329). One exciting example of the applicability of these models is the use of patient-derived organoids to predict drug responses for cystic fibrosis treatment (222, 231, 250, 307, 329, 330).

Limitations and future directions of 3-D organoids. As for other models, organoids have limitations that researchers are working to overcome. Variability and quality control challenges between experimental preparations includes (i) heterogeneity in size, shape, and viability of organoids within a culture and across different samples, (ii) batch-to-batch variability in Matrigel or other ECM, and (iii) batch-to-batch variability in growth factor sources. Organoid infection presents challenges as described above. Medium cost is high if scaling up due to reliance on specific growth factors. Incorporation of the full array of cell types found *in vivo*, including the diverse collection of immune cells and microbiota, has not been attained. Organoid models also lack spontaneous M cell formation (251, 331). Pretreatment of *in vitro* models with RANKL, exposure to lymphocytes, or infection with pathogens like *S*. Typhimurium can induce M cell formation (331–333). Although the mechanism by which M cells spontaneously differentiate in RWV models (11, 152, 171, 175) is unknown, it is possible that the low-fluid-shear suspension culture environment is important, since flotation of ECM scaffolds was more permissive for differentiation than surface-attached ECM (12, 155, 156). Since organoid models are typically ECM embedded, another limitation is that the application of the range of biomechanical forces found *in vivo* is limited; however, an iHIO model containing functional neurons that enabled peristalsis-like contractions was reported (256). Combinations of technologies, including organoid-derived 3-D models developed using the RWV bioreactor (202) and organ-on-a-chip (OAC) (334), are further expanding these capabilities. TEER measurements are also not currently possible with intact organoid models due to their size and structure and because they are ECM embedded. Some studies have dissociated organoids into 2-D on Transwells to facilitate these measurements, although there can be disadvantages to using this approach, as discussed.

Organ-on-a-chip models. Advanced microfluidic and microfabrication technologies are being broadly applied to develop organ-on-a-chip models that mimic key aspects of *in vivo* microenvironments. Rather than focusing on recreating the 3-D structure of the entire tissue, this technology aims to recreate a microscale model of the local 3-D architecture and spatial distribution of dynamic tissue interfaces to mimic tissue- and organ-level functions (335). These devices are designed with micrometer-sized fluidic channels separated by thin, flexible porous membranes that enable development of different tissues in adjacent chambers while retaining their ability to interact (Fig. 1B, panel c) (335–339). These features allow flexibility to model active processes within a tissue, such as vascular-like perfusion. One exciting functional feature engineered into the design of many of these devices is the capability to apply dynamic forces across the tissue to model fluid shear and peristalsis (334, 340–343).

OAC models vary in complexity, ranging from simple systems containing a single perfused chamber and cell type to more advanced chips that contain several microchannels, membranes, and assorted cell types, thereby allowing the reconstruction of multiple tissue interfaces (335). Microengineering techniques for these devices have been extensively reviewed (335, 338, 344–349). Chips are commonly made of a silicone polymer called polydimethylsiloxane (PDMS), which is compatible with many cell types and has several advantages, including optical transparency for easy imaging, low cost, flexibility, and high gas permeability (335, 339, 344, 350). PDMS does carry some disadvantages (discussed below), so other options are being explored (350, 351). Depending on experimental requirements, chip design and approaches for tissue development can be altered. Porous membranes can be coated with a variety of matrices/scaffolds (335, 339, 344, 345, 352). Moreover, 3-D bioprinting techniques are facilitating complex spatial patterning of cells and scaffolds (352). Although traditional electrodes used for TEER measurements do not accommodate the small culture area of most OAC models (220), recent studies have integrated custom electrodes (353).

A variety of OAC platforms have been derived from cell lines, stem cells, and/or primary cells, including small and large intestine (334, 340–342, 353–356), lung (357–361), liver (362–369), kidney (370–372), heart (373–377), cornea (378), skin (379),

nervous tissue (380–383), bone (384, 385), reproductive tract (386), and blood/endothelium and blood-brain barrier (387–393), among others. Once developed, these models typically retain their structural and functional integrity for several weeks (model specific), further lending to their experimental tractability. Similar to the other 3-D models discussed, OAC models exhibit *in vivo*-like characteristics, including spontaneous differentiation into multiple cell types, polarity/barrier function, formation of local 3-D structures (Fig. 1B, panel f), responses to biophysical forces, cytokine production, gene expression, mucus production, responses to nanoparticles and drugs, support of commensals, responses to microbial components (e.g., LPS), and/or susceptibility to microbial infections (334, 335, 339–342, 354–377, 379–394). The application of physical forces across several of these models alters physiological responses, including changes in expression/localization of tight junctions, barrier integrity/function, polarity and differentiation, cell viability, size, morphology, ECM production, integrin expression, enzyme activity, cytokine responses, chemical/gas exchange gradients, molecular transport, drug responses, bacterial colonization, virion-related cytopathic effects, and/or formation of 3-D structures (e.g., villi) (334, 340–343, 345, 358, 359, 361, 371, 372, 376, 377, 384, 387, 388, 395). Importantly, several models have been advanced to incorporate immune cells (342, 359, 396). Below we discuss examples of gut-on-a-chip models that have been applied to study pathogens or commensals.

Gut-on-a-chip models. The Ingber laboratory developed a series of "mechanically active" gut-on-a-chip models and applied them to study host-microbe interactions (340, 342, 343). They initially constructed a PDMS chip containing two microfluidic channels separated by a flexible, porous ECM-coated membrane (340). Colonic cells were seeded in the upper channel under low-fluid-shear stress (0.006 to 0.06 dyne/cm^2), and medium also flowed in the bottom chamber. The chip was engineered with dual vacuum chambers on either side of the main microchamber to enable application of a physiological cyclic strain across the membrane to mimic intestinal peristalsis. This led to a highly polarized columnar epithelium and spontaneous formation of 3-D villus-like folds with basal proliferative cells in the crypt region. Model characterization revealed well-formed tight junctions, mucus production, and multiple intestinal epithelial cell types (absorptive, goblet, enteroendocrine, and Paneth cells) (340, 341). The ability of this model to support commensal colonization was assessed using *Lactobacillus rhamnosus* LGG. Colonization of LGG improved barrier function and was supported for greater than a week without impacting model integrity, consistent with previous *in vivo* observations for probiotics. The model was also applied to study host-virus interactions using CVB (Fig. 1B, panel i) (343). Exposure of CVB to the apical surface led to successful viral replication, induction of cytopathic effects (CPE), and polarized (apical) release of proinflammatory cytokines. Infection of the basal side led to decreased viral titers and lower CPE, with apical secretion of proinflammatory cytokines.

The above-described gut-on-a-chip model was further advanced to include immune cells (peripheral blood mononuclear cells [PBMCs]) and/or endothelial cells (vascular or lymphatic) (342). This combination of models enabled exploration of the interplay between these factors (and others) in bacterial overgrowth and inflammation in the onset of intestinal injury. Synergistic effects between PBMCs and either nonpathogenic *E. coli*, pathogenic enteroinvasive *E. coli* (EIEC), or purified lipopolysaccharide (LPS) led to altered barrier function and changes in villus architecture. Similarly, the presence of both PBMCs and LPS led to polarized secretion of basal proinflammatory cytokines, which stimulates recruitment of additional immune cells in an *in vivo* scenario. Exposure of the PBMC-containing model to a therapeutic formulation of probiotic bacteria increased barrier function. The formulation reduced EIEC-induced intestinal damage in the model lacking PBMCs but in the presence of immune cells only delayed injury onset. Cessation of cyclic stretching led to enhanced bacterial overgrowth, even under constant medium flow.

Limitations and future directions of OAC models. While there are many advantages to OAC models, there are limitations. Many of these models have multiple cell types which exhibit enhanced 3-D architecture; however, the vast array of native heterogeneous cell types found *in vivo* still need to be incorporated and different laboratories are optimizing ECM composition and structure. Along these lines, to our knowledge, no one has yet reported the presence of M cells in gut-on-a-chip models. There is also a strong push for physically linked multiorgan models, or "humans-on-chips" (338, 397). Another limitation is the PDMS material commonly used for chip construction, which can absorb small hydrophobic molecules and interfere with drug screening and cell signaling analysis (338, 350, 351). There are also risks of uncross-linked PDMS leaching into the culture if the curing process is incomplete, causing cell damage (350, 351). While the small number of cells required can be considered advantageous, in some cases, larger numbers of cells (106, 107) may be required depending on the experiment. Infection studies typically involve many permutations, and it is not uncommon to use several multiwell plates within a single experiment. For example, during colonization assays, samples are harvested at different times and plated for viable bacteria, while others are processed for downstream analyses. Thus, it will be beneficial to incorporate multiple 3-D model systems into infectious disease research depending on the experimental question being addressed, as no single model system is sufficient to address all infectious disease experimental scenarios.

CONCLUSIONS

Over the past 2 decades, a multidisciplinary consortium of researchers has been creative in developing 3-D intestinal models of increasing complexity that better mimic the biological, chemical, and physical microenvironments of the endogenous tissue for studying host-microbe interactions. These models have been developed using a variety of approaches and are being applied to understand the dynamic relationship between the host, pathogens, and commensals that dictate infection outcome and for development of new treatment/prevention strategies. Collectively, these models have ushered in a new era for infectious disease research by offering predictive *in vitro* translational platforms. Indeed, the establishment of 3-D intestinal models and their application as human surrogates for infectious disease research have provided specific examples of how the study of microbial pathogenesis can be advanced by using appropriate, biologically meaningful models.

We are still in the infancy of learning how to build more realistic 3-D tissue models, and there remain an endless number of questions and hypotheses to test about how infection actually happens in the body. Continued model advancement to better recapitulate the *in vivo* tissue microenvironment coupled with the application of multiple 3-D model systems will lead to increased translation of research discoveries to practical and significant outcomes. Such advances will be pivotal for the success of personalized medicine approaches using patient-specific normal and diseased cells and of incorporation of the full repertoire of immune cells to predict clinical correlates of protection for vaccine development.

Toward this goal, we must deeply comprehend 3-D tissue/organ structure and function, the associated microenvironment, and the microorganisms to be studied. It is likewise important that we are aware of and acknowledge the rich history and work of researchers who have long applied 3-D tissue modeling to study host-pathogen interactions. Accordingly, we should revisit past research in the field to help us understand and guide our direction. While it remains a daunting task to gain a complete understanding of infectious disease, the alignment of multidisciplinary research teams dedicated to the establishment of 3-D models that reconstruct the architecture and function of the *in vivo* organ and their application for host-pathogen interaction studies make this an exciting time to be a scientist!

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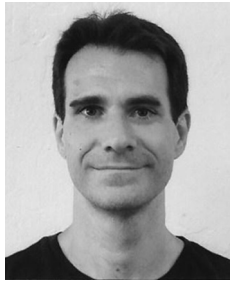
Karla Franco received her bachelor's degree in general science from Pontificia Universidad Catolica de Puerto Rico. She became interested in microbial pathogenesis during a year-long NIH training fellowship (ASU PREP). She enrolled in the microbiology Ph.D. program at Arizona State University, where she received a 2-year fellowship from the NIH IMSD program. As a member of the Nickerson laboratory, she has spent the last 3 years investigating the role of mechanotransduction in regulating the phenotypic and molecular genetic responses of *Salmonella* Typhimurium.



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Sandhya Gangaraju received her master's degree in biochemistry in 2003 from the University of Ottawa, Canada. In 2003, she joined the National Research Council, Canada, as a research technical officer and viral facility manager in the department of neurogenesis and brain repair. She implemented lentiviral technology to deliver neurotropic factors to neural cells and developed Transwell assays to study neutrophil transmigration through endothelial cells. In 2014, she joined the Biodesign Institute, Arizona State University, as a principal research specialist and compliance officer for the Center for Biosignatures Discovery Automation, where she managed the cell culture facility, led experimental design for students and junior staff members, and optimized working protocols for microfluidic devices for studying three-dimensional tissue environments. Most recently, she joined the Nickerson laboratory as a principal research specialist; at this laboratory, she uses the RWV and the spaceflight platform to study microbial pathogenesis and for 3-D tissue engineering.

C. Mark Ott received his B.S. in chemical engineering from the University of Texas at Austin in 1982, his M.B.A. from Louisiana State University in 1989, and his Ph.D. in microbiology from Louisiana State University in 1998. He has published extensively in the areas of microbial ecology in spacecraft, human and microbial responses to spaceflight, and the development of advanced tissue culture models to investigate infectious disease. For the past 20 years, Dr. Ott has served as a technical lead in the Johnson Space Center Microbiology Laboratory, which is responsible for mitigating infectious disease risk during human spaceflight. His responsibilities include the assessment of microbial risk and development of spaceflight requirements based on vehicle and mission architecture as well as crewmember, food, and environmental monitoring.

Carolyn B. Coyne completed her Ph.D. at the University of North Carolina at Chapel Hill, where she studied the human respiratory epithelium. She then carried out her postdoctoral fellowship at the Children's Hospital of Philadelphia (CHOP), Philadelphia, PA, and the University of Pennsylvania, Philadelphia, PA, where her research focused on identifying the mechanisms by which enteroviruses invade the gastrointestinal epithelium and blood-brain barrier endothelium. She joined the University of Pittsburgh, Pittsburgh, PA, as a faculty member in 2007; at this institution, her work continued to focus on defining the mechanisms by which viruses breach cellular barriers. Dr. Coyne's laboratory also studies how the human placenta restricts viral infections. Her research interests also include the development of primary and cell line-based models of cellular barriers, focusing on both the gastrointestinal tract and placenta. Her research interests include enteroviruses and flaviviruses, with a particular emphasis on the strategies by which these viruses bypass cellular barriers.

Mina J. Bissell is a Distinguished Scientist, the highest rank bestowed at Lawrence Berkeley National Laboratory, and serves as Senior Advisor to the Laboratory Director on Biology. She is also Faculty of four graduate groups in UC Berkeley: Comparative Biochemistry, Endocrinology, Molecular Toxicology, and Bioengineering (UCSF/UCB joint). Having challenged several established paradigms, Bissell is a pioneer in breast cancer research, and her body of work has provided much impetus for the current recognition of the significant role that extracellular matrix signaling and the microenvironment play in gene expression regulation in both normal and malignant cells. Her laboratory developed novel 3-D assays and techniques that demonstrate her signature phrase: after conception, "phenotype is dominant over genotype." Bissell has received numerous honors and awards and is an elected fellow of most U.S. honorary scientific academies. She has published over 400 publications and continues to engage in full-time research, among other scientific activities.

Cheryl A. Nickerson is a Professor in the School of Life Sciences at the Biodesign Institute, Arizona State University. She received her Ph.D. in microbiology from Louisiana State University. Her postdoctoral training in *Salmonella* pathogenesis was done with Dr. Roy Curtiss III at Washington University in St. Louis, MO. She initiated her ongoing studies into the connection between cellular biomechanics/mechanotransduction and host-pathogen systems biology after joining the faculty at the Tulane University School of Medicine in 1998. Her development of innovative model pathogenesis systems includes 3-D organotypic tissue culture models to study host-pathogen interactions and approaches that characterize pathogen responses to physiological fluid shear forces encountered in the infected host and in the microgravity environment of spaceflight. She received the Presidential Early Career Award for Scientists and Engineers, NASA's Exceptional Scientific Achievement Medal, is an American Society for Microbiology Distinguished Lecturer, and was selected as a NASA astronaut candidate finalist.