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Physiologically Relevant Human Tissue Models for Infectious Diseases

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Abstract

Limitations of animal infection models have engendered longstanding obstacles in basic science and translational research. Lack of suitable animal models, the need for better predictors of human immune responses, and pathogens that grow poorly or not at all outside the human host impact our ability to study infectious agents that cause human disease, generation of essential tools for genetic manipulation of microbial pathogens, and development of vaccines, therapeutics, and host-targeted immunotherapies. The advent of conceptual and methodological advances in tissue engineering along with collaborative efforts between the bioengineering and infectious diseases scientific communities holds great promise to overcome these significant barriers.

Teaser sentence

Bioengineered human tissue models demonstrate the need to balance ease of use for basic science research with recapitulation of human tissue architecture in organotypic models for infectious disease and preclinical product development studies.

Keywords

tissue engineering; infectious diseases; microbial pathogenesis; 2D; 3D; stem cells

The use of animal models in infectious diseases research enabled historic developments in vaccines and therapeutics, and contributed greatly to our understanding of microbial pathogenic mechanisms. However, limitations inherent to animal models of infection also hindered progress in these areas. Some pathogens have a uniquely human host range; their inability to infect any animal species confounds the development of products to combat these infections. Moreover, animal infections inadequately reproduce human disease pathophysiology [1–4]. Classical two dimensional (2D) *in vitro* culture systems typically used in research laboratory settings lack tissue-like architecture and mirror the inadequacies of animal models, *i.e.*, the failure to mimic human disease or to reflect the immune response.

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Thus, preclinical studies in animal models and traditional *in vitro* cell culture models that use primarily unicellular, transformed cells cannot accurately predict clinical trial outcomes, a significant obstacle that underscores the need for better predictors of efficacy, host toxicity, and host-targeted therapies. Lastly, some pathogens cannot be cultured routinely, which precludes the development of genetic tools for these microbes and impedes insights into their pathogenesis.

The potential to generate improved infection models evolved with breakthroughs in understanding embryogenesis, developmental biology and stem cells, in conjunction with technological advances in tissue engineering, a field that initially focused on regenerative medicine [5]. Biomimetic infection models applicable to basic and translational research represent critical advances for the infectious diseases field, and collaborations between bioengineers and infectious disease experts to generate new models represent a significant scientific frontier. Early studies of bacterial pathogenesis, some of which combined advances in tissue engineering with infectious diseases research, generated intriguing results [6–8]. Now, collaborations between tissue engineers and infectious diseases experts are poised to realize in-depth studies in human-pathogen interactions that address longstanding scientific obstacles. Here, we review selected, three dimensional (3D) and derived 2D nontransformed human experimental *ex vivo* tissue-like models developed to recapitulate major organ systems (Figures 1 and 2, Table 1) and their related pathogens. This exploding area of research explores many questions of normal human cell biology, physiology and pathophysiology, and these new tools are being applied to precision medicine, regenerative medicine and tissue engineering. Many types of human organs are being modeled, and varied nomenclature causes some confusion in the field. Pluripotent stem cell (PSC)-derived tissues and structures are most frequently called organoids. However, “organoids” may have different origins. For example, different models of “mini-intestine” cultures of the small intestinal tract can be distinguished; tissue-derived epithelial-only cultures, tissue-derived epithelial-mesenchymal cultures that are grown in air-liquid interface conditions, and PSC-derived cultures [9]. Although the Intestinal Stem Cell Consortium [10] proposed using the term organoid to refer to cultures derived from PSC and using the term enteroids to refer to cultures derived from crypts (containing intestinal stem cells) isolated from biopsy or surgical tissues (Figure 1), this has not been uniformly followed. We use these terminologies herein but also discuss specific models following the nomenclature in individual publications. A few methodology and review articles are included here [11–13] and in pertinent sections.

Pathogenesis Models

Brain

3D brain-like tissues were developed with primary rat cortical neuron [14,15] or human pluripotent stem cell (hPSC) cultures [16,17]. Models generated with primary cortical neuron cultures used biomaterials of differing mechanical properties, *i.e.*, silk protein-based stiff, porous scaffolds for neuronal anchoring and softer extracellular matrix (ECM) gels for axon penetration and connectivity. This 3D model formed functional brain-like cortical tissue that was maintained for months *in vitro*. The modular format provides a flexible

platform approach that can be used to fabricate other scaffold shapes and incorporate additional extracellular matrix (ECM) components, soluble factors, and cell types to achieve more complex brain-like organizations. This physiologically relevant biomimetic responded to traumatic injury induced via the weight-drop method and exhibited neuronal damage, injury-induced surge of the excitatory Glu (glutamate) neurotransmitter, and changes in electrophysiological activity consistent with *in vivo* responses to concussive brain injury [15]. Although these studies did not involve infectious agents, recently, organoid models have been used to study Zika virus and demonstrate viral tropism for human brain cells and disrupted neurogenesis [18–20]. Progress in recreating blood-brain barrier physiology in a neurovascular unit may also facilitate new therapeutics to overcome the challenge of delivery of drugs for neurotropic pathogens [21,22].

3D cerebral organoids generated from *in vitro* culture of hPSCs recapitulate fundamental mechanisms of mammalian neurodevelopment and display characteristics of human brain development. Within 1–2 months in culture, the brain-like tissue revealed essential features (developing cerebral cortex, ventral telencephalon, choroid plexus and retinal identities); these organoids could be maintained in long-term culture for > a year. Studies with cerebral organoids derived from a microcephalic patient modeled aspects of microcephaly; results showed smaller neuroepithelial tissues and a large degree of neuronal outgrowth compared to controls, findings that suggest premature neural differentiation at the expense of early progenitors, supporting a model wherein the failure of progenitor population expansion leads to smaller brain size and smaller skull formation [16].

Gastrointestinal Tract

Selected review articles relevant to novel gastrointestinal tract models and pathogenesis studies are provided [9,23–28].

Gastric Models—Human gastric organoid (hGO) systems were developed by several investigative groups and used to study gastric physiology, signaling pathways involved in proliferation of the epithelium, and the pathogenesis of *Helicobacter pylori*, the causative agent of peptic ulcer disease and gastric adenocarcinoma; the latter of which led to WHO designation of *H. pylori* as a Class 1 Carcinogen [29–34]. In one study, human pluripotent stem cells were used to develop *de novo* 3D human gastric tissue through directed differentiation. Organoid development was similar to *in vivo* gastric organogenesis; cellular complexity mimicked that of the human gastric antrum, and, importantly, via this first *in vitro* model of human stomach development, the investigators reported a new role for EGF receptor signaling during embryonic stomach formation. *H. pylori* microinjected into hGO lumens showed translocation of the CagA virulence factor, thought to be involved in malignant transformation, into epithelial cells; association of CagA with the host c-Met receptor; and a twofold increase in epithelial cell proliferation [29]. Another study showed that the stem cell marker, cluster-of-differentiation (CD44), plays a functional role in *H. pylori*-induced epithelial cell proliferation, and this is associated with the bacteria expressing a functional CagA [34]. Taken together, these data suggest that hGO models reflect physiological changes expected upon *H. pylori* infection.

Another group generated hGOs with multipotent stem cells isolated from adult human gastric tissue for studies of the epithelial response to *H. pylori* [30,31]. These hGOs were grown from single stem cells, and organoids could be directed into the four different lineages of the stomach (pit mucous cells, gland mucous cells, chief cells, and enteroendocrine cells), reflecting normal attributes of the human stomach. This system demonstrated long-term (>1 year) cultivation without loss of essential features, and organoids could be frozen and thawed similar to cell lines, characteristics that permit studies of long duration, and the ability to culture equivalent organoids for later experiments [30,31]. *H. pylori*-infected hGOs demonstrated robust NF- κ B activation, revealing the capacity of this hGO model to mount an innate inflammatory response to *H. pylori*, as is seen in the human host. Interleukin (IL)-8 expression in hGOs was independent of bacterial viability, the bacterial cytotoxicity associated gene pathogenicity island (*cagPAI*), and of TLR4, 5, and 9 signalling [30], unlike earlier studies that showed *cagA*-dependent activation of NF- κ B in transformed human epithelial cell lines [35–37]. The differential findings related to *cagA* and the inflammatory response from these two model systems highlights the changing landscape of information arising with the use of physiologically relevant tissue models.

Recently, hGOs developed with gastric glands isolated from normal human stomach tissue were used to identify the mechanism whereby *H. pylori* locates the gastric epithelia prior to infection (Fig. 1 photo inset shows an inside-in polarity hGO) [33]. Inside-out polarity hGOs allowed access to apical surfaces; bacteria were observed swimming towards organoids and adhering to tight junctions. These studies revealed that *H. pylori* detects minute quantities of urea metabolite via its chemoreceptor TlpB and rapidly responds in a chemotactic-dependent manner; TlpB is a high affinity chemoreceptor that binds urea directly. Importantly, the pathogen's virulence factor urease plays a role in chemosensation. Urea concentration in human stomach ranges from 1–5mM, but TlpB detects urea in nanomolar quantities, suggesting that bacterial urease, which lowers urea concentrations, might be connected to urea sensing. Thus, the hGOs produced urea and the TlpB chemoreceptor was sufficiently sensitive to detect it in the minute quantities that diffused from the organoid epithelia, and the use of this novel model revealed a chemoreception system linked to a known virulence factor [31,33].

More recently, organoids were generated from gastric glands isolated from healthy human stomach tissue and shown to recapitulate aspects of normal gastric physiology [32]. Sheared gastric organoid cultures inoculated with a CagA+ *H. pylori* strain exhibited translocation and phosphorylation of CagA, as well as the hallmark 'hummingbird phenotype' of infection. A robust inflammatory response was observed via microarray analyses; here too, highly upregulated genes were associated with the NF- κ B signaling pathway, highlighting another model that can potentially define novel mechanisms of gastric disease pathogenesis.

Intestinal Models—Human intestinal culture systems representing compartments throughout the intestine have been developed and used to study normal human gastrointestinal physiology as well as pathophysiology after infection with viral and bacterial pathogens [38–47]. A Transwell® system was used to establish cultures (called spheroids, but these would be called enteroids per the ISSC nomenclature proposal [10])

from a patient-specific panel of intestinal crypt units from biopsy specimens [38]. These cultures displayed a relatively rapid differentiation rate (~2–3 weeks) well suited to personalized medicine. Banked cultures include lines from healthy patients, patients with inflammatory diseases, and patients with prior intestinal surgery. Duodenal, ileal, and rectal cultures retained regional-specific cell markers, confirming other studies showing small intestinal adult stem cells are intrinsically programmed to recapitulate location-specific function [44]. For *in vitro* studies of physiological processes, the ileal and rectal enteroid cultures were dissociated and seeded onto coated Transwell® membranes to form polarized epithelial monolayers, which were shown to retain their complex multicellular phenotype and to possess a mucus layer and barrier function based on measuring transepithelial resistance. Adherence of diarrheagenic *Escherichia coli* (EC) strains [enteroaggregative (EaggEC), enteropathogenic (EPEC), and enterohemorrhagic (EHEC)] and a nonadherent EC control strain were compared in 2D cultures with HeLa cell monolayers. The control strain did not adhere to any cell line; EPEC and EaggEC adhered to ileal and rectal epithelial cells in patterns similar to those in HeLa cells; diffuse EHEC adherence was visualized only in ileal and rectal cell cultures. The latter result suggests that primary human epithelial enteroids are more suitable for adherence assays of diarrheagenic *E. coli*. An unexpected finding was that EPEC, a small bowel pathogen, adhered to rectal epithelial cells, suggesting there may be a role for this bacterial pathogen in the colonic pathology occasionally reported in patients with EPEC or EAggEc who have bloody diarrhea [38]. The ability to make monolayer enteroid cultures facilitates infections with many enteric pathogens and commensals [25,38,41]. Most importantly, the ability to bank host-specific tissue-derived enteroids supports patient-directed experimental approaches that not only are an important step toward the goal of personalized medicine, but also provide the potential to dissect the impact of host genotype on host-pathogen interactions and deleterious sequela.

Another novel 3D *in vitro* model of the human large intestine was bioengineered to mimic the structure and function of native intestine [39]. Modular intestinal constructs comprised functional intestinal epithelium cultured on silk-based protein scaffolds with hollow lumens; primary human intestinal myofibroblasts were seeded within scaffold bulk spaces, and human enterocyte-like and goblet-like cells from the cancer-derived Caco-2 and HT29-MTX cell lines were cultured on the surface of scaffold lumens. To explore whether cells in the hollow lumens would produce oxygen profiles different from the same cells grown on open surfaces of Transwells®, an oxygen probe was used to quantify oxygen levels. Unlike confluent 2D monolayers, which maintained an oxygen tension of 13%, cells lining interior surfaces of 3D scaffolds experienced decreased oxygen levels that mimicked *in vivo* conditions and exhibited depth-graded oxygen profiles in the luminal direction that ranged from microaerobic to anaerobic to anaerobic conditions. The use of wild type pathogenic *Yersinia pseudotuberculosis* strains engineered as oxygen-sensing reporters confirmed the presence of low oxygen levels in 3D models, and interactions with these enteric pathogens supported the feasibility of using this 3D system in pathogenesis studies. Further, co-culture of a probiotic *Lactobacillus rhamnosus GG* strain with luminal epithelial cells demonstrated that these novel models can be colonized by pathogens and may also support gut microbiota growth. This 3D system was viable for months, although tissue functions decreased a few weeks post-seeding, a limitation that might be overcome in a perfusion bioreactor system

[39]. This model has not yet been reported with nontransformed human enteroid or organoid cultures.

Human intestinal enteroid (HIE) cultures represent a novel model that recapitulates properties of human rotavirus (HRV) infection [40], models small intestinal pathophysiology and ion transport physiology [43], and potentially aids in personalized medicine, as clinical samples from individuals retain genotypic and phenotypic characteristics [25,40,45]. HIEs were established with tissue samples from the duodenum, jejunum, and ileum of different individuals [40]. HIEs adopted either multilobular or cystic morphologies and comprised a single, continuous lumen surrounded by epithelial cells that could be differentiated into absorptive enterocytes, enteroendocrine cells and goblet cells. Undifferentiated and differentiated HIEs contain Paneth cells within the stem cell niche [25]. Functional relevance of these models was demonstrated first by robust replication of HRV strains, with fewer cells infected by animal rotavirus strains, which corresponds to the host range specificity of HRV. In addition, HRV was found to replicate in differentiated enteroids in both enterocytes and enteroendocrine cells, which is consistent with a proposed model in which rotavirus stimulates serotonin production/release from enteroendocrine cells to activate the enteric nervous system [48]. HRV-infected HIEs displayed structures involved in the viral replication cycle (viroplasm and lipid droplets), as well as virus-induced swelling (mimicking fluid secretion during diarrhea) after viral infection or exposure to the rotavirus enterotoxin. Importantly, viral replication of RV1 vaccine strain was attenuated in all HIEs from secretor-positive patients, providing the first *in vitro* demonstration of RV1 attenuation [40]. Differential replication in HIEs from different patients supports the utility of these cultures to understand genetically-determined host differences in susceptibility and resistance to infection. Clinical isolates of HRV can be cultured in both HIEs and iPSC-derived organoids, and antivirals (ribavirin, interferon- α) can be evaluated [49,50]. HIEs also were used to examine ion transport, and key ion transporters were expressed and localized similarly to human intestine. A duodenal enteroid model showed that cAMP stimulated duodenal HCO_3^- secretion; that CFTR, the cystic fibrosis transmembrane conductance regulator, and NBCe1, the electrogenic $\text{Na}^+/\text{HCO}_3^-$ cotransporter 1, are essential transporters for HCO_3^- transport; and that NBCe1 might be a target for treatment of secretory diarrheas [43]. A novel finding from these studies was the finding that Na absorption in villus and colonic surface and upper crypt cells and anion secretion in crypt cells may not be as restricted as previously thought.

Human enteroids derived from ileal or colonic biopsies from blood type O or blood type A patients were generated to model and elucidate clinical and epidemiological associations of blood group O with the severity of cholera [51]. Enteroid enterocytes expressed blood group antigens appropriately on their cell surface, and cholera toxin (CT) treatment of enteroids from type O patients elicited higher levels of cAMP, a known cellular response to CT, compared to enteroids from type A patients. *Vibrio cholerae* infects the small intestine, but both ileal- and colonic-derived enteroids responded to CT with cAMP activation. These data reinforce the observed correlation between severe cholera disease in individuals with blood type O, and again support the use of human enteroids to model bacterial infections with the potential to gain mechanistic insight into pathogenesis. [51]

Human Colonoid Monolayer cultures (HCMs) derived from proximal colon were used to model Shiga toxin-producing EHEC infection [41]. Cultures of 3D Matrigel-grown colonoids were converted into 2D monolayers for access to apical and basolateral surfaces during studies of EHEC-epithelial interactions. HCM differentiation was characterized by the appearance of mucus-covered brush borders (BB) with numerous intermicrovillar bridges and tightly packed microvilli. Differentiated HCM were colonized by higher numbers of EHEC than undifferentiated HCM, and EHEC seemed to target the mucus layer. Reduction of mucus layer thickness and attachment of EHEC to glycocalyx remnants suggests that the colonic mucus layer might promote initial colonization. Sites of attachment to the epithelium displayed apical and basolateral membrane perturbation with characteristic formation of actin pedestals and attaching and effacing lesions. Mucin 2, a main component of colonic mucus, and Protocadherin 24 (PCDH24), an essential building block of intermicrovillar bridges, were targeted at early stages of infection, and EHEC-secreted EspP serine protease initiated BB damage through reduction of PCDH24. These data reflected and confirmed earlier findings with *in vitro* and *in vivo* models. Converting and culturing 3D colonoids into 2D monolayers enabled mechanistic pathogenesis studies, suggesting this approach represents a “next-generation” gut model system [38,42,45].

Human Intestinal Organoids (HIOs) that resembled the human proximal colon and derived from hPSCs were used to delineate further results obtained from patients with *Clostridium difficile* infections (CDI) [23,52–54]. Studies with clinical isolates of *C. difficile*, fecal samples, colonic biopsies and surgical resections investigated multiple aspects of CDI pathogenesis. *C. difficile* toxin B production inhibited expression of the Na⁺/H⁺ exchanger isoform 3 (NHE3), which led to elevated stool [Na⁺] and more alkaline fecal pH; these alterations in the intestinal tissue milieu were associated with changes in the gut microbiota, i.e., decreased presence of Firmicutes and increases in both Bacteroidetes and Proteobacteria. Microinjection of HIOs with *C. difficile* or CDI stool supernatant (or fecal supernatant from healthy subjects) confirmed that NHE3 inhibition was specific to *C. difficile* [52]. Additional studies from the same group with the same specimen types found that intestinal mucus in CDI patients is more acidic, consists primarily of MUC1 with decreased MUC2 expression, and a presumable mucus barrier defect. The composition of oligosaccharides in intestinal mucus was also changed, with GlcNAc and galactose increased and decreased levels of GalNAc. HIOs injected with *C. difficile* alone showed decreased MUC2 production, but injection of CDI stool supernatant was necessary to change mucus oligosaccharide levels, suggesting that another factor, possibly related to the changes observed in the gut microbiota, was required. Thus, the HIO model not only replicated results obtained with human specimens, but also elucidated finer details of CDI pathogenesis [53]. Microinjection and colonization with *C. difficile* or its toxin TcdA, but not toxin TcdB, also resulted in disruption of the epithelial paracellular barrier, which contributes new insight into bacterial and toxin pathogenesis [54].

Human intestinal organoids (iHOs) cultured from human iPSCs were tested as a model for studies of *Salmonella enterica* Serovar Typhimurium pathogenesis. Microinjection of this microbial pathogen resulted in *Salmonella* invasion of the organoid, and formation of *Salmonella*-containing vacuoles. A *Salmonella* invasion-defective mutant was unable to invade, illustrating the ability to assess the pathogenesis of different bacterial mutants. RNA

sequencing characterized significantly changed iHO gene expression; upregulation (1448 genes) and downregulation (577 genes) of host transcription provided the first information about the early innate human epithelial response to bacterial infection with altered cytokine expression predominating. Thus, this iHO infection model demonstrated established steps of *Salmonella* pathogenesis and provided clues about how epithelial cells may participate in defense against this infection [55].

The importance of building more complex models of the intestine is well recognized and a human gut-on-a-chip microdevice shows potential to analyze how gut microbiome, inflammatory cells and peristalsis-associated mechanical motion contribute to intestinal infection and inflammation [46,47]. A model of bacterial overgrowth established in a microengineered device showed that immune cells and endotoxin stimulate a proinflammatory response that induced villus injury and compromised intestinal barrier function. Lack of motion also contributed to bacterial overgrowth [47]. The application of this system to human intestinal organoid cultures is of high interest.

These gastrointestinal models are beginning to be utilized to test antimicrobial drugs and therapeutics; a recent publication illustrates the feasibility of testing drugs in tissue models. Multiple model systems were utilized, including small intestinal organoids generated from mouse intestinal crypts and human duodenal organoids generated from human biopsies; both mammalian intestinal organoid models exhibit increased growth when treated with recombinant mouse or human interleukin-22 (IL-22) after tissue damage. Recombinant IL-22 treatment targeted intestinal stem cells (ISCs) in the organoids, subsequently increasing proliferation and expanding ISCs. These and other findings suggest that components of the mammalian immune system play important roles in restoring intestinal epithelial barrier function, which is a critical host defense [56]. Other studies have shown that high throughput drug screening can be done using enteroids from colorectal carcinomas; these studies demonstrated the role of Wnt signaling in colorectal cancer (CRC) development and the potential for Wnt inhibition as a treatment option in a subset of CRC patients [45,57]. Importantly, enteroids exhibited a range of sensitivities to the drugs, and compounds with the same molecular targets showed similar activity profiles. These studies demonstrated that patient-derived enteroids could be used for high-throughput drug screening to test the efficacy of clinically available drugs and to identify new therapeutic targets in a “personalized or precision medicine” approach. This may be important for antimicrobial drugs where increasing genetic susceptibilities to infectious disease are being recognized.

Kidney

A 3D human renal model provided the first assessment of damage to kidneys caused by Shiga toxin and new insight into hemolytic uremic syndrome (HUS), characterized by renal failure, hemolytic anemia, and thrombocytopenia [58]. Shiga toxin and its variants are bacterial cytotoxins secreted by *Shigella dysenteriae* and the Shiga toxin-producing *E. coli* during bacterial intestinal infection. HUS is a serious sequela, especially in young children. No animal models of infection reproduce HUS or the various diarrheal diseases caused by the human-specific shigellae. Thus, the mechanism(s) whereby Shiga toxin, which gains

access to the circulatory system after damaging intestinal epithelium, injures human kidneys are unknown. The 3D model encompasses immortalized renal proximal tubule cells combined with a collagen I - Matrigel mixture cultured in Transwells®. Upon exposure to purified, LPS-free Shiga toxin (Stx2), the 3D model and renal cells grown in 2D displayed similar dose-dependent protein synthesis inhibition. Stx2-induced cytotoxicity was demonstrated in both systems, and Stx2 treatment of 3D tissue resulted in elevated levels of the kidney injury marker 1 (Kim-1) compared to treated 2D cultures. Inflammatory cytokine secretion was seen in Stx2-treated 3D and 2D models with increasing levels of IL-6, IL-8, and TNF- α , despite toxin-induced protein synthesis inhibition in cells, which seemed consistent with Shiga toxin's capacity to induce protein production via activating translational regulation pathways. This is the first use of a 3D bioengineered human kidney tissue model to study renal damage by Stx2, and results imply that Kim-1 might be an early biomarker of renal injury. The cellular complexity and functional diversity of the kidney are substantial challenges to achieving functional maturation of the organ [59]. However, progress is being made on individual kidney cell types such as the proximal tubule [60] that can be used for initial studies of the response of nontransformed human kidney cells to infection.

Liver

3D and 2D *in vitro* human liver models were established that are applicable to basic science studies of hepatotropic pathogens and translational research to facilitate product development [61–67]. Human 3D hepatic sinusoid biomimetics were derived from cultures of liver sinusoidal endothelial cells (LSEC) and Huh-7 hepatoma cells [or Huh7-NTCP hepatocytes that stably express the HBV receptor sodium taurocholate co-transporter polypeptide (NTCP)] within a collagen 1 matrix. These cultures were used as infection models for the protozoan parasite *Entamoeba histolytica*, a human-only pathogen that causes amoebiasis, and hepatitis B virus (HBV), which causes chronic infections that can lead to cirrhosis and liver cancer [61,62]. Four variations of layered models were built with the scaffold and established cell lines in order to study aspects of pathogen invasion, i.e. migration, hepatocyte barrier crossing, endothelial and hepatocyte barrier crossing, and human HBV replication quantification. These easily assembled models combined simplicity and reproducibility with enhanced physiological relevance compared to standard 2D cultures. LSECs were essential for matrix architecture, hepatocyte differentiation, and proinflammatory responses. *E. histolytica* infection of the construct comprised of single layers of LSECs and hepatocytes in a collagen matrix demonstrated, for the first time, dependence of amoebic Gal/GalNAc lectin for endothelial crossing and a crucial role for human galectins in parasite adhesion to endothelia. Additionally, galectins-1 and -3 stimulated pro-inflammatory cytokine release. Protozoan infection of a single hepatocyte layer construct showed directional migration; galactose, a known inhibitor of *E. histolytica* adhesion, reduced migration distance. Amoebae behavior in a collagen-only model showed that hepatocytes were needed to maintain protozoan viability, correlating with earlier findings that hepatic cells alter medium composition and scaffold structure and composition. Infection of a double hepatocyte/single LSEC layered construct, representing liver sinusoid endothelial and hepatocyte barriers, revealed amoebae squeezing between hepatic cells to cross that barrier. HBV infection of tissue comprised of single LSEC and hepatocyte (Huh7-

NTCP cells) layers showed viral replication in the absence of PEG-8000, necessary in standard HBV infection protocols; replication rates were comparable to those seen in conventional 2D cultures. The 3D model demonstrated that HBVx protein is required for productive infection, as in 2D cultures and *in vivo* experiments [61,62].

Micropatterned cocultures (MPCCs) of primary human hepatocytes organized into microspheroid-like 2D islands surrounded by mouse stromal cells produced miniature *in vitro* models used to study infections caused by hepatitis C virus (HCV) and HBV [63,65,66]. MPCCs express all the required factors for HCV entry and support HCV infection for several weeks [66]. MPCCs enabled sustained expression of HBV NTCP receptor and supported productive HBV infection throughout the 3 week long culture. HBV infection was enhanced by inhibition of the innate immune pathway via treatment with the Janus kinase inhibitor (JAKi) that diminishes expression of Interferon (IFN)-stimulated genes or an inhibitor of the TANK-binding kinase 1 (TBK1), an upstream activator of the IFN response pathway. MPCCs also were used as anti-HBV drug-testing tools in experiments with the HBV reverse transcriptase inhibitor entecavir and IFN- β , an alternative antiviral [65]. Thus, the MPCC platform potentially can be used for drug and vaccine development. Newer models to generate tissue-derived epithelial organoids from adult human liver bile duct cells demonstrated long-term viability and improved hepatocyte function when compared to the standard/reference HepG2 cell line [68]. This model can produce cultures from diverse patients but comparisons of these cultures for their ability to support hepatitis virus replication remains to be reported.

Studies designed to generate metabolically active hepatocyte cells from hPSCs [HES2 human embryonic stem cell line (hESC)] identified key steps in hepatocyte maturation. Maturation of hESC-derived hepatocyte-like cells required an initial aggregation step, which increased the expression of genes associated with liver function, epithelial characteristics, such as E-Cadherin, and the hepatocyte cell surface marker asialo-glycoprotein receptor 1 (ASGR1). Cyclic adenosine monophosphate (cAMP) signaling constituted the second step and induced changes associated with increased metabolic activity, reduction in fetal gene expression and concomitant increase in adult gene expression, and global expression of changes that indicate hepatocyte maturation [67]. Development of a strategy for production of metabolically active mature hepatocytes from hPSCs holds great promise for numerous applications, including their use in human liver tissue mimics. Other groups have shown that human induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (iHLCs) can serve as *in vitro* models for liver-stage malaria infection [64]. iHLCs express genes encoding malaria parasite CD81 and SRB1 host entry factors for liver-stage disease, and their proteins localize appropriately on cell surfaces. Differentiated iHLCs were phenotypically stable and could be infected with human-specific *Plasmodium falciparum* parasite sporozoites up to 55 days after differentiation initiation. Human and rodent (*P. berghei* and *P. yoelii*) malaria parasite sporozoites infect iHLCs, and these *in vitro*-derived host cells supported growth and maturation of EEFs (HSP70-expressing exoerythrocytic forms) [64].

Lung

Human 3D lung tissue models were developed and used to study *Mycobacterium tuberculosis*, staphylococcal pneumonia, and Andes Hantavirus pathogenesis [69–71]. Indeed, the adaptability of these human lung tissue models to studies of multiple pathogens, both viral and bacterial, suggests their potential for broad application and impact on the field. One model consisted of type 1 collagen-embedded human lung fibroblasts cultured on porous membranes followed by the introduction of uninfected human primary monocytes and *M. tuberculosis*-infected human primary macrophages. Human bronchial epithelial cells were then seeded on top of the collagen-fibroblast matrix; this multi-cell tissue was subsequently exposed to air to initiate production of extracellular matrix proteins, mucus secretion, and stratification of the epithelial layer. This lung infection model displays clustering of macrophages and migration of monocytes into the tissue at the site of infection to yield a granuloma-like formation. Although the authors cite limitations such as the use of cell lines, the absence of vascularization, and the lack of neutrophils and lymphocytes, the model does exhibit the early stages of TB granulomas [69].

A similar 3D human lung tissue construct was used to study staphylococcal pneumonia pathogenesis; it was generated on Transwell® inserts with lung fibroblasts in a collagen mixture as a first layer, followed by seeding of bronchial epithelial cells, and subsequent air exposure [70,72]. *Staphylococcal aureus* strains from patients with severe pneumonia, including necrotizing pneumonia and lung empyema, were used here, as were bacterial supernatants and purified α -toxin; supernatants contained pore-forming α -toxin and PVL (Panton-Valentine leukocidin) cytotoxins. Supernatants from necrotizing pneumonia and lung empyema strains were highly cytotoxic to primary human neutrophils; only the former demonstrated severe toxicity to lung epithelial cells. Lung tissue models responded similarly to bacterial supernatants; the necrotizing pneumonia isolates secreted high levels of α -toxin and PVL and caused significant α -toxin-mediated lung epithelium damage, PVL-mediated neutrophil cytotoxicity, and inflammation via toxin-mediated release of cytokines and chemokines in lung epithelia. Toxin-mediated damage was inhibited by polyspecific intravenous immunoglobulin that contained antibodies to α -toxin, PVL, and other factors present in the clinical isolate supernatants [70].

To study Andes virus (ANDV), which causes hantavirus pulmonary syndrome (HPS), an analogous organotypic lung tissue model was developed that was comprised of a collagen-coated porous membrane with sequential cultivation of, first, a mixture of human lung fibroblast cells (MRC-5 cell line) in collagen and, second, a layer of human bronchial epithelial cells (16HBE14o- cell line), which was then air exposed. ANDV was applied to the apical, or air-exposed, surface, and viral replication was observed with a peak of progeny virus occurring ~ 1 week post-infection (p.i.). Moreover, the capacity of the tissue model to sustain a long-term viral infection (1 month p.i.) was demonstrated by the presence of infected cells and progeny virus production. Peak production of progeny virus was coincident with the induction of innate immune responses as detected by increased expression of IFN- λ 1, IFN- λ 2, IFN- β , and the IFN-stimulated gene ISG56 mRNAs with later production of the proinflammatory cytokines IL-6 and IL-8 ~ 3 weeks p.i. Thus, a transient antiviral response preceded a proinflammatory response that might reflect the

clinical course of asymptomatic infection transitioning to an inflammatory state, which might parallel the development of HPS. Of crucial importance was the capacity of the 3D model to enable *in vitro* studies not permissible in cell monolayers as well as studies of infection over a long period of time [71].

Skin

3D skin equivalent models would represent another critical advance for infectious diseases research; recently, human skin tissue models have been developed and used to study both bacterial and viral infections. An *in vitro* herpes simplex virus type 1 (HSV-1) skin model was developed for studies of viral latency and reactivation that might provide insights not permitted via traditional cell culture monolayers. This skin mimic incorporated the human keratinocyte cell line HaCaT (epidermal layer) grown on a collagen substrate containing human primary fibroblasts (dermal layer); a critical improvement was addition of a component designed to mimic further the *in vivo* environment, i.e., a quiescent HSV-1 infected neuronal equivalent (latency component). Epidermal stratification and epidermal-dermal interactions were attained via cultivation at an air-liquid interface. The authors described successful reactivation of latent virus via UVB-light with a caveat about reproducibility issues that may be resolved by optimizing infection conditions. Importantly, this skin model shows potential for investigating mechanisms related to latency and possibly new therapeutics [73]. A related organotypic skin tissue model, also comprised of stratified epithelium and a fibroblast dermal layer, was used to investigate the pathogenesis of *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) strains, three of which were invasive strains that cause necrotizing soft tissue infections and one non-invasive strain. All SDSE strains were able to infect this model with bacterial loads resembling those seen in tissue biopsies also utilized in this study, but the invasive strains disseminated throughout the epithelium and caused substantial tissue damage evidenced by disruption and detachment of the epithelium [74].

Conclusions and Future Directions

Microphysiological systems such as modular human organs-on-a-chip and integration of organs into a human-on-a-chip are under development [21,22,46,47,75]. These chips have great potential to facilitate product development; their use in pharmacokinetic and toxicology assays can provide more accurate predictors of clinical trial outcomes. The goal of integrating modules to understand physiologically relevant interactions is essential, although a common culture medium and optimized platforms to accommodate needs of each organ system for integrated modules are yet to be developed.

Expertise and methodologies afforded to infectious diseases researchers working with experienced bioengineers conversant in areas such as biomaterials, cell sources, microtissue platforms, and nondestructive imaging catalyze development of urgently needed models for host-pathogen interactions studies. There have been many advances as summarized above, but there is more to be accomplished (Key Advances and Challenges). Limitations are acknowledged by the authors for many of the models presented here; limitations include the lack of mechanical or force stimulation, such as peristalsis; vascularization, i.e., to simulate

blood and/or lymph circulation; innervation in the non-neural models; and components of the immune system, such as the mucosal-associated lymphoid tissue and circulating or resident immune cells; to name a few crucial features of a whole body system. A practical limitation is the current cost of these systems. Research is progressing to address these limitations but the full complexity and maturity of tissues remains to be achieved. Nonetheless, the existing models provide sufficient features to enable discovery of new insights in microbial pathogenesis and host interactions. These models demonstrate the need to balance simplicity and ease of use for basic science research with the recapitulation of human tissue architecture in organotypic model systems for preclinical therapeutic and vaccine studies. Generation of specialized tissues with multiple cell types in precise geometric locations that engender a high level of spatial organization would provide optimal biomimetic models that could asymptotically approach organogenesis. Here, the development of an *in vivo*-like restricted O₂ gut microenvironment [39]; the identification of pathways, such as the new role for EGF signaling [29]; the capacity for long-term cultivation without loss of architectural features, function, and cellular markers in some models; and the remarkable similarity to organogenesis observed in many constructs point to the feasibility of developing 3D models of infection that more closely mimic human physiology and human diseases pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Seok J, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A*. 2013; 110(9):3507–3512. [PubMed: 23401516]
2. Warren HS, et al. Mice are not men. *Proc Natl Acad Sci U S A*. 2015; 112(4):E345. [PubMed: 25540422]
3. Bolker J. Model organisms: There's more to life than rats and flies. *Nature*. 2012; 491(7422):31–33. [PubMed: 23128209]
4. Martic-Kehl MI, et al. Can animal data predict human outcome? Problems and pitfalls of translational animal research. *Eur J Nucl Med Mol Imaging*. 2012; 39(9):1492–1496. [PubMed: 22790876]
5. Langer R, Vacanti JP. Tissue engineering. *Science*. 1993; 260(5110):920–926. [PubMed: 8493529]
6. Birkness KA, et al. A tissue culture bilayer model to study the passage of *Neisseria meningitidis*. *Infect Immun*. 1995; 63(2):402–409. [PubMed: 7822003]
7. Kerneis S, et al. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science*. 1997; 277(5328):949–952. [PubMed: 9252325]
8. Nickerson CA, et al. Three-dimensional tissue assemblies: novel models for the study of *Salmonella enterica* serovar Typhimurium pathogenesis. *Infect Immun*. 2001; 69(11):7106–7120. [PubMed: 11598087]
9. Dedhia PH, et al. Organoid Models of Human Gastrointestinal Development and Disease. *Gastroenterology*. 2016; 150(5):1098–1112. [PubMed: 26774180]

10. Stelzner M, et al. A nomenclature for intestinal in vitro cultures. *Am J Physiol Gastrointest Liver Physiol.* 2012; 302(12):G1359–G1363. [PubMed: 22461030]
11. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp Biol Med (Maywood).* 2014; 239(9):1061–1072. [PubMed: 25187571]
12. Ramanan V, et al. New Methods in Tissue Engineering: Improved Models for Viral Infection. *Annu Rev Virol.* 2014; 1:475–499. [PubMed: 25893203]
13. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science.* 2014; 345(6194):1247125. [PubMed: 25035496]
14. Chwalek K, et al. In vitro bioengineered model of cortical brain tissue. *Nat Protoc.* 2015; 10(9): 1362–1373. [PubMed: 26270395]
15. Tang-Schomer MD, et al. Bioengineered functional brain-like cortical tissue. *Proc Natl Acad Sci U S A.* 2014; 111(38):13811–13816. [PubMed: 25114234]
16. Lancaster MA, et al. Cerebral organoids model human brain development and microcephaly. *Nature.* 2013; 501(7467):373–379. [PubMed: 23995685]
17. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc.* 2014; 9(10):2329–2340. [PubMed: 25188634]
18. Dang J, et al. Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3. *Cell Stem Cell.* 2016
19. Garcez PP, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science.* 2016; 352(6287):816–818. [PubMed: 27064148]
20. Qian X, et al. Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. *Cell.* 2016; 165(5):1238–1254. [PubMed: 27118425]
21. Brown JA, et al. Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor. *Biomicrofluidics.* 2015; 9(5):054124. [PubMed: 26576206]
22. Alcendor DJ, et al. Neurovascular unit on a chip: implications for translational applications. *Stem Cell Res Ther.* 2013; 4(Suppl 1):S18. [PubMed: 24564885]
23. Leslie JL, Young VB. A whole new ball game: Stem cell-derived epithelia in the study of host-microbe interactions. *Anaerobe.* 2016; 37:25–28. [PubMed: 26549696]
24. Wells JM. Regenerative medicine in 2015: Generating and regenerating the digestive system. *Nat Rev Gastroenterol Hepatol.* 2016; 13(2):65–66. [PubMed: 26758784]
25. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science.* 2013; 340(6137):1190–1194. [PubMed: 23744940]
26. Zachos NC, et al. Human Enteroids/Colonoids and Intestinal Organoids Functionally Recapitulate Normal Intestinal Physiology and Pathophysiology. *J Biol Chem.* 2016; 291(8):3759–3766. [PubMed: 26677228]
27. Sinagoga KL, Wells JM. Generating human intestinal tissues from pluripotent stem cells to study development and disease. *Embo j.* 2015; 34(9):1149–1163. [PubMed: 25792515]
28. Sato T, Clevers H. SnapShot: Growing Organoids from Stem Cells. *Cell.* 2015; 161(7) 1700-1700.e1701.
29. McCracken KW, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature.* 2014; 516(7531):400–404. [PubMed: 25363776]
30. Bartfeld S, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology.* 2015; 148(1):126–136. e126. [PubMed: 25307862]
31. Bartfeld S, Clevers H. Organoids as Model for Infectious Diseases: Culture of Human and Murine Stomach Organoids and Microinjection of Helicobacter Pylori. *J Vis Exp.* 2015; (105)
32. Schlaermann P, et al. A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. *Gut.* 2016; 65(2):202–213. [PubMed: 25539675]
33. Huang JY, et al. Chemodetection and Destruction of Host Urea Allows Helicobacter pylori to Locate the Epithelium. *Cell Host Microbe.* 2015; 18(2):147–156. [PubMed: 26269952]
34. Bertaux-Skeirik N, et al. Co-culture of Gastric Organoids and Immortalized Stomach Mesenchymal Cells. *Methods Mol Biol.* 2016; 1422:23–31. [PubMed: 27246019]
35. Ferrero RL. Innate immune recognition of the extracellular mucosal pathogen, Helicobacter pylori. *Mol Immunol.* 2005; 42(8):879–885. [PubMed: 15829277]

36. Bartfeld S, et al. High-throughput and single-cell imaging of NF-kappaB oscillations using monoclonal cell lines. *BMC Cell Biol.* 2010; 11:21. [PubMed: 20233427]
37. Fischer W, et al. Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol.* 2001; 42(5):1337–1348. [PubMed: 11886563]
38. VanDussen KL, et al. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. *Gut.* 2015; 64(6):911–920. [PubMed: 25007816]
39. Chen Y, et al. Robust bioengineered 3D functional human intestinal epithelium. *Sci Rep.* 2015; 5:13708. [PubMed: 26374193]
40. Saxena K, et al. Human Intestinal Enteroids: a New Model To Study Human Rotavirus Infection, Host Restriction, and Pathophysiology. *J Virol.* 2015; 90(1):43–56. [PubMed: 26446608]
41. In J, et al. Enterohemorrhagic *Escherichia coli* reduce mucus and intermicrovillar bridges in human stem cell-derived colonoids. *Cell Mol Gastroenterol Hepatol.* 2016; 2(1):48–62. e43. [PubMed: 26855967]
42. Tyska MJ. Brush Border Destruction by Enterohemorrhagic *Escherichia coli* (EHEC): New Insights From Organoid Culture. *Cellular and Molecular Gastroenterology and Hepatology.* 2(1): 7–8.
43. Foulke-Abel J, et al. Human Enteroids as a Model of Upper Small Intestinal Ion Transport Physiology and Pathophysiology. *Gastroenterology.* 2016; 150(3):638–649. e638. [PubMed: 26677983]
44. Middendorp S, et al. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. *Stem Cells.* 2014; 32(5):1083–1091. [PubMed: 24496776]
45. van de Wetering M, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell.* 2015; 161(4):933–945. [PubMed: 25957691]
46. Ingber DE. Reverse Engineering Human Pathophysiology with Organs-on-Chips. *Cell.* 2016; 164(6):1105–1109. [PubMed: 26967278]
47. Kim HJ, et al. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc Natl Acad Sci U S A.* 2016; 113(1):E7–E15. [PubMed: 26668389]
48. Hagbom M, et al. Towards a human rotavirus disease model. *Curr Opin Virol.* 2012; 2(4):408–418. [PubMed: 22722079]
49. Finkbeiner SR, et al. Stem cell-derived human intestinal organoids as an infection model for rotaviruses. *MBio.* 2012; 3(4) e00159-00112.
50. Yin Y, et al. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. *Antiviral Res.* 2015; 123:120–131. [PubMed: 26408355]
51. Kuhlmann FM, et al. Blood Group O-Dependent Cellular Responses to Cholera Toxin: Parallel Clinical and Epidemiological Links to Severe Cholera. *Am J Trop Med Hyg.* 2016
52. Engevik MA, et al. Human *Clostridium difficile* infection: inhibition of NHE3 and microbiota profile. *Am J Physiol Gastrointest Liver Physiol.* 2015; 308(6):G497–G509. [PubMed: 25552580]
53. Engevik MA, et al. Human *Clostridium difficile* infection: altered mucus production and composition. *Am J Physiol Gastrointest Liver Physiol.* 2015; 308(6):G510–G524. [PubMed: 25552581]
54. Leslie JL, et al. Persistence and toxin production by *Clostridium difficile* within human intestinal organoids result in disruption of epithelial paracellular barrier function. *Infect Immun.* 2015; 83(1):138–145. [PubMed: 25312952]
55. Forbester JL, et al. Interaction of *Salmonella enterica* Serovar Typhimurium with Intestinal Organoids Derived from Human Induced Pluripotent Stem Cells. *Infect Immun.* 2015; 83(7): 2926–2934. [PubMed: 25964470]
56. Lindemans CA, et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature.* 2015; 528(7583):560–564. [PubMed: 26649819]
57. Madan B, et al. Wnt addiction of genetically defined cancers reversed by PORCN inhibition. *Oncogene.* 2016; 35(17):2197–2207. [PubMed: 26257057]

58. DesRochers TM, et al. Effects of Shiga toxin type 2 on a bioengineered three-dimensional model of human renal tissue. *Infect Immun*. 2015; 83(1):28–38. [PubMed: 25312954]
59. Little MH. Growing Kidney Tissue from Stem Cells: How Far from "Party Trick" to Medical Application? *Cell Stem Cell*. 2016; 18(6):695–698. [PubMed: 27257757]
60. Kelly EJ, et al. Innovations in preclinical biology: ex vivo engineering of a human kidney tissue microperfusion system. *Stem Cell Res Ther*. 2013; 4(Suppl 1):S17. [PubMed: 24564863]
61. Petropolis DB, et al. A new human 3D-liver model unravels the role of galectins in liver infection by the parasite *Entamoeba histolytica*. *PLoS Pathog*. 2014; 10(9):e1004381. [PubMed: 25211477]
62. Petropolis DB, et al. Human Liver Infection in a Dish: Easy-To-Build 3D Liver Models for Studying Microbial Infection. *PLoS One*. 2016; 11(2):e0148667. [PubMed: 26863526]
63. March S, et al. Micropatterned coculture of primary human hepatocytes and supportive cells for the study of hepatotropic pathogens. *Nat Protoc*. 2015; 10(12):2027–2053. [PubMed: 26584444]
64. Ng S, et al. Human iPSC-derived hepatocyte-like cells support Plasmodium liver-stage infection in vitro. *Stem Cell Reports*. 2015; 4(3):348–359. [PubMed: 25660406]
65. Shlomai A, et al. Modeling host interactions with hepatitis B virus using primary and induced pluripotent stem cell-derived hepatocellular systems. *Proc Natl Acad Sci U S A*. 2014; 111(33):12193–12198. [PubMed: 25092305]
66. Ploss A, et al. Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures. *Proc Natl Acad Sci U S A*. 2010; 107(7):3141–3145. [PubMed: 20133632]
67. Ogawa S, et al. Three-dimensional culture and cAMP signaling promote the maturation of human pluripotent stem cell-derived hepatocytes. *Development*. 2013; 140(15):3285–3296. [PubMed: 23861064]
68. Huch M, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell*. 2015; 160(1–2):299–312. [PubMed: 25533785]
69. Braian C, et al. A 3D Human Lung Tissue Model for Functional Studies on Mycobacterium tuberculosis Infection. *J Vis Exp*. 2015; (104)
70. Mairpady Shambat S, et al. Modelling staphylococcal pneumonia in a human 3D lung tissue model system delineates toxin-mediated pathology. *Dis Model Mech*. 2015; 8(11):1413–1425. [PubMed: 26398950]
71. Sundstrom KB, et al. Andes Hantavirus-Infection of a 3D Human Lung Tissue Model Reveals a Late Peak in Progeny Virus Production Followed by Increased Levels of Proinflammatory Cytokines and VEGF-A. *PLoS One*. 2016; 11(2):e0149354. [PubMed: 26907493]
72. Nguyen Hoang AT, et al. Technical advance: live-imaging analysis of human dendritic cell migrating behavior under the influence of immune-stimulating reagents in an organotypic model of lung. *J Leukoc Biol*. 2014; 96(3):481–489. [PubMed: 24899587]
73. Hogk I, et al. 3D-tissue model for herpes simplex virus-1 infections. *Methods Mol Biol*. 2013; 1064:239–251. [PubMed: 23996262]
74. Siemens N, et al. Increased cytotoxicity and streptolysin O activity in group G streptococcal strains causing invasive tissue infections. *Sci Rep*. 2015; 5:16945. [PubMed: 26601609]
75. Fabre KM, et al. Organs-on-chips (microphysiological systems): tools to expedite efficacy and toxicity testing in human tissue. *Exp Biol Med (Maywood)*. 2014; 239(9):1073–1077. [PubMed: 24962171]

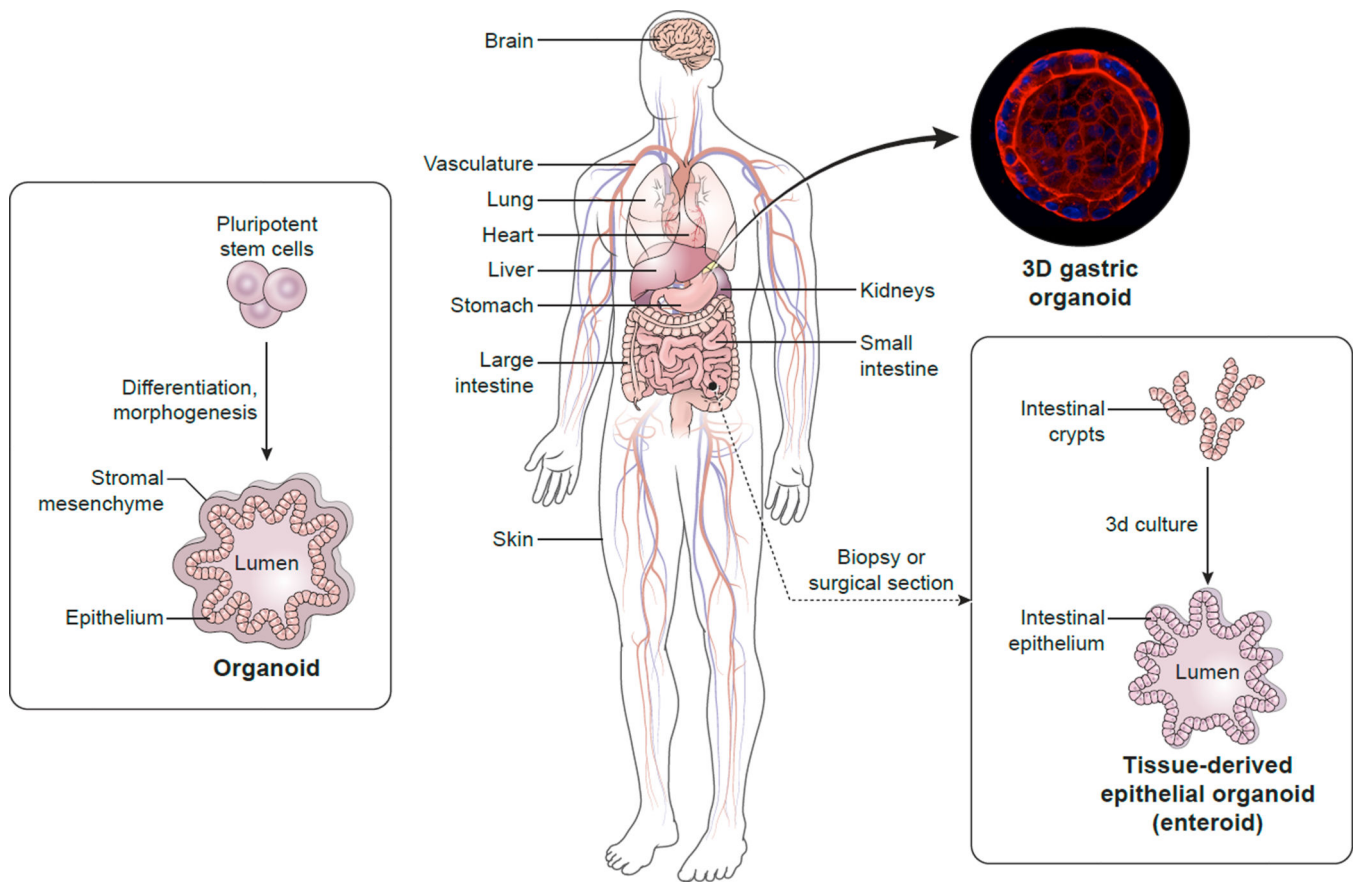


Figure 1. Generating human tissue biomimetics for studies of infectious diseases

(Left) Stem cells can undergo directed differentiation, morphogenesis, and, potentially, organogenesis to develop human organ-specific organoids. The organoid shown contains both mesenchymal and epithelial tissue with an open interior to which may be added microbes, immune cells or other substances, e.g., therapeutics. An alternative method uses biopsy or surgical sections to generate organoids comprised of epithelial tissue; the tissue-derived epithelial organoid shown (Right) shows the use of a small intestinal tissue sample to obtain intestinal crypts that ultimately form an “inside-out” enteroid with an outer epithelial layer and an interior lumen. The photo inset shows an “inside-in” polarity gastric epithelial organoid derived from human stomach tissue and stained for nuclei and the actin cytoskeleton. Permission to use this confocal reconstruction image was generously provided by Drs. Calvin J. Kuo and Manuel R. Amieva (Huang, J.Y. et al. (2015) *Cell Host Microbe* 18 (2), 147–156. See [33] in main reference list.

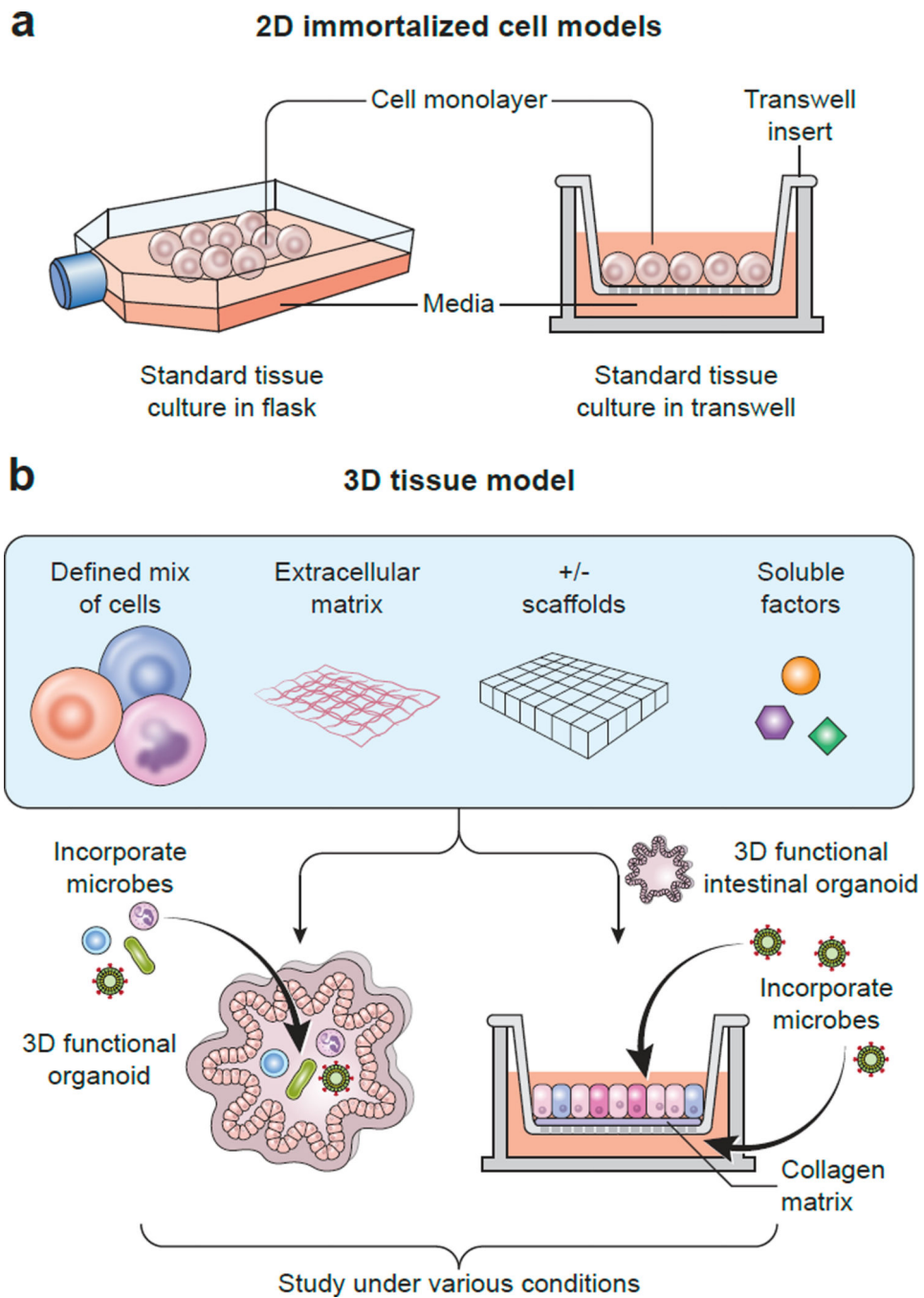


Figure 2. 2D and 3D tissue culture models differ in cell sources, additives, methods, and model complexity

Panel A. This illustration shows a classical 2D cell culture model for studies of infectious diseases. An immortalized cell line was cultured in flasks, cells were harvested, recombined with media, and then plated and grown as monolayers comprised of a single cell type in various cell culture dishes, e.g, microtiter plates. The Transwell™ culture system represented a technological advance. Cell monolayers can be assessed for polarization; and microbes, immune cells, or other substances can be added either to the apical or basolateral

side of the monolayer. **Panel B. Increased complexity of 3D tissue culture models is depicted in this schematic.** Cell mixtures contain different cell types; an extracellular matrix may consist of collagen or more complex materials, e.g., Matrigel; soluble factors, e.g., growth factors, are added to the growth medium; and these components may be cultured in the presence or absence of scaffolding materials, which can serve as structural guides for tissue development. (Left) Microbial organisms (commensals and/or pathogens) or immune cells, or other factors, can be added to functional 3D organoids, which then can be studied under various conditions, e.g., by differing the pH or oxygenation status, or by adding mechanical or electrical stimulation. (Right) 3D functional intestinal epithelial organoids, or enteroids, may be converted into 2D monolayers for culturing in Transwell™ systems; a matrix (matrigel or collagen) may be added to the porous membrane to serve as an extracellular matrix; pathogens or commensal microbes may be added (here shown as viruses added to medium in the apical and basolateral compartments); immune cells or other factors can be added, and then the model can be exposed to varying conditions, e.g., differing pH or oxygenation.

Table 1

Pathogenic Models: Characteristics and Features

Models	Cell Types	Matrix / Scaffold	Stability	Responses	References
BRAIN					
Cortical brain tissue	Primary cortical neurons (rat)	Collagen 1 and Silk-collagen protein	Weeks to months	Physiological: Axon growth Neuronal clusters/networks Neuronal gene expression Electrophysiological function Pharmacophysiological response Pathophysiological: Traumatic brain injury: Neuronal damage Impact-induced hyperactivity Increased Glu levels post-injury	Chwalek <i>et al.</i> 2015. <i>Nat Protoc</i> Tang-Schomer <i>et al.</i> 2014. <i>Proc Natl Acad Sci U S A</i>
Cerebral organoid	hPSCs ^a or iPSCs ^b or Patient-specific iPSCs (microcephaly) fibroblast feeder cells	Matrigel or Growth factor-reduced Matrigel	6–12 months	Physiological: Discrete, interdependent brain regions Fluid-filled ventricle-like structure Glutamatergic receptor activity Neural activity Pathophysiological: Microcephaly model: Smaller neural tissues Premature neural differentiation Neurogenic non-proliferative divisions Imprecise glial spindle orientation	Lancaster <i>et al.</i> 2013. <i>Nature</i> Lancaster and Knoblich. 2014. <i>Nature Protocols</i>
Brain organoid	iPSCs	Matrigel	Up to 10 months	Physiological: Mimics 1 st trimester development Pathophysiological: Zika virus infection: Growth rate reduction	Garcez <i>et al.</i> 2016. <i>Science</i> Lancaster <i>et al.</i> 2013. <i>Nature</i>
Forebrain organoids	iPSCs	Matrigel	Months	Physiological: Embryonic cortical development Markers for 6 cortical layers Complex neuronal morphology Neuronal maturation features Pathophysiological: Zika virus infection: (differing fetal stage models) Cell type tropism Decreased organoid size Increased lumen/ventricle size Reduced neuronal layer thickness	Qian <i>et al.</i> 2016. <i>Cell</i>
Cerebral organoid	hESC ^s ^c H9 hESC ^s (WA09) Irradiated mouse embryonic fibroblasts	Matrigel	Npd	Physiological: Complex morphology Fluid-filled ventricle-like structure Regional specificity	Dang <i>et al.</i> 2016. <i>Cell Stem Cell</i> Lancaster <i>et al.</i> 2013. <i>Nature</i>

Models	Cell Types	Matrix / Scaffold	Stability	Responses	References
STOMACH					
Gastric epithelial organoid	hPSCs	Matrigel	> 1 month	<p>Neural activity</p> <p>Mimics early trimester fetal tissue</p> <p>Pathophysiological: Zika virus infection (MR766 strain): Organoid shrinkage Increase in viral copy number Neurogenesis dysregulation Activation of apoptosis TLR3 network perturbation</p>	McCracken <i>et al.</i> 2014. <i>Nature</i>
Gastric epithelial organoid	Human adult tissues: (gastric glands from normal/cancerous stomach tissue)	Matrigel	> 1 year - may be frozen and thawed indefinitely	<p>Physiological: Recapitulate embryonic development Differentiated antral epithelial cells High degree of cellular complexity</p> <p>Pathophysiological: <i>H. pylori</i> infection: Epithelial responses CagA translocation CagA-dependent proliferation</p>	Bartfeld <i>et al.</i> 2015. <i>Gastroenterology</i> Bartfeld and Clevers. 2015. <i>J Vis Exp</i>
Gastric epithelial organoid	Human adult tissues: (gastric glands from normal stomach tissue)	Matrigel	> 1 year - may be frozen and thawed indefinitely	<p>Physiological: Metabolite production (urea)</p> <p>Pathophysiological: <i>H. pylori</i> infection: Chemoreception Chemotaxis Host urea destruction</p>	Bartfeld and Clevers. 2015. <i>J Vis Exp</i> Huang <i>et al.</i> 2015. <i>Cell Host Microbe</i>
Gastric epithelial organoid	Human adult tissues: (gastric glands from normal stomach tissue)	Matrigel	~Indefinite	<p>Physiological: Polarized epithelial cells Gland-like structures Mucus secretion Epithelial proliferation</p> <p>Pathophysiological: <i>H. pylori</i> infection: Morphological changes Inflammatory response</p>	Schlaermann <i>et al.</i> 2016. <i>Gut</i>
(GASTRO) INTESTINAL					

Models	Cell Types	Matrix / Scaffold	Stability	Responses	References
Gastrointestinal epithelial spheroids: Stomach Duodenum Ileum Colon	Human adult tissues: (Patient-specific intestinal crypts/stem cells)	Matrigel	Spheroid cell lines amenable to freezing and storage	Physiological: Barrier integrity Mucus layer production Pathophysiological: Pathogenic <i>E. coli</i> infections: (EAggEC, EHEC, EPEC); Adherence	VanDussen <i>et al.</i> 2015. <i>Gut</i>
Intestinal epithelial tissue	Human intestinal cell lines: Caco-2 Ht29-MTX H-InMyoFib (Intestinal Myofibroblasts)	Collagen I Silk fibroin protein	2 months	Physiological: Mucus layer production Intraluminal oxygen gradients Pathophysiological: <i>Y. pseudotuberculosis</i> interaction: Colonization <i>L. rhamnosus GG</i> (probiotic): Colonization	Chen <i>et al.</i> 2015. <i>Sci Rep</i>
Small intestinal enteroids: Duodenum Jejunum Ileum	Human adult tissues: (Patient-specific intestinal crypts/stem cells)	Matrigel	Amenable to freezing and storage	Physiological: NP Pathophysiological: Human Rotavirus (HRV) and Animal Rotavirus (ARV) infection: Host range restriction HRV replication Fluid secretion mimicry Vaccine strain attenuation	Saxena <i>et al.</i> 2015. <i>J Virol</i>
Duodenal enteroids	Human adult tissues: (intestinal crypts/stem cells)	Matrigel	Up to 8 months	Physiological: Ion transport Absorptive processes Fluid secretion Pathophysiological: Cholera toxin responses	Foulke-Abel <i>et al.</i> 2016. <i>Gastroenterology</i>
Ileal and colonic enteroids	Human adult tissues: (intestinal crypts/stem cells)	Matrigel	NP	Physiological: Blood group antigen expression Pathophysiological: Cholera toxin treatment: cAMP activation	Kuhlmann <i>et al.</i> 2016. <i>Am J Trop Med Hyg</i>
Intestinal Organoids	hPSC line: AI/ATD-1	Matrigel	NP	Physiological: Intestinal cell type markers Crypt-like and microvilli structures Pathophysiological: <i>Salmonella</i> infection: Global transcriptional changes Bacterial invasion <i>Salmonella</i> -containing vacuoles	Forbester <i>et al.</i> 2015. <i>Infect Immun</i>
Colonoids (HCMs)	Human adult tissues: (intestinal crypts/stem cells)	Matrigel	NP	Physiological: Organized brush borders Mucus production Pathophysiological: EHEC infection: Colonization	In <i>et al.</i> 2016. <i>Cell Mol Gastroenterol Hepatol</i> Tyska, M.J. 2016. <i>Cell Mol Gastroenterol Hepatol</i>

Models	Cell Types	Matrix / Scaffold	Stability	Responses	References
Large intestine organoids	hPSCs	Matrigel	NP	Brush border damage Mucus layer destruction Intermicrovillar bridge reduction Physiological: Secretory/absorptive functions Mucus production Pathophysiological: <i>C. difficile</i> infection: Inhibition of NHE3 Decreased MUC2 levels	Engvik <i>et al.</i> 2015. <i>Am J Physiol Gastrointest Liver Physiol</i> 308:G497-G509 Engvik <i>et al.</i> 2015. <i>Am J Physiol Gastrointest Liver Physiol</i> 308:G510-G524
KIDNEY					
Renal tissue	Human renal proximal tubule cell line: NK1-2	Matrigel- Collagen 1	NP	Physiological: NP Pathophysiological: Shiga toxin Type 2 treatment: Inhibition of protein synthesis Cytotoxicity Kim-1 secretion (injury marker)	DesRoches <i>et al.</i> 2015. <i>Infect Immun</i>
LIVER					
Hepatic sinusoid tissue	Human hepatocyte cell line: Huh-7 Huh-NTCP Liver sinusoidal endothelial cell lines (unknown designation)	Collagen 1	NP	Physiological (select examples): LSEC barrier function Hepatic adhesive proteins Hepatic ECM release Pathophysiological: <i>E. histolytica</i> infection: Parasite adhesion Parasite migration Increased cytokine levels HBV infection: Viral replication	Petropolis <i>et al.</i> 2014. <i>PLoS Pathog</i> Petropolis <i>et al.</i> 2016. <i>PLoS One</i>
MPCs (Micropatterned Cocultures)	Human primary hepatocytes Mouse embryonic fibroblast cell lines: 3T3-J2 or NIH-3T3	Collagen 1 on MP plates	4-6 weeks	Physiological (select examples): CYP450 activity Albumin/urea secretion NTCP (HBV receptor) Pathophysiological: HBV/HCV entry and infection	March <i>et al.</i> 2015. <i>Nat Protoc</i> Shlomai <i>et al.</i> 2014 <i>Proc Natl Acad Sci U S A</i> Ploss <i>et al.</i> 2010 <i>Proc Natl Acad Sci U S A</i>
Hepatocyte-like cells	iPSC lines: RC2 iPS.C2a LN4 Subject-specific iHLCs ^e	Matrigel	Months	Physiological: Polygonal morphology Hepatic markers Host entry factors (parasites) Pathophysiological: Plasmodium infection: Supports liver-stage malaria Antimalarial drug sensitivity	Ng <i>et al.</i> 2015. <i>Stem Cell Reports</i>
LUNG					

Models	Cell Types	Matrix / Scaffold	Stability	Responses	References
Lung tissue	Human cell lines: Lung fibroblasts: MRC-5 Bronchial epithelial: 16HBE14o	Collagen 1	Weeks	Physiological: ECM protein synthesis Pathophysiological: <i>M. tuberculosis</i> infection: TB-infected macrophage migration Granuloma-like formation	Braian <i>et al.</i> 2015. <i>J Vis Exp</i>
Lung tissue	Human cell lines: Lung fibroblasts: MRC-5 Bronchial epithelial: 16HBE14o- Alveolar epithelial: A549	Collagen 1	NP	Physiological: Morphology Pathophysiological: <i>S. aureus</i> infection: Cytotoxicity Neutrophil-mediated damage Inflammatory response	Shambat <i>et al.</i> 2015 <i>Dis Model Mech</i> Nguyen Hoang <i>et al.</i> 2014. <i>J Leukoc Biol</i>
Lung tissue	Human cell lines: Lung fibroblasts: MRC-5 Bronchial epithelial: 16HBE14o-	Collagen 1	NP	Physiological: NP Pathophysiological: Andes Hantavirus infection: Viral replication Immune responses	Sundström <i>et al.</i> 2016. <i>PLoS One</i>
SKIN					
Skin tissue	Human primary fibroblasts Human cell line: Keratinocyte: HaCaT Pheochromocytoma cell line: PC12	Collagen	Weeks	Physiological: Epidermal stratification Epidermal-dermal interactions HSV-1 infected neuronal cells Pathophysiological: HSV-1 infection: Viral latency Reactivation of latent HSV-1	Hogk <i>et al.</i> 2013. <i>Methods Mol Biol</i>
Skin tissue	Human cell lines: Keratinocytes: N/TERT-1 HaCaT Human primary cells: Neutrophils PBMCs ^f	Collagen	Weeks	Physiological: Epidermal stratification Epidermal-dermal interactions Pathophysiological: SDSE ^g infection: Colonization Bacterial replication Tissue damage	Siemens <i>et al.</i> 2015. <i>Sci Rep</i>

^a hPSCs (human pluripotent stem cells)^b iPSCs (induced pluripotent stem cells)^c hESCs (human embryonic stem cells)^d NP Not provided^e iHLCs (iPSC-derived hepatocyte-like cells)

SDSE (*Streptococcus dysgalactiae* subsp. *equisimilis*)

PBMCs (Peripheral blood mononuclear cells)

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