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Microfluidic Organs-on-a-Chip for Modeling Human Infectious Diseases

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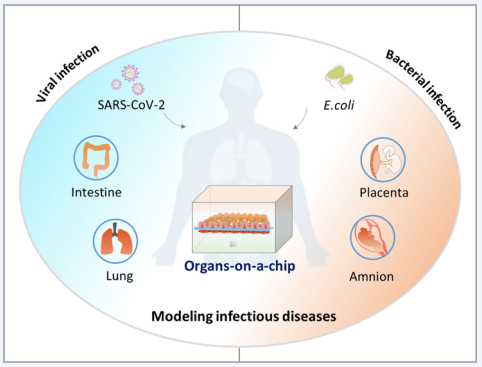
CONSPECTUS: Infectious diseases present tremendous challenges to human progress and public health. The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus disease 2019 (COVID-19) pandemic continue to pose an imminent threat to humanity. These infectious diseases highlight the importance of developing innovative strategies to study disease pathogenesis and protect human health. Although conventional *in vitro* cell culture and animal models are useful in facilitating the development of effective therapeutics for infectious diseases, models that can accurately reflect human physiology and human-relevant responses to pathogens are still lacking. Microfluidic organs-on-a-chip (organ chips) are engineered microfluidic cell culture devices lined with living cells, which can resemble organ-level physiology with high fidelity by rebuilding tissue−tissue interfaces, mechanical cues, fluidic flow, and the biochemical cellular microenvironment. They present a unique opportunity to bridge the gap between *in vitro* experimental models and *in vivo* human pathophysiology and are thus a promising platform for disease studies and drug testing. In this Account, we first introduce how recent progress in organ chips has enabled the recreation of complex pathophysiological features of human infections *in vitro*. Next, we describe the progress made by our group in adopting organ chips and other microphysiological systems for the study of infectious diseases, including SARS-CoV-2 viral infections and intrauterine bacterial infections. Respiratory symptoms dominate the clinical manifestations of many COVID-19 patients, even involving the systemic injury of many distinct organs, such as the lung, the gastrointestinal tract, and so forth. We thus particularly highlight our recent efforts to explore how lung-on-a-chip and intestine-on-a-chip might be useful in addressing the ongoing viral pandemic of COVID-19 caused by SARS-CoV-2. These organ chips offer a potential platform for studying virus−host interactions and human−relevant responses as well as accelerating the development of effective therapeutics against COVID-19. Finally, we discuss opportunities and challenges in the development of next-generation organ chips, which are urgently needed for developing effective and affordable therapies to combat infectious diseases. We hope that this Account will promote awareness about *in vitro* organ microphysiological systems for modeling infections and stimulate joint efforts across multiple disciplines to understand emerging and re-emerging pandemic diseases and rapidly identify innovative interventions.



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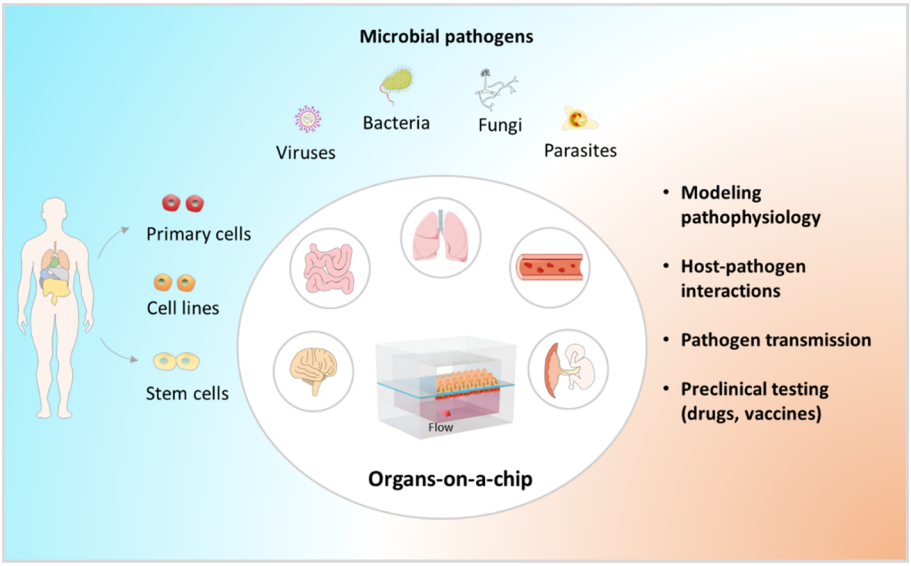

Figure 1. Illustration of human organs-on-a-chip for studies of infectious diseases. Infectious diseases are commonly caused by a variety of microbial pathogens (e.g., bacteria, fungi, viruses, and parasites) that infect human bodies. The organs-on-a-chip is a bioengineered microfluidic culture device containing a variety of living human cells (e.g., primary cells, cell lines, and stem cells). The chip can be used to model the pathogenesis of human infectious diseases (e.g., human-relevant pathophysiology, host−pathogen interactions, and pathogen transmission) and to develop therapies (e.g., drugs and vaccines).

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1. INTRODUCTION

Infectious diseases pose significant challenges to public health and the economic stability of societies worldwide. They are commonly caused by a variety of microbial pathogens (e.g., bacteria, fungi, viruses, and parasites) that invade or infect the human body.[5,6] Viral infections, including those caused by human immunodeficiency virus and hepatitis B virus (HBV), have caused severe outcomes in patients and led to high morbidity and mortality worldwide. Bacterial and parasitic diseases have a similar impact. Epidemics of new and old infectious diseases emerge periodically, greatly magnifying the global health burden. For example, influenza viruses have caused multiple outbreaks in the 20th century, including the 1918 H1N1 flu pandemic.[7] In the 21st century, more than 10 major viral disease epidemics have emerged in human populations, including infections caused by the coronavirus, alphavirus, norovirus, and flavivirus families.[8]

Human respiratory tracts are susceptible to pathogen infections, and a majority of infections are caused by viruses, such as influenza virus, coronavirus, adenovirus, and respiratory syncytial virus. Severe acute respiratory syndrome (SARS), one of the coronaviruses, emerged in late 2002 with a fatality rate of roughly 10%.[9] Another novel zoonotic coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in 2012 with a high mortality rate approaching 35%.[9] The new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the current COVID-19 pandemic, has led to tremendous economic and social losses globally.[10] Unlike the common respiratory infectious diseases, COVID-19 is both lethal and contagious, with a wide spectrum of symptoms ranging from mild to severe and in some cases causing even systemic injury to several organs.[10,11] Human enteroviruses infections are common throughout the world, and the well-known members include the coxsackieviruses, polioviruses, and echoviruses.[12] The replication of enteroviruses often occurs within the gastrointestinal tract or other mucosal surfaces, and the primary transmission is the fecal−oral route. Coxsackievirus B (CVB), as a representative group of enteroviruses, can cause fever, rash, mild respiratory illness, and even myocarditis and aseptic meningitis. Human intestinal cell lines and animal models have been used to study enteric virus infection.[13] At present, there are no approved drugs or vaccines for enteroviruses treatment other than polioviruses. Other enteric pathogens, such as *Escherichia coli (E. coli)* and the vibrios causing cholera, have adapted to fecal−oral transmission through environmental cues (e.g., water and food).

Most of these important pathogens exhibit specific human tropism. However, the development of novel intervention strategies has been largely hindered by the lack of robust, cost-effective, and reliably predictive models that can replicate the major hallmarks of human infections. While rodent and nonhuman primate models have been used for studying organism responses to infections and drug testing, most of

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these models have also exhibited varying susceptibility as well as substantially different symptoms from the human condition. In addition, human cell lines can be used to culture viruses and for the high-throughput screening of drugs, but these simplistic monolayer cell cultures differ from native human tissues in terms of gene profiles, epigenetics, and organ functions. The limitations of the existing models highlight an urgent need for the concurrent development of robust model systems for infectious disease studies and effective therapies.

Human organs-on-a-chip (henceforth "organ chips") are bioengineered microfluidic devices inhabited by living cells that offer the possibility of replicating the major functional units of human organs.[14−17] Organ chips may present alternatives or complements to certain animal models as well as conventional 2D cell culture by providing a higher degree of human mimicry and human-relevant responses. They can recapitulate the pathophysiology of complex organs under dynamic conditions and facilitate the study of disease pathogenesis. In this Account, we first describe the conventional methods and the progress made in emerging organ chip technology with regard to the study of microbial pathogen-induced infections. Next, we highlight the projects and efforts by our group to explore the use of organ chips and other microphysiological systems to model human infectious diseases, including COVID-19. We also discuss the opportunities and challenges for developing robust *in vitro* model systems to advance infectious disease research to address both ongoing and future pandemics (Figure 1).

2. CONVENTIONAL METHODS FOR STUDYING INFECTIOUS DISEASES

Conventional methods for studying infections include transformed cell lines, primary tissue-derived human cells, stem cells, and animal models. Culturing microbial pathogens directly from host animals has been a longstanding challenge. Owing to the ease of handling and the low experimental costs involved, culturing host cells in a Petri dish may be a better alternative to studying infectious diseases and screening antimicrobial agents.[8] In general, cell line cultures provide an important basis for understanding host−pathogen interactions, such as infectious diseases caused by herpes viruses, hepatitis viruses, malaria parasites, and *Mycobacterium tuberculosis*.[18] However, most of the cell lines in 2D cultures are immortalized and differ from native tissues in terms of gene profiles, epigenetics, and functions. Moreover, because these cell cultures often lack complex tissue architectures and the proximate physiological tissue microenvironment, they cannot replicate human organ physiology and functional responses to external stimuli. While the primary tissue-derived human cells can retain the phenotypic and genotypic features of the original tissues, the limited sources and the low replication potential of primary cells often restrict their widespread use. Recently, human pluripotent stem cell (PSC)-derived tissue-specific cells and organoids have becoming potential models for recapitulating organ physiology and pathology. While stem cells and human organoids have been used to study pathogen infections, they do not accurately reflect the structures or functionalities of organs *in vivo*, partly due to the lack of biomimetic fluid flow and a controlled tissue microenvironment.

Small- and large-animal models, such as laboratory rodents, ferrets, and cynomolgus macaques, have been used widely in preclinical testing for studying organism responses to pathogens, viral pathogenesis, transmission and testing the efficacy of various drugs and vaccines. Although rodents are among the most frequently used animal models in studies of infections, they are evolutionarily distant from humans. For example, mice are not the natural hosts for many human viruses, including SARS-CoV-2.[19] Owing to high evolutionary relatedness, nonhuman primates and humans possess similar physiology and immunology. Nonetheless, the ethical issues, high financial and logistical costs, and complex husbandry requirements associated with studies involving nonhuman primates greatly limit their scalability. In sum, the existing models often exhibit different pathological changes and clinical manifestations from humans, which hamper their effective translation potential.

3. ADVANCES IN MICROENGINEERED ORGANS-ON-A-CHIP TECHNOLOGY

Microfluidic organ chips have emerged as *in vitro* microphysiological systems that possess the potential for recreating functional units of human organs from native counterparts.[14,20] They are able to mimic the *in vivo*-like tissue microenvironment by the precise control of fluidic flow, mechanical cues, and the tissue−tissue interface, thereby facilitating the creation of physiologically relevant 3D tissues or organs with functional hallmarks. Owing to these features, organ chips can simulate human physiology and organ-specific responses and are thus becoming a promising alternative to conventional 2D cell cultures and animal models. *In vivo* tissue or organ formation is regulated by a dynamic microenvironment involving blood flow. Implementing microfluidic perfusion flow into the microchannels of chip devices facilitates sufficient nutrient exchange or provides controlled shear stress to maintain the morphology and functionality of 3D tissues. Moreover, the microfluidic chips allowed the manipulation of cell behavior by spatiotemporal control over the delivery of biochemical compounds or oxygen gradients. As tissue interfaces are crucial to maintaining the tissue barrier function between various human tissues and organs, the compartmentalized nature of organ chips can be leveraged to produce similar tissue−tissue interfaces by cocultures of distinct cell types. In addition, the mechanical cues, which include stretching and deformation, have broadened the horizon for the in-depth study of cell behaviors. By incorporating cyclic mechanical deformation into tissues, organ chips, such as engineered lung[16] and gut chips,[21] provide significant models for studying human organ physiology and pathology. Besides mechanical signals, organs-on-a-chip can also introduce electrical or optical stimuli (e.g., optogenetics), for instance, those used for the pacing of cardiac tissue and the functional assessment of cardiac contraction.[22,23] As such, toward advanced 3D tissues and organs, it is essential to improve the generation of 3D tissues and organs by the precise spatiotemporal control of the morphogen gradients and physiological microenvironment cues.

Organ chip technology has made remarkable progress in the past decade, such as in successfully reconstructing different biomimetic models of human organs including the lung,[16,24,25] heart,[26] liver,[27] and gut.[21] These organ chips have been applied to model diseases and evaluate drugs. Recently, organ chip platforms have been devoted to explore pathogen infections as well as to study infection kinetics and host immune responses. For example, a microvessel-on-a-chip was designed to model hemorrhagic syndrome induced by Ebola, which was further used for testing antiviral agents.[28] Hepatitis B is a severe

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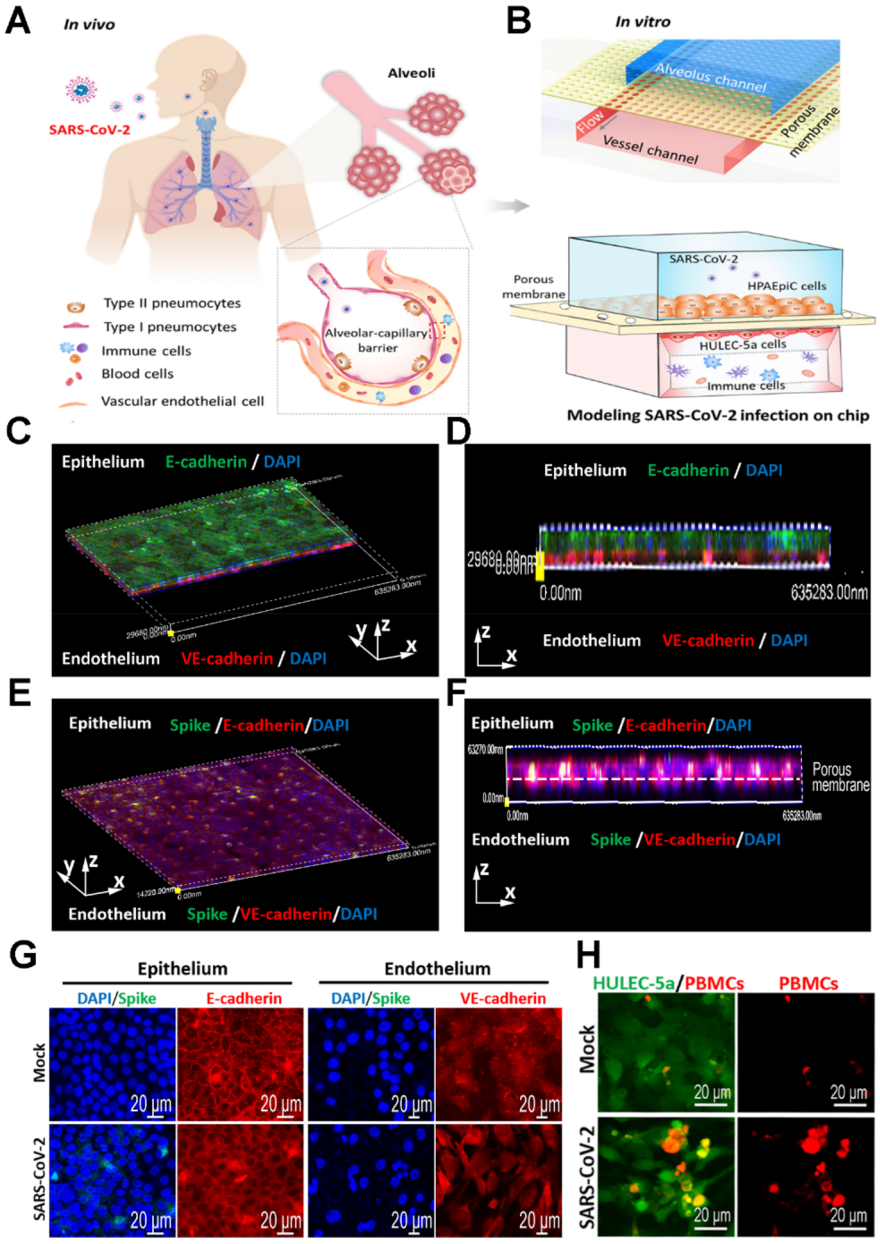

Figure 2. Characterization of SARS-CoV-2-induced lung injury using the human lung-on-a-chip. (A) Illustration of the human alveolar−capillary barrier *in vivo*. (B) Configuration of the human lung-on-a-chip model with SARS-CoV-2 infection. The microengineered lung chip is composed of an alveolar epithelium in the upper layer and a pulmonary microvascular endothelium in the lower layer, separated by a porous PDMS membrane. It mimics the human alveolar−capillary barrier *in vivo* by the coculture of HPAEpiC and HULEC-5a cells under fluid flow. (C) Three-dimensional reconstructed confocal image of the alveolar epithelium (E−cadherin) and endothelium (VE−cadherin) on a chip. (D) Side view showing the interface of the alveolar epithelium (E−cadherin) and endothelium (VE−cadherin). (E) Three-dimensional reconstructed confocal image showing the alveolar−capillary barrier on the third day postinfection. (F) Side view showing the alveolar barrier after viral infection. SARS-CoV-2 infection was predominantly identified in the epithelium layer by spike protein expression. (G) Confocal images showing the effects of SARS-CoV-2 infection (spike) on the epithelium (E−cadherin) and the endothelium (VE−cadherin) on the third day postinfection. (H) Confocal immunofluorescence microscopy images showing the recruitment and adhesion of immune cells (PBMCs) (red) to the surface of the HULEC-5a layer (green) after viral infection.

infectious liver disease caused by HBV, whose chronic infection may lead to liver fibrosis and cirrhosis or even cancer.[29] A human liver chip derived from primary hepatocytes was used for the study of HBV infections, which exhibited similar biological responses (e.g., immune cell activation) to HBV as a real human liver.[30] In addition, lung chips have been used in research on respiratory viral infections, virus transmission, and antiviral drug assessment, such as in the case of influenza viruses.[31] It appears that organ chip technology provides an opportunity to model human organ-specific responses to microbial infections and to study host−pathogen interactions in a more physiologically relevant environment.

4. MODELING HUMAN INFECTIOUS DISEASES IN ORGAN CHIPS

Viruses and bacteria are the two main types of infectious pathogens that seriously threaten human health. In the past few years, we have made attempts to explore the utility of

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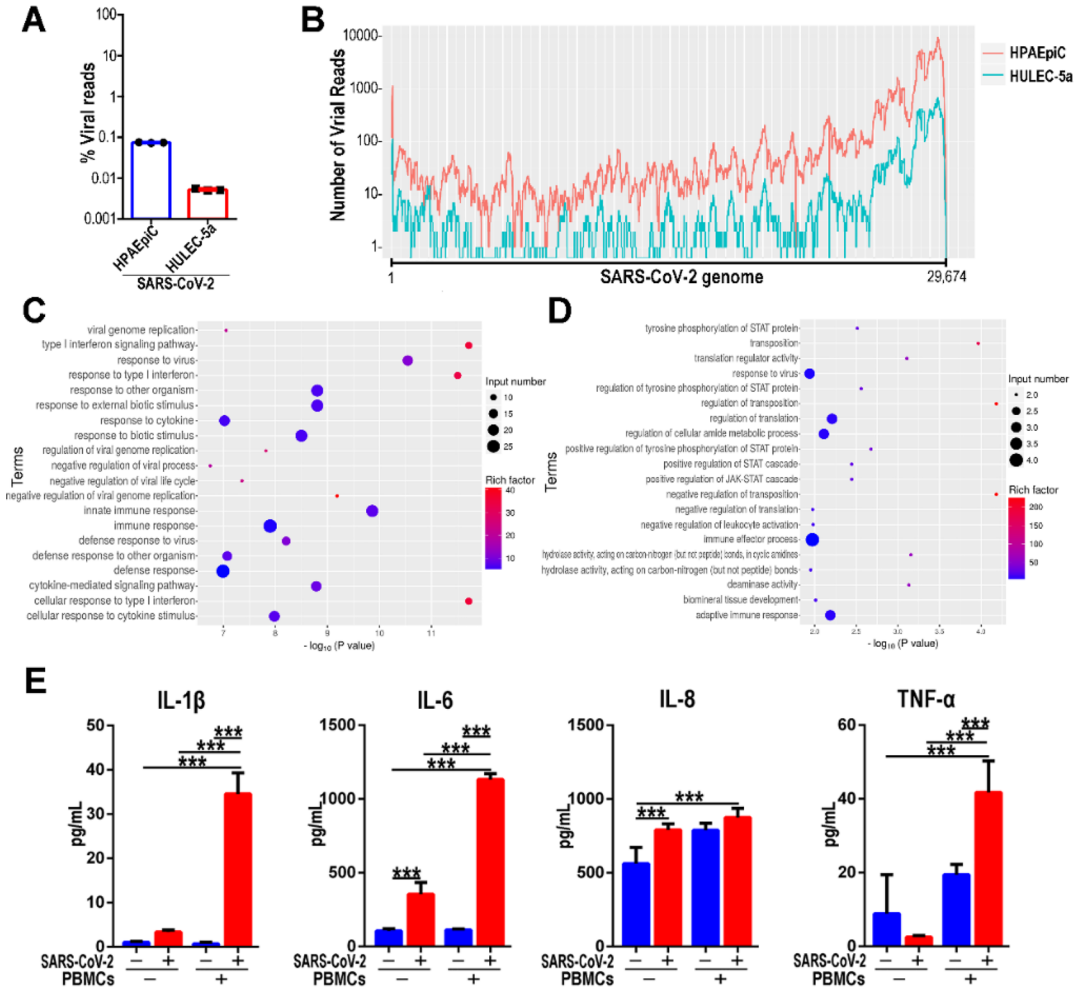

Figure 3. Transcriptome analysis reveals viral tropism and inflammatory responses after SARS-CoV-2 infection. (A) Bar graph showing viral replication levels in the HPAEpiC and HULEC-5a cells on the third day following SARS-CoV-2 infection based on RNA-seq analysis. (B) Read-coverage graph showing viral reads along the SARS-CoV-2 genome for the infected epithelial and endothelial cells. The graph indicates the number of viral reads at every position of the viral genome in the infected cells. (C and D) GO enrichment analysis of upregulated DEGs associated with immune responses in HPAEpiC (C) or HULEC-5a (D) cells. (E) ELISA results showing the inflammation cytokines (IL-1β, IL-6, IL-8, and TNF-α) released from the medium in the vascular channel under different conditions.

complex *in vitro* microphysiology systems for modeling infectious diseases, testing drugs, and studying underlying pathogenesis. In particular, spurred by the challenges faced during the early phases of the COVID-19 pandemic, we have quickly leveraged human lung chips to investigate the potential of this platform for studying host−pathogen responses and for identifying potential therapeutics against SARS-CoV-2 under challenging conditions.

4.1. SARS-CoV-2 Viral Infection in Organ Chips

4.1.1. Lung-on-a-Chip. In the current COVID-19 pandemic, the lung serves as the primary target organ for the infection and replication of SARS-CoV-2 through the respiratory transmission route. In some severe COVID-19 cases, the patients progress to acute respiratory distress syndrome, coagulopathy, and multiple organ dysfunction.[32] Monolayer cell-based models (e.g., Vero E6, Calu-3, and Huh-7) have been widely used in the isolation and identification of the SARS-CoV-2 virus,[19,33] which played vital roles during the early stages of the COVID-19 outbreak. Animal models such as rhesus macaques and cynomolgus macaques have been instrumental in efforts to understand the virus pathogenesis and drug testing.[34,35] Despite the significant progress made by these conventional models for COVID-19 research, models that can accurately reflect human pathophysiological responses to this virus are still lacking.

4.1.1.1. Alveolar Barrier Infection by Native SARS-CoV-2. To simulate the lung barrier in a human relevant manner, we initially established a lung-on-a-chip composed of human pulmonary alveolar epithelial type II cells (HPAEpiC), pulmonary microvascular endothelial cells (HULEC-5a), and peripheral blood mononuclear cells (PBMCs). The lung chip device contains two compartmentalized chambers, with the coculture of epithelial cells in the upper alveolar channel and endothelial cells in the lower vascular channel with circulating immune cells, in which both chambers are separated by a porous membrane (Figure 2A,B). The barrier integrity was examined by the expression of adherent junction proteins (E−cadherin and VE−cadherin) and the formation of confluent tight monolayers in these two types of cells under fluid flow conditions (Figure 2C,D). Moreover, the barrier permeability was identified by the diffusion rate of FITC-dextran in the chip. By synergistically integrating cell cocultures, the peripheral immune system, and fluid flow, this lung chip

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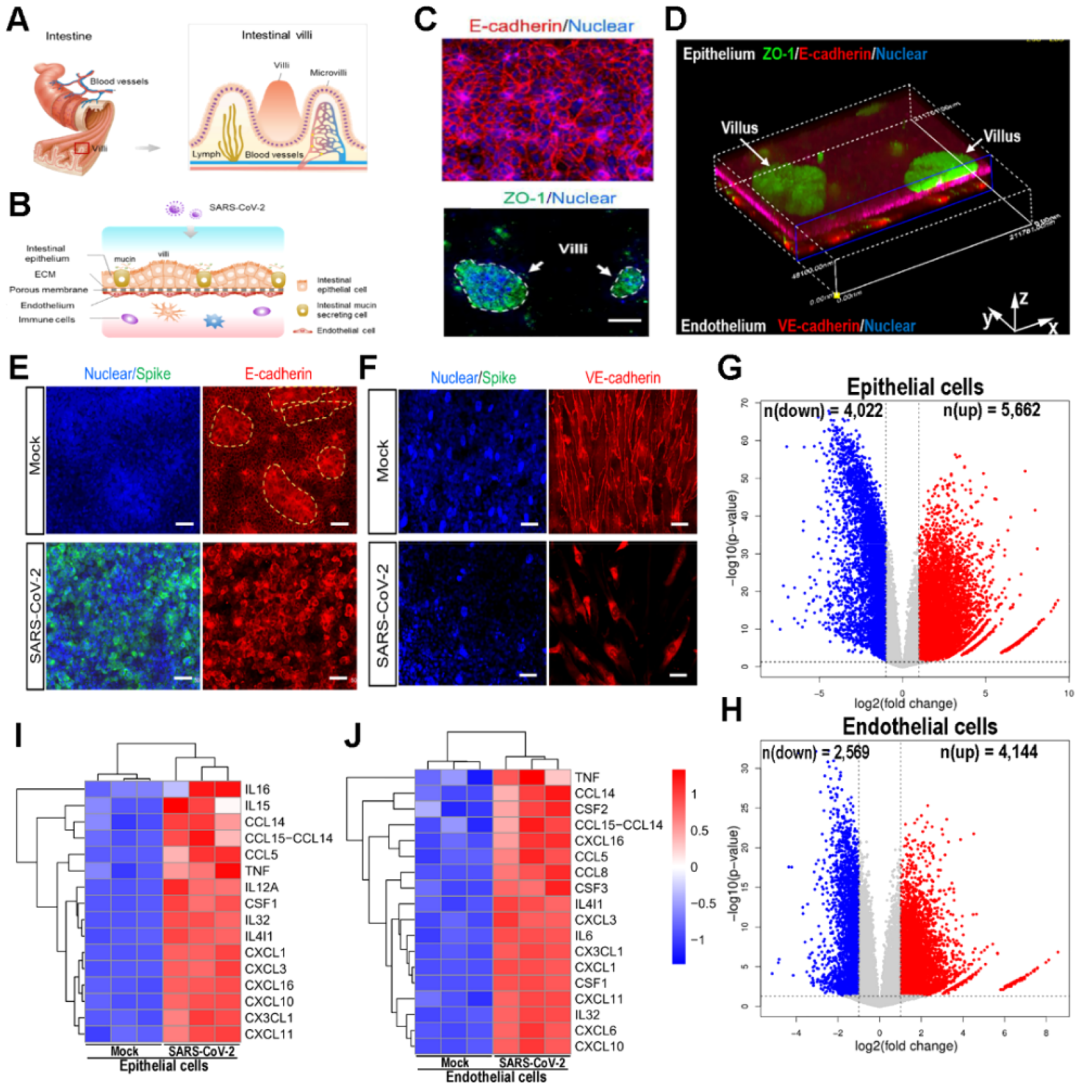

Figure 4. Modeling SARS-CoV-2 infection with the human intestine-on-a-chip. (A) Illustration of the human intestinal barrier *in vivo*. (B) Configuration of the intestine-on-a-chip infected by SARS-CoV-2. The chip device contains upper and lower layers separated by an ECM-coated porous PDMS membrane. The intestinal barrier was constructed by the upper intestinal epithelial layer (Caco-2 and HT-29) and lower vascular layer (HUVECs and immune cells) under fluid flow. (C and D) Chip model showing an integrated intestinal epithelial barrier and villus-like structure characterized by the junction proteins (E−cadherin and ZO-1) expression (scale bars: 50 μm). (E and F) Confocal immunofluorescence microscopy images showing that the intestinal epithelium was more permissive to SARS-CoV-2 than the endothelium, as identified by the predominant expression of the spike protein in the epithelial layer on the third day postinfection (scale bars: 50 μm). (G and H) Volcano plots of the dysregulated genes induced by viral infection. (I and J) Heat maps depicting the significant upregulation of cytokine genes in the intestinal epithelial and endothelial cells.

system can recapitulate the essential physiological features of the human alveolar−capillary barrier. To further study the response of this lung chip to viral infection, we inoculated native SARS-CoV-2 particles in the upper alveolar channel to mimic respiratory viral infection. The results showed the obvious infection and replication of this virus in the alveolar epithelium but not in the vascular endothelium (Figure 2E,G).

4.1.1.2. Lung Vascular Damage and Immune Responses. Severe symptoms of COVID-19 cases involve hypercoagulopathies and systemic endothelialitis.[36,37] We next examined the effects of SARS-CoV-2 infection on endothelial cells in the lung chip. The results showed that viral infection induced endothelial impairment accompanied by injury to the alveolar barrier, even though the virus did not primarily infect the endothelial cells. Moreover, this damage was exacerbated in the presence of PBMC infusion (Figure 2H). We propose that the microvascular endothelial injury was caused by cytokines released from immune cells or adjacent alveolar epithelial cells but not the SARS-CoV-2 itself. In addition, RNA-seq analysis was performed to identify the host response to viral infection. The results indicated that the viral load of epithelial cells was much higher than that of endothelial cells (Figure 3A,B). Further analysis showed that immune-related pathways, such as the IFN-I signaling pathways and the JAK-STAT signaling pathways, were significantly activated in the infected alveolar epithelium and endothelium, respectively (Figure 3C,D).

Clinical trials suggested that immune cells are closely associated with systematic inflammatory responses, which may lead to the occurrence of "cytokine storms" in some COVID-19 patients.[38] We studied the SARS-CoV-2 infection-induced inflammatory responses in the lung chip. In the presence of circulating immune cells, the levels of several pro-

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inflammatory cytokines (e.g., IL-6 and IL-1β) were significantly increased in both vascular and alveolar channels following infection (Figure 3E). This suggests that immune cells may play a crucial role in mediating lung injury and exacerbating the inflammatory response. Our disease model reveals the complicated cross-talk among the alveolar epithelium−endothelium and the host immune response, which is not easily achieved by traditional cell cultures and animal models. To our knowledge, this work represents the first attempt to build a human lung disease model for native SARS-CoV-2 infections using organ chips that can reflect human lung injury and immune responses involved in COVID-19 progression at the organ level.

4.1.1.3. Assessment of an Antiviral Drug. To investigate the feasibility of the *in vitro* lung chip model for assessing the potential of antiviral therapeutics against SARS-CoV-2, we leveraged this disease model to test the FDA-approved drug Remdesivir as a candidate antiviral agent. Remdesivir is considered to be a promising antiviral compound against various RNA viruses (e.g., SARS-CoV-2, SARS, and MERS-CoV).[39,40] Following inoculation of the chip with native SARS-CoV-2, Remdesivir was added to the epithelial channel. The drug showed obvious therapeutic efficacy by inhibiting viral replication after its administration for 3 days and further alleviated the barrier disruption caused by SARS-CoV-2. Because of the limits in the tense environment during the beginning of the COVID-19 epidemic, only antiviral agents were tested using the lung disease model. In the future, the capabilities and potentials of this platform make it possible to test other candidate drugs, such as anti-inflammatory cytokine inhibitors, and repurpose potential drugs against COVID-19.

Although the organ chips demonstrate promising potentials for modeling lung infections, this work still has some limitations due to the challenging experimental conditions during the pandemic. For instance, this lung chip lacks stretch stress to resemble the breathing of the lung alveolar barrier. And it is limited by the low throughput for drug screening. The advances in biofabrication and microengineering technology can be easily integrated with microfluidic elements to further improve the function of the lung chip and increase the chip flux. Additionally, the PDMS material can absorb small molecules or drugs to some extent, which might influence the determination of accurate drug doses. As such, hydrophilic biomaterials or hydrogels could be applied in organ chips to render PDMS nonabsorbent. Nevertheless, the established lung infection model on a chip displays its unique value to reflect the human-relevant lung pathophysiology and host response to SARS-CoV-2, which is not easily achieved by existing cell-based systems.

4.1.2. Intestine-on-a-Chip. Besides respiratory symptoms, obvious gastrointestinal symptoms (e.g., abdominal pain, diarrhea, and acute hemorrhagic colitis) were observed in 20 to 50% of COVID-19 patients.[41] The viral RNA was examined in the stool samples of multiple patients. Typical presentation of the coronavirus was also observed in rectal tissue. These clues indicate that SARS-CoV-2 may be transmitted via the fecal−oral route. However, the response of the human intestine to SARS-CoV-2 infection remains unclear. Thus, we constructed a bioengineered human intestine infection model by SARS-CoV-2 in a perfusable multilayer microfluidic chip device and explored the viral infection-induced intestinal injury and immune response (Figure 4A,B).

4.1.2.1. Disrupted Intestinal Barrier. The integrity of the tissue barrier is essential to maintaining the function of intestinal tissue. The integrity of the epithelial−endothelial barrier in this intestinal chip was assessed by the expression of tight junctions (ZO-1 and E−cadherin) in the epithelium and the conjunction (VE−cadherin) in endothelial cells, which displayed the intact distribution of these junction proteins. The established intestinal chip exhibited the integrity of an intestinal barrier with villus-like structures and the mucus secretion function. By integrating multicellular components, intestinal epithelium−endothelium interactions, and fluid flow, the chip system recapitulated the critical features of the intestinal barrier (Figure 4C and D). Upon SARS-CoV-2 infection, the intestinal chip showed viral infection and replication in the epithelium (Figure 4E), suggesting that the intestinal epithelium serves as a portal for viral transmission. Notably, adherent junctions in the endothelium were severely disrupted, whereas no obvious spike-positive cells were observed (Figure 4F). This indicates that the cross-talk between the epithelium and endothelium may play a crucial role in vascular endothelial cell injury. In addition, a series of pathological features were observed, such as the destruction of intestinal villi, the disruption of the barrier integrity, and the disruption of mucosal secretion. Studying these features in the intestinal chip may advance our understanding of the intestinal pathological processes involved in COVID-19.

4.1.2.2. Intestinal Immune Responses. Biopsy samples from COVID-19 patients showed the infiltration of many plasma cells and lymphocytes into the stomach, duodenum, and rectum.[42] To investigate the intestinal immune response to viral infection, we further identified the host biological pathways related to the immune response by RNA-seq. The transcriptome analysis data indicated that the pathways associated with the abnormal metabolism of RNA and protein and the immune responses were significantly modulated in both the epithelium and endothelium following viral infection (Figure 4G,H). In particular, multiple cytokine- and chemokine-related genes were significantly upregulated; this might contribute to gastrointestinal symptoms associated with intestinal tissue injury. These findings may provide new insights into the mechanisms, involving intrinsic cross-talk among human host cells and immune cells, that underlie SARS-CoV-2-induced tissue inflammation and damage.

This study still has some limitations. While an intestinal epithelial cell line (Caco-2) has been widely used, this tumor-derived cell line cannot fully reflect the nearly physiological functions of the human intestine *in vivo*. The primary intestinal stem-cell-derived intestinal epithelium may be selected for future studies. Several previous studies have revealed the role of the intestinal microbiota in conferring antiviral immunity as well as its potential therapeutic value in SARS-CoV-2 infections.[43] In the next stage, we will attempt to integrate the intestinal microbiota into the current intestinal model and further study the effects of the intestinal microbiota on COVID-19 progression.

4.2. Intrauterine Bacterial Infection in Organ Chips

Maternal infection caused by pathogens and the associated host inflammatory response are risk factors for preterm birth, which is the predominant cause of neonatal morbidity and mortality.[44] Clinically, preterm labor often occurs in cases involving inflammation induced by bacterial or viral infections within the feto-maternal tissues, which are associated with

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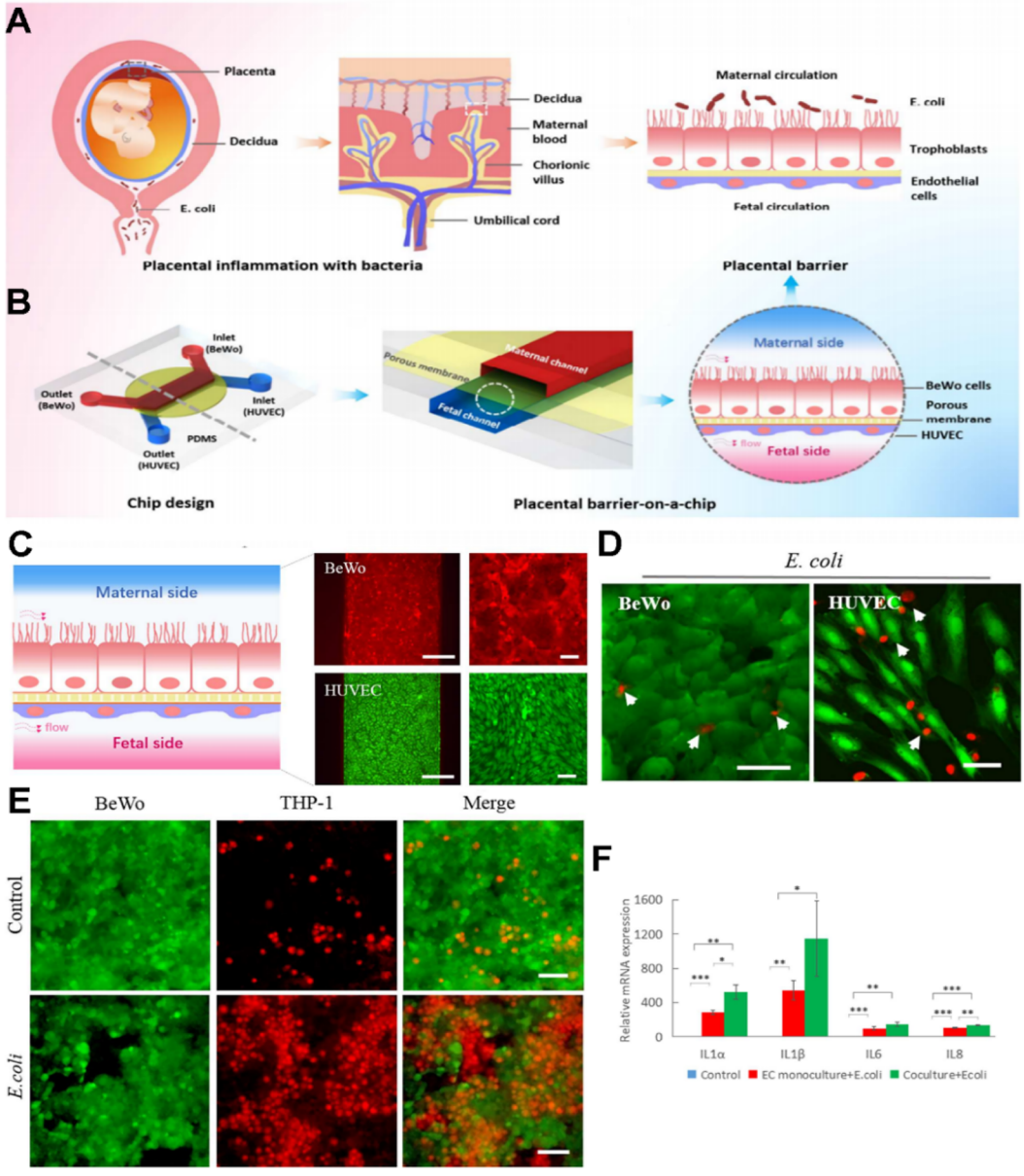

Figure 5. Modeling intrauterine infection caused by bacteria with the placenta-on-a-chip. (A) The placenta is an organ that links the fetus to the maternal uterine wall via the umbilical cord. Bacteria commonly pass by the ascending route in the lower genital tract into the uterus, causing placental inflammation. (B) Diagram of the placenta-on-a-chip consisting of the top maternal layer and the bottom fetal layer separated by a porous membrane. The placental barrier was constructed from a BeWo epithelial layer and a HUVEC layer on the opposite side of the membrane-on-a-chip. (C) Confocal immunofluorescence microscopy images showing the formed confluent trophoblasts (occludin) and endothelial cell (VE–cadherin) layers (scale bars: 100 μm). (D) Examination of cell viability following *E. coli* infection. Dead cells are shown in red, and live cells are shown in green (scale bars: 50 μm). (E) Confocal immunofluorescence microscopy images showing THP-1 monocyte adhesion to trophoblast cells following *E. coli* infection (scale bars: 100 μm). (F) qPCR analysis showing the expression of proinflammatory cytokine genes in endothelial cells.

placental damage and adverse fetal outcomes.[45] The placenta is an extraembryonic organ that provides nutrients and oxygen to the fetus, playing a pivotal role in the health of both the fetus and the mother. Fetal membranes (e.g., amniochorionic membrane), which form the innermost lining of the intrauterine cavity that surrounds the fetus, provide mechanical and immune protection throughout pregnancy.[46] A knowledge of intrauterine infections is vital to safeguarding reproductive health. However, in-depth studies on the propagation of intrauterine infections and their underlying mechanisms are highly challenging to undertake due to the limitations of animal models and conventional cell cultures in recapitulating the biology and physiology of human organs. In addition, the use of the human primary placenta or fetal tissues is limited owing to ethical and practical concerns.

4.2.1. Placenta-on-a-Chip. In the human body, the placental barrier is a multilayered structure that consists of trophoblast cells and endothelial cells in the maternal–fetal interface, separating maternal and fetal circulation in the placenta.[46] To model ascending bacterial infection and determine inflammatory responses in the placenta tissue, we built a bioengineered human placental barrier (the "placental chip") model to study the placental responses to bacterial infection (Figure 5A). The multilayered chip design could enable the construction of microarchitecture of the placental barrier by cell coculture, thus simulating the fetal–maternal

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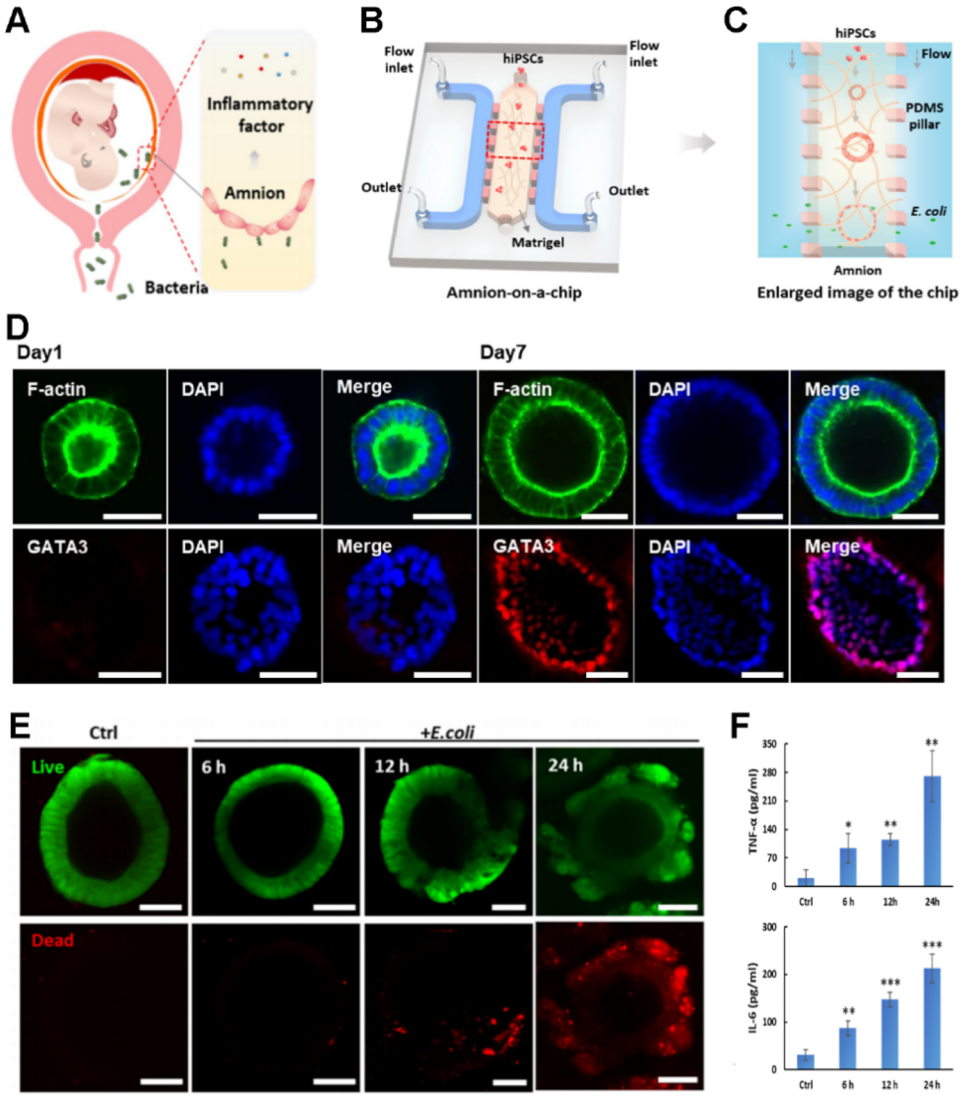

Figure 6. Modeling intrauterine bacterial infection with the amnion-on-a-chip. (A) Illustration showing that amniotic infection is related to the inflammatory responses of amniotic tissue to bacterial exposure during pregnancy. (B and C) Amnion chip design and enlarged images of the procedures used to generate the hiPSC-derived amniotic cavity on the chip. (D) Confocal immunofluorescence images showing the expression of F-actin and the early amnion marker GATA3 in the amniotic cell cavities at days 1 and 7 (scale bars: 50 μm). (E) Examination of cell viability of the amniotic tissue without or with *E. coli* exposure at different time points. Dead cells are shown in red, and live cells are shown in green (scale bars: 50 μm). (F) ELISA results showing that the levels of TNF-α and IL-6 released from amnion organoids were elevated following bacterial infection.

interface. The fluid flow in the upper and lower channels resembled dynamic blood flow (Figure 5B). The integrity of the placental barrier was assessed by the protein expression of the tight junction (occludin) in the trophoblastic epithelium and the intercellular junction (VE−cadherin) in the endothelium. The results indicated the formation of the polarized microvilli on the apical surface of trophoblasts and intact tight junctions in endothelial cells under flow in the placenta-on-a-chip (Figure 5C).

Escherichia coli is one of the most common bacterial pathogens found in fetal organs.[47] We introduced *E. coli* onto the maternal side of the chip to model acute placental inflammation. The results showed that bacterial infection induced the secretion of inflammatory cytokines by trophoblasts, cell death, and activated maternal macrophages (Figure 5D−F). In particular, bacterial infection aggravated the inflammatory response in the coculture chip system, indicating the potential role of trophoblasts in contributing to fetal inflammation syndrome in clinics. The placental chip evidently provides a useful platform for exploring the complicated cross-talk between placental cells and placental inflammation and thus may improve our understanding of the pathogenesis of reproductive diseases and preterm birth. In addition to placental inflammation caused by bacterial infections, other pathogens such as cytomegalovirus, herpesviruses, and the Zika virus (ZIKV) have been reported to infect the maternal placenta and further affect the fetus potentially via vertical transmission.[48] Thus, placental chips could also be extended to wider research fields of placental infection caused by viral pathogens.

4.2.2. Amnion-on-a-Chip. A common uterine infection by infectious organisms (e.g., *E. coli*) may lead to intra-amniotic inflammation and premature rupture of the membranes.[49] Amniotic infection (known as acute chorioamnionitis) as an

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important factor can increase the risk of preterm labor (Figure 6A). The amniotic membrane, the most elastic component of the fetal membrane, performs essential immune and endocrine functions that are critical to the maintenance of pregnancy. With the rapid development of stem cell biology, human-induced pluripotent stem cells (hiPSCs) have been used to simulate postgastrulation development. The hiPSC-derived amniotic sac, which recapitulates the human amnion development in peri-implantation, has been generated in previous work.[50]

To reconstruct the complex physiological microenvironment of the amniotic tissue *in vivo*, we developed a microengineered amniotic tissue on a chip derived from hiPSCs. The amnion chip contained two parallel perfusion channels separated by a 3D matrix channel, which offers a permissive dynamic microenvironment for amnion development (Figure 6B,C). Under perfused 3D culture on a chip, a cell cavity was initially formed by the self-organization of hiPSCs, followed by the differentiation of polarized squamous amniotic epithelium (Figure 6D). We found that fluid flow is beneficial to the differentiation and maturation of amniotic tissue, resembling human amniotic development in midgestation. When exposed to *E. coli*, the amnion-on-a-chip exhibited obvious functional impairments, including cell apoptosis, a disrupted integrity of cell junctions, and an increased secretion of inflammatory factors (Figure 6E,F). This model thus reflects human-relevant pathological features, including the inflammatory responses and tissue damage of the human amniotic cavity when exposed to bacterial infection during early pregnancy. The 3D amnion chip provides an attractive tool for investigating amnionitis caused by bacterial infection in a physiologically relevant manner. We contend that this model can be applied to the study of intrauterine infection caused by other microbial pathogens, thereby potentially contributing to our understanding of infections and inflammation associated with adverse pregnancy and gestational disorders.

5. CONCLUDING REMARKS

Microfluidic organ chips are emerging as complements or alternatives to conventional *in vitro* cell cultures and animal models, largely owing to their ability to reproduce many aspects of human organ physiology and disease states. In particular, by incorporating the immune system and blood vessel cells into human-relevant settings, organ chips can emulate the pathogen-induced responses of multiple human organs and allow for the visualization of these dynamic changes in real time. In comparison, such features are not possible with conventional models. The recent advances in organ chips have demonstrated their utility for studying human infectious diseases, including the recent COVID-19 pandemic, by reproducing the complexities of human pathophysiology at the organ level. This technology facilitates the exploration of the interactions between human host cells and pathogens, which will likely aid clinical diagnosis and therapies for the next pandemic.

It is worth noting that organs in the human body do not exist in isolation. In clinical settings, infectious diseases often have systemic pathological symptoms. For example, SARS-CoV-2 has been shown to affect several organs in an interconnected manner. The cross-talk between organs can reflect a systemic response to pathogen infections. To truly understand what happens when an individual is infected with pathogens and the efficacy of distinct therapies, more complex systems that include multiple organ types are needed. Therefore, "multi-organs-on-a-chip" may provide a unique opportunity to model the systemic responses of tissues and organs to infections.[51] The ultimate goal of organ chips is to create a "body-on-a-chip" based on human biology and to accelerate studies on disease pathogenesis, preclinical drug development, and innovative therapies by reducing the use of animals.

Recently, human stem cell-derived organoids have become valuable *in vitro* organ models because they are capable of retaining the complex multicellular architectures, functions, and genetic backgrounds of their original donors.[52−54] Organoids have provided useful information for the study of viral tropism and pathogenesis, such as ZIKV,[55] HBV,[56] and even SARS-CoV-2.[57,58] However, as existing organoid models still lack immune cells, they cannot faithfully represent the immune responses of target organs upon pathogen infections. A combination of stem cells and microfluidic organ chips can improve organoid systems with enhanced tissue functions and a controllable microenvironment,[59−61] thus reflecting more realistic host−pathogen interactions *in vitro*.

Despite the significant advances in organ chip technology, the field of infectious disease studies using organ chips is still relatively new. Future challenges will likely pertain to the demand for online read-outs and biosensors. Multiplex biosensors can be integrated within organ chips to enable real-time monitoring of cell behavior, environmental parameters (e.g., pH and oxygen), infection dynamics, and biological processes. Organ chips can also be integrated with other technologies, such as 3D printing, gene editing, and high-resolution imaging, to create more complicated and high-throughput organ model systems. Overall, emerging and re-emerging infectious diseases are likely to lead to unpredictable epidemics and significant challenges to public health. The complex challenges of modeling major human diseases can be achieved by a concerted effort across multiple disciplines. We envision that the next generation of organ chips will help advance the understanding of infections and enhance the readiness and accessibility of these devices for human diseases with epidemic potential.

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Notes

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