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ABSTRACT

Respiratory viral infections are leading causes of death worldwide. A number of human respiratory viruses circulate in all age groups and adapt to person-to-person transmission. It is vital to understand how these viruses infect the host and how the host responds to prevent infection and onset of disease. Although animal models have been widely used to study disease states, incisive arguments related to poor prediction of patient responses have led to the development of microfluidic organ-on-chip models, which aim to recapitulate organ-level physiology. Over the past decade, human lung chips have been shown to mimic many aspects of the lung function and its complex microenvironment. In this review, we address immunological responses to viral infections and elaborate on human lung airway and alveolus chips reported to model respiratory viral infections and therapeutic interventions. Advances in the field will expedite the development of therapeutics and vaccines for human welfare.

I. INTRODUCTION

Respiratory viruses are the most frequent cause of disease in humans, with significant morbidity and mortality on a global scale, particularly in children. Approximately 20% of all childhood deaths worldwide are related to acute respiratory infections (ARIs), particularly in developing countries. A number of human respiratory viruses [e.g., rhinoviruses (HRVs), enteroviruses, influenza viruses, respiratory syncytial viruses (RSVs), and adenoviruses] circulate commonly in all age groups and are recognized as adapted to efficient person-to-person transmission. In addition to these, severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) and avian influenza virus H5N1, and more recently SARS-CoV2, have emerged as threats to public health.[1,2] Respiratory viruses can lead to severe symptoms such as acute respiratory distress syndrome (ARDS)[3] and even death. Despite the potential to cause severe disease, over 70% of viral infections remain asymptomatic.[4] This is due to a wide range of factors such as virulence and the immune competence of the host.[5] Therefore, it is vital that we understand how these viruses infect the host and how the host responds to prevent infection and onset of disease. Animal models have been used to study pathogenesis, molecular mechanisms underlying these infections, and effects of potential drug candidates. Various human cell types have been transplanted into immunodeficient mice to develop models with expanded tropism for human pathogens.[6,7] Wahl *et al.*[8] engrafted lung cells with autologous immune cells to study respiratory viruses. However, data translated to the clinic based on animal models have not always yielded favorable outcomes with humans[9,10] due

to interspecies variations in physiology and genetics.[11] Therefore, more sophisticated disease models are required to mimic the pathology in humans,[12] and lung-on-chips serve as microphysiologically relevant models to predict human responses in respiratory diseases. In this review, we provide a critical assessment on immune response to viral infections and evaluate lung-on-chip platforms used for modeling viral infections, while sharing our perspective for the ongoing era of lung-on-chips.

II. A SNAPSHOT OF RESPIRATORY INFECTION OUTBREAKS

In recorded history, the most devastating pandemics are related to airway infections as the lungs are highly vulnerable to viral assaults.[13] For instance, the most severe and deadliest pandemic, also known as the Spanish flu, which spread worldwide between 1918 and 1919, was caused by the subtype of H1N1 influenza A.[14] The occurrence of 1957 Asian flu caused by the influenza A H2N2 virus[15] blocked the human circulation of 1918 pandemic strain of H1N1 viral descendants until 1968, and then the pandemic of H2N2 was replaced by H3N2 Hong Kong influenza. These outbreaks were followed by H5N1 in 2003 and a novel influenza A H1N1 virus in 2009.[16]

Looking at the 21st century, the first non-influenza global respiratory disease outbreak occurred in late 2002, which originated from SARS-CoV.[17] In 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) emerged and caused a major crisis in global public health.[18] Following the two deadly coronavirus outbreaks, the coronavirus disease 2019 (COVID-19) was declared by the World Health Organization as a pandemic in 2020, and SARS-CoV-2 virus, alongside the SARS-CoV and MERS-CoV, became another zoonotic coronavirus affecting humans.[19] Respiratory viruses caused the deadliest events in the recorded human history, and they are also a major cause of morbidity. Apart from both influenza and coronaviruses, there are a number of viral pathogens causing various respiratory infections. For instance, inducing the self-limiting upper respiratory tract infection in healthy individuals, HRV is also known as the major agent of asthma exacerbations in children and adults.[20] Similarly, RSV,[21] parainfluenza virus,[22] and metapneumovirus[23] lead to life-threatening pneumonia by progressing to the lower respiratory tract infection in children, immunocompromised patients, or elderly adults. Measles[24] and adenoviruses,[25] on the other hand, are the other viruses that cause several respiratory illnesses (e.g., cold-like symptoms, bronchiolitis, pneumonia, etc.). As the viruses spread globally, possible mutations of the existing viruses need to be considered to avoid mortality and morbidity. For an accurate prediction of future outbreaks and rapid preventions regarding possible threats, the host–pathogen relation and the underlying mechanisms of the related immune responses must be better understood.

III. IMMUNE RESPONSE TO VIRAL INFECTIONS

Respiratory infections are prevalent due to the respiratory tract being a major entry point into the host.[26] The epithelial tissue lining respiratory tract is the largest interior surface that comes into contact with the external environment[27] and vital for the sustainability of physiological homeostasis. As for pathophysiology, different viruses interact with and infect the respiratory tract in different ways depending on the virus type [Fig. 1(a)]. For example, influenza A infects the alveolar epithelial cells that have sialylated glycans as receptors.[28] Injury to these cells can lead to gas exchange failure and ARDS.[3,28] However, different strains of influenza have different infection mechanisms and targets. H1N1 adsorbs ciliated epithelial cells and goblet cells,[5] whereas H5N1 targets alveolar macrophages.[29]

The innate immune system is the first line of defense against pathogens and is composed of physical barriers such as epithelial cells and mucosal membranes and cellular components such as phagocytes. The respiratory tract contains innate immune cells such as dendritic cells inside the pulmonary interstitium and between epithelial cells to detect pathogenic inhaled particles such as viruses.[26] As early as a week after birth,[30] CD45+ alveolar macrophages are present in the respiratory tract and are the first cells that encounter any viral antigens in the alveolar space.[26] The first event leading to activation of the immune system is the recognition of the viral particles. These are first sensed by the respiratory epithelium [Fig. 1(b)].[31]

A healthy airway tract is equipped with a number of innate immune defense mechanisms including a thin layer of mucus, ciliated cells, anti-microbial peptides, and cytokines (e.g., β-defensins, lactoferrin, and type I and type III interferons). Collectively, these elements contribute to maintaining airway hemostasis by providing a physical barrier, clearance of foreign particles, and killing pathogens. Epithelial cells also secrete chemokines and cytokines to recruit and activate immune cells for response to pathogens when they are detected. These respiratory tract defenses can be triggered by viruses and cause a response by the airway epithelium. This leads to the loss of homeostasis of the airway environment resulting in barrier disruption and delay of epithelial repair [Fig. 1(c)].

Viruses have diverse pathogen-associated molecular patterns (PAMPs) such as DNA, RNA, and surface glycoproteins[33] that are recognized by a group of pattern recognition receptors (PRRs). There are three main types of PRRs that play important roles in viral recognition: Toll-like receptors (TLRs), NOD (nucleotide-binding oligomerization domain) like receptors, and RIG-I-like receptors (RLRs).[34] For example, viral DNA is recognized by TLR9, single strand (ss)RNA by TLR7 and TLR8, and double strand (ds) RNA by RLRs and TLR3.[33] Melanoma differentiation-associated gene 5, an RLR, can also sense non-self RNA and the transcriptional products of a virus in the host cell cytoplasm.[35] Upon viral recognition, the type I interferon response is the first innate response against viral infection.[36] It is a highly optimized response that is effective against a plethora of viral infections.[37] These cytokines activate the antiviral response in the adjacent cells by binding to the IFNAR(α/β) receptor and activating the JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) pathway.[38–41] Viral evolution against interferon response is crucial for viral replication and transmission.[42,43] This interferon response upregulates the effector function of the innate immune cells.[44] Interferon is not the only cytokine involved in the innate response. IL-1β is produced through mRNA synthesis and pro-IL-1β cleavage through caspase-1.[34] IL-1β and IL-18[45] can induce pyroptosis that eradicates the virus-infected cell from the host.[46]

The adaptive immune system is a slower but a more specific response than the innate immune system. The adaptive immune response is composed of cellular (CD4+ and CD8+ T cells) and

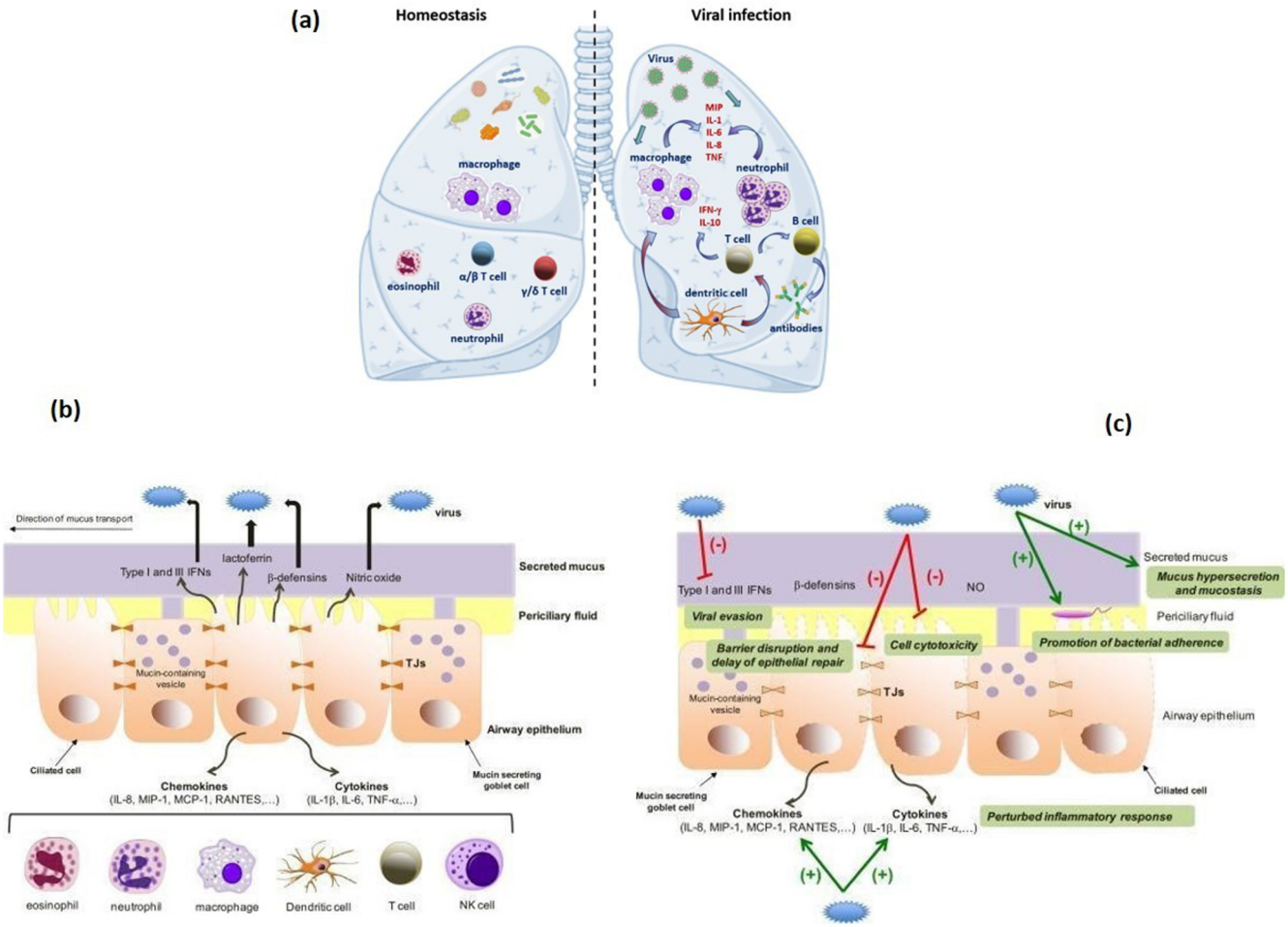

FIG. 1. (a) Schematic illustration of lung homeostasis and immune response toward viral infection in the respiratory system. The image was created by using the visuals in the SMART Servier Medical Art (https://smart.servier.com/) program licensed under a Creative Commons Attribution 3.0 Unported License. (b) Shows the healthy epithelial airway and its defenses against pathogens. Secretion of antiviral substances regulates the innate and adaptive immune system. In the submucosa, secretion of cytokines and chemokines recruits immune cells. (c) Shows the response of the airway epithelium when exposed to pathogens. Viruses can evade the interferon response and cause damage to the epithelium to infect the host and can cause an exaggerated inflammatory response. (b) and (c) Reproduced with permission from Vareille *et al.*, Clin. Microbiol. Rev. **24**(1), 210–229 (2011). Copyright 2011 American Society for Microbiology.[32]

humoral (driven by B cells) arms. T cells destroy virus-infected cells either directly or through release of a tumor necrosis factor that damages the cell.[47] CD8+ T cells are of particular importance in the response to viral infection as they differentiate into cytotoxic T lymphocytes that kill virus-infected cells and inhibit viral replication through the secretion of cytokines and antiviral proteins.[5] During respiratory infection, these cells are activated in the lymphoid tissues and translocate to the site of viral infection. These cells secrete perforin to permeabilize the membrane and granzymes A and B to induce apoptosis in the infected cell.[48] CD4+ T cells aid the CD8+ T cells by promoting engagement of CD8+ T cells with the dendritic cells. CD4+ T cells are also crucial for assisting in the highly specific neutralizing antibody response from B cells.[49]

Antibodies play a vital role in clearing infection[50] and are the product of the adaptive humoral responses.[51] Enveloped viruses can be neutralized by antibodies binding to the viral glycoprotein and preventing viral replication or viral egress.[52] Neutralizing antibodies bind to the virus and result in a reduction of infectious titer.[53] Antibodies can also work through making immune complexes to clear viral infection. Antibody-dependent cellular phagocytosis is one such complex that clears the virus and infected cells, activates other adaptive immune responses through antigen presentation, and promotes secretion of inflammatory mediators.[54] Antibody-dependent cell cytotoxicity is primarily undertaken by natural killer cells, but with respiratory diseases such as influenza, it has also been shown to incorporate lymphocytes,

monocytes, and neutrophils.[55] These cells actively lyse the target cell marked by the antibodies on the cell surface membrane.[56] Antibody-dependent cell mediated viral inhibition is involved in viral clearance and is the measure of Fcγ receptor mediated antiviral activity.[57] IgA antibodies are pivotal in the immune response in mucous membranes and, therefore, against respiratory viruses and are produced in local cells and transported via mucus through transepithelial transport and can kill intracellular viruses.[48] Overstimulation of antibody production can have negative consequences; however, a paradoxical phenomenon can occur with antibodies where they actually promote viral entry and replication, and this is known as antibody-dependent enhancement.[58]

IV. HUMAN LUNG CHIPS FOR VIRAL INFECTION MODELS

The main function of the respiratory system is to supply oxygen and remove carbon dioxide, while maintaining blood pH, filtering xenobiotics from the blood, and serving as a blood reservoir within the pulmonary vasculature. Bioengineering approaches have been applied to fabricate lung-on-chip platforms enabling cultured cells with physiologically related biophysical signals.[59] Existing lung-on-chip models recapitulate many aspects of the small airways, alveolus,[60] and alveolar–capillary interface[61] along with the complex microenvironment. These microphysiological models enabled the study of not only respiratory viral infections but also a number of different respiratory diseases such as lung inflammation,[62] asthma, chronic obstructive pulmonary disease,[63] cystic fibrosis,[64] pulmonary edema,[65] and intravascular thrombosis.[66]

A. Small airway-on-chips

Including the trachea and alveoli, the airways consist of 23 generations of dichotomously branching tubes.[67] The small, non-cartilaginous airways refer to distal airways with an internal diameter of less than 2 mm, and they are located from the eighth generation of airways to terminal bronchioles [Figs. 2(a) and 2(b)].[68] Being the major site of airflow makes small airways vulnerable to the accumulation of infectious agents (e.g., chemicals, viruses, etc.) from the inhaled air during the ventilation. Although sampling from human subjects is quite challenging, small airways are still the major focus in many airway diseases related to the airflow limitation. Moreover, as their contribution to the total respiratory resistance is relatively low, symptoms related to certain pathologies are observed subsequent to extensive damage of small airways. These major challenges motivated researchers to explore the underlying mechanisms regarding the complex functions of these "silent zones" of the airways.[69] One of the first attempts was focused on

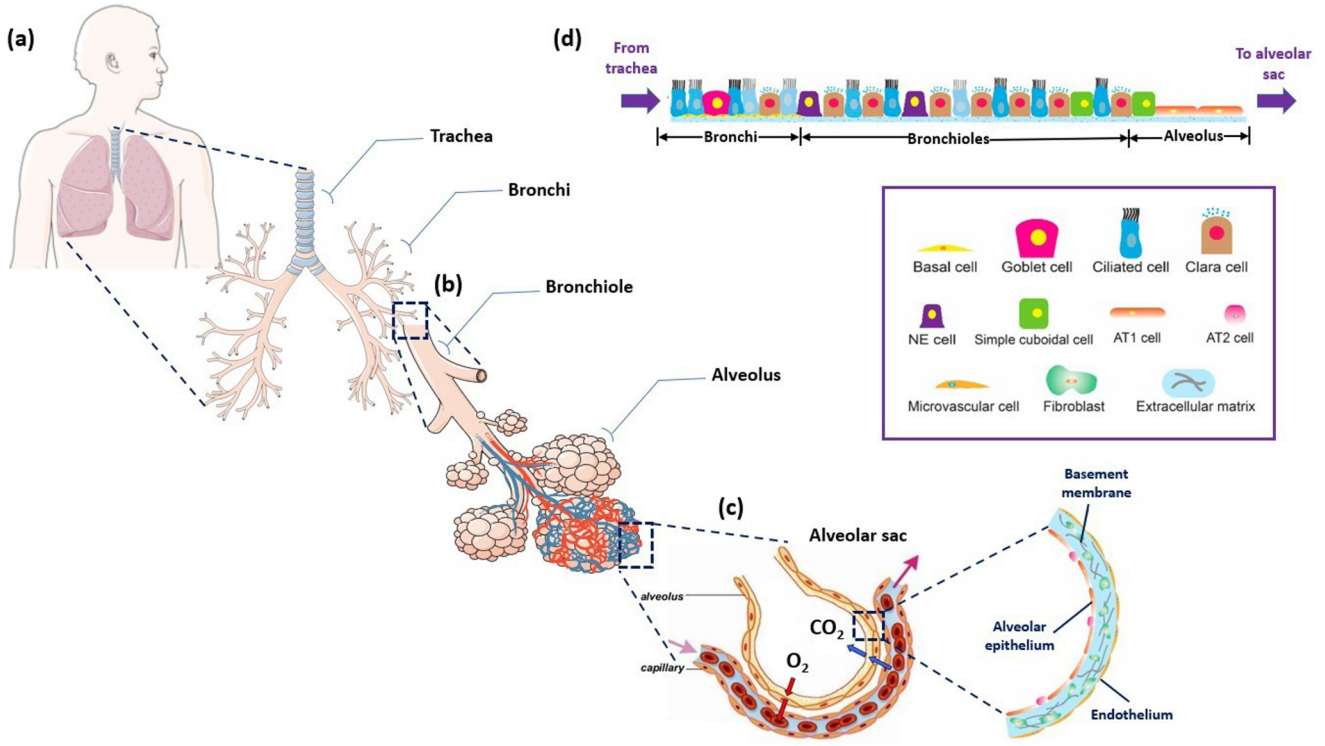

FIG. 2. (a) Schematic depicting human lung, (b) respiratory airways including the bronchioles and the alveolus, (c) gas exchange at the alveolar-capillary membrane of the alveolar sac, and (d) the distribution of the predominant cell types of the human lung. The images were created by using the visuals in the SMART Servier Medical Art (https://smart.servier.com/) program licensed under a Creative Commons Attribution 3.0 Unported License.

demonstrating the biomimetic and multilayered microfluidics to predict the outcomes of the human clinical trials. Huh *et al.*[61] conducted an experimental investigation on lung injuries through microfluidics-based lung-on-chip devices. To generate the air–liquid interface (ALI) where the gas exchange with the blood occurs through, the team designed a compartmentalized microfluidic device where the polydimethylsiloxane (PDMS) membrane was sandwiched between two PDMS microchannels. This reductionist approach provided both *in vivo*-like multicellular structure and physicochemical environment of the lung, and this seminal study became a pioneer for further studies related to the viral and bacterial infections.

As such, Benam *et al.*[62] modified this platform to study small airways, in particular. In their small airway-on chip design, the differentiated, mucociliary bronchiolar epithelial cells were seeded on the top of the membrane exposing to air, whereas microvascular endothelial cells were seeded to the other site of the porous membrane where fluid flows. The developed platform emulated the innate immune cell recruitment in the circulation and allowed researchers to investigate neutrophil recruitment due to the cross-talk between the human epithelium and endothelium along with the inflammatory responses. The patent disclosures, on the other hand, have shown the pattern of tendencies such as reconstitution of the salient structure and the mimicry of function of the living human lung through the platform design, to diverse challenges (e.g., modular sensing apparatus integration) facing modeling and tracking of the virus-induced lung infections.[70–73] One of the most recent examples is the COVID-19 pandemic, which highlights the urgent need for development of humanized models to evaluate novel treatment strategies. With a better understanding of the complexity of microbial infections and related disease mechanisms owing to the experiences in either *in vivo* or 3D *in vitro* studies, researchers have leveraged the airway lung-chip platform to mimic human infection by airborne SARS-CoV-2. Si *et al.*[74] conducted a study to investigate the SARS-CoV-2 viral entry in the human hepatocellular carcinoma cell line, Huh-7, using the pseudoparticles, which express the SARS-CoV-2 spike protein and repurposed the clinically approved drugs to inhibit viral expression. Although Huh-7 is the common cell line to study SARS virus infection,[75] Airway Chip (Emulate, Inc.) where the human lung airway epithelium was layered between air and blood channels was selected to continue, as the target of the SARS-COV-2 is lungs. Following the entry of pseudoparticles into Airway Chip, along with the neutrophil responses, high expression levels of both serine protease TMPRSS2 and entry receptor angiotensin-converting enzyme 2 (ACE2), consistent with the results of native SARS-COV-2 entry,[76] were recorded.

Influenza is another serious viral infection resisting existing drugs due to rapid and continuous evolution ability. Although animal models for influenza virus evolution exist, 3D humanized systems are needed in order to reflect real pathologic milieu. Hence, airway lung-chips have emerged as effective preclinical tools for the development of new antivirals. As such, researchers demonstrated the Emulate's Airway Chip for viral replication of current subtypes of influenza A virus that routinely circulate among people: influenza A/WSN/33 (H1N1) and human influenza A/Hong Kong/8/68 (H3N2). The disruption of the endothelial layer due to the influenza infections was found to be consistent with the clinical data as well as the vascular leakage caused by the *in vivo* virus induction. Moreover, researchers came up with a new approach to mirror patient-to-patient transmission by passaging the influenza virus from one chip to another. Following, the infected airway-chips were successfully treated by the repurposed antiviral therapeutics, proving that the airway chips enable one to identify the potential anti-influenza drugs.[77] Respiratory track defenses against viruses lead to an initial response of the airway epithelium as mucosal hypersecretion to increase the thickness of the mucus layer providing a thicker physical barrier for the viral particles. If the virus evades this barrier along with the defense molecules such as β-defensins, the epithelial barrier can be damaged resulting in the prolongation of the repair process. Subsequently, the evasion can cause an exaggerated inflammatory response by the epithelial cells leading to the recruitment of immune cells to the airway epithelium in an attempt to clear the infection, a process that might also cause further damage to the airway lining. A recent study successfully represented this barrier disruption along with the immune cell recruitment responding to the H1N1 and H3N2 viral infections within the airway epithelium on-chip system.[78] Another seminal study has focused on HRV, which is commonly associated with asthma exacerbation. Although its effects on contractility of airway smooth muscle cells have been proved by several studies,[79,80] the lack of small animal models limited the investigation of the pathogenesis of HRV infection. Thus, Nawroth *et al.*[81] demonstrated the micro-engineered airway lung-chip, similar to the small-airway-on-chip design,[62] which stimulates human mucociliary airway epithelium to study HRV-induced asthma exacerbation. As for the development of novel therapeutics, testing of antibodies in these *in vitro* models might reveal invaluable information as antibodies play a vital role in clearing infection. With an overall evaluation, despite the lack of vascularity and an external immune cell integration, which make organ-chip platforms remain in their infancy for viral infections, these studies proved that the airway chips might be useful tools for virus-caused infections.

B. Lung alveolus chips

The adult human lung has around 300×10^6 alveoli, with a surface area of up to 100 m^2 for efficient gas exchange. A capillary network [Fig. 2(c)] surrounds each of the alveolar sacs, and the alveolar-capillary membrane of the alveolar sac is covered with two types of pneumocytes one side along with the microvascular endothelium on the other side [Fig. 2(d)].[82] Recapitulation of the alveolar structure and its mechanism along with the *in vitro* models of alveolar diseases has been presented not only in the context of publications but also through patent disclosures.[73,83,84] An *in vitro* microfluidic "organ-on-chip" model that was patented by Levner *et al.*[83] consists of co-culture of epithelial and endothelial cells between lamina propria-derived cells similar to other current systems. Inventors declared that this system could be used for modeling inflammatory diseases along with differentiation of cells on chip into lung cells, lung pathophysiology, and pulmonary drug testing. In another patent, Guenat *et al.*[84] improved an *in vitro* model system that simulates *in vivo* conditions of the lung in order to recapitulate tissue interfaces between alveoli epithelium and

vascular endothelium, mechanical breathing process, and blood perfusion. For this, two microchannels were separated by porous, flexible membrane, and pressure was applied in connected channels to cyclically stretch the membrane to mimic respiratory movements, in particular, of lung alveoli. The device has enabled mimicry of the structure and function of an alveolar-capillary system and organ-level responses under physiological and pathological conditions. Pioneering studies have been performed to simulate cyclic stretching of the alveolar membrane induced by breathing movements and resulted mechanical stress on lung cells.[60,61] Douville *et al.*[85] developed a microfluidic alveolar model comprised of a cell culture chamber and an underlying actuation channel separated by a thin flexible membrane where the alveolar epithelial cells were cultivated. The fluid was withdrawn from the actuation channel to induce downward deflection of the membrane and stretching of the epithelium to simulate ventilation-induced deformation of the alveolar epithelial tissue. With this structural deformation, researchers simulated the propagation of airway occluding liquid plugs often found in patient lungs with ventilator-induced lung injury.

Starting from the working mechanisms of the first pioneers of on-chip alveolus platforms in the literature, a great deal of work has been done with microengineered biomimetic microfluidic systems, in which disease models related to viral infections have

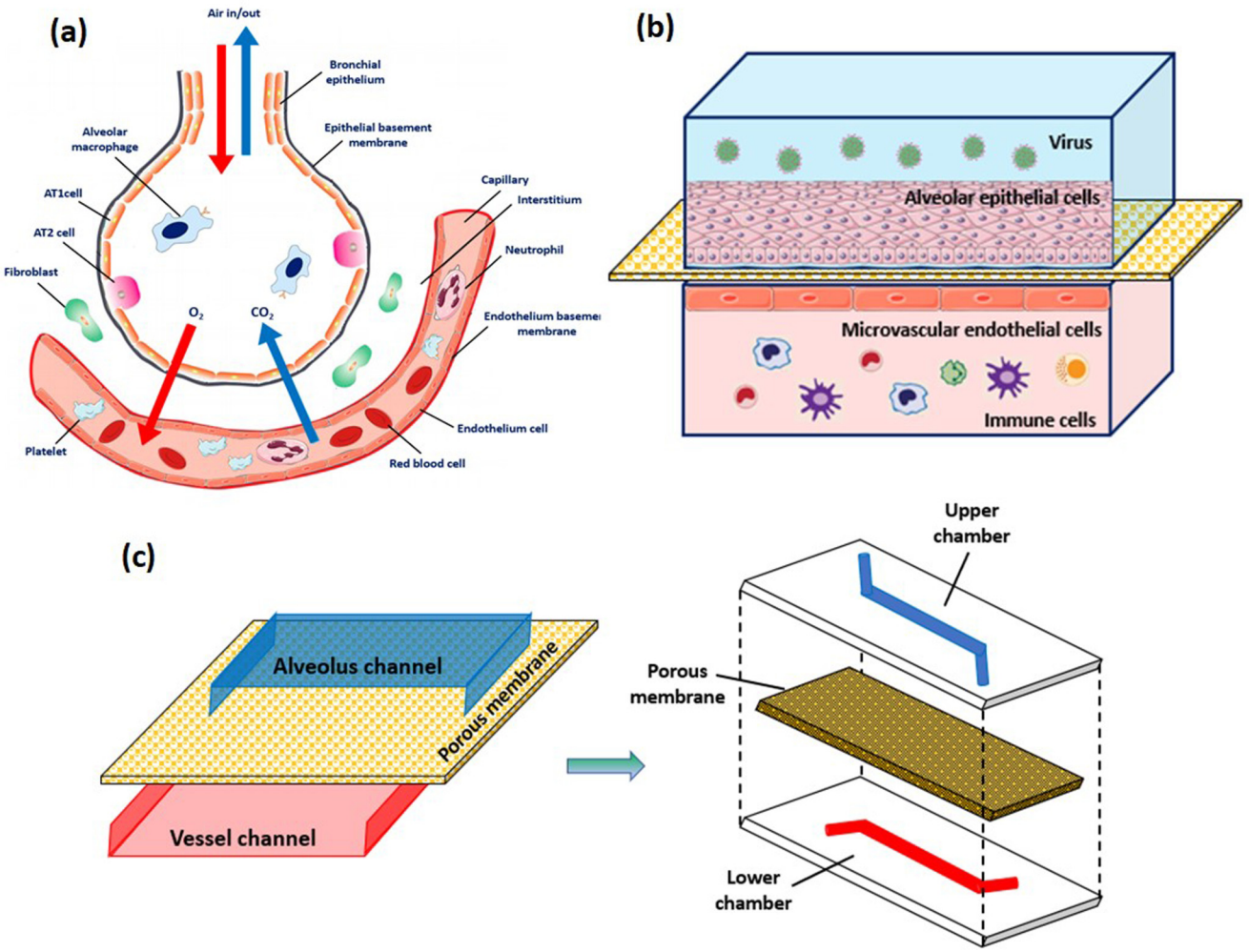

FIG. 3. Bioengineered human alveolus-on-a-chip infected by virus. (a) Schematic of alveolar-capillary membrane pulmonary cells. The alveolar epithelium is composed of two cell types: alveolar epithelial type 1 (AT1) and type 2 cells (AT2). (b) Modeling viral infection on the lung alveolus platform. The developed chip is exposed to virus on the epithelium. Human immune cells secreted by the lung microvascular endothelium are infused into the lower vascular channel during the progression of virus. The viral infection activates the innate immune responses in epithelial and endothelial cells. (c) The configuration of the biomimetic human alveolus chip. The platform consists of an upper alveolar epithelial channel and a lower microvascular endothelial channel separated by a porous membrane. The images were created by using the visuals in the SMART Servier Medical Art (https://smart.servier.com/) program licensed under a Creative Commons Attribution 3.0 Unported License.

been studied in recent years (Fig. 3). Within the framework of viral infection studies, Zhang *et al.*[86] constructed an *in vitro* bioengineered human lung alveolus chip model in which the immune response to SARS-CoV-2 infection was investigated. To generate dynamic culture conditions and form alveolar epithelium–endothelium tissue interface, the culture chambers were suitable for the perfusion of media flow from both upper alveolus and lower microvascular endothelial channels. Subsequent to SARS-CoV-2 virus inoculation to the upper alveolus channel, recruitment and adhesion of circulating immune cells [peripheral blood mononuclear cells (PBMCs) and CD14+ monocytes] along with the increased production of inflammatory cytokines (IL-6, IL-8, IL-1β, and TNF-α) from the culture medium in the vascular channel were observed in a physiologically relevant manner. Moreover, to discover the potential antiviral therapeutic drug against SARS-CoV-2 infection, the team treated the virus-infected human alveolus chip with remdesivir, a promising antiviral against many RNA viruses, and the results demonstrated the potential of the drug in suppressing virus replication and minimizing virus-induced injury of alveolar-capillary barriers.

In another immune-responsive model established by Deinhardt-Emmer *et al.*,[87] the single and co-infections of influenza virus and *Staphylococcus aureus* (*S. aureus*) were used to measure the potential of alveolus-on-chips to mimic pneumonia. To resemble the alveolar tissue in the chip system, vascular and epithelial cells with cocultured macrophages were used, and the presence of macrophages within the system along with the optimized flow conditions resulted in an increased barrier function up to 14 days. Following the single and co-infections of *S. aureus* and the influenza virus, the loss of barrier function associatively led to a significant endothelial cell damage. Notably, their findings verified the pulmonary surfactant secretion from the alveolus chip, which is crucial for the integrity of the epithelial barrier along with the host defense. In another study conducted by Deinhardt-Emmer *et al.*,[88] an *in vitro* human-specific alveolus-on-a-chip model was utilized to recapitulate alveolar architecture and function along with SARS-CoV-2 alveolar infection. To investigate the interaction between endothelial and epithelial cells along with the viral effects on alveolar barrier integrity and inflammatory responses, Calu-3 epithelial and human umbilical vascular endothelial cells with cocultured mononuclear cells (macrophages isolated PBMCs) were used. Subsequent to SARS-CoV-2 virus infection, the virus was attached on the lung epithelium for efficient replication with high viral loads. Their findings demonstrated that although the microvascular endothelium was not invaded by SARS-CoV-2, epithelial cytokines have been released, giving rise to alveolar barrier dysfunction and viral propagation. With these promising pioneer studies, alveolus-on-chip models took their places among the alternative preclinical tools for *in vitro* human viral infections and evaluation of new antiviral therapeutics.

C. Therapeutic interventions

The near-complete picture of human antiviral arsenal has been elucidated by the basic immunology up to now, and this achievement has paved the way for current clinical treatments as well as the development of novel antiviral drugs.[89] Nevertheless, viruses, having the ability to mutate over time, may render treatments less effective and the development of drugs against emerging viruses with pandemic potential may remain infeasible. Therefore, it is imperative to follow a proactive approach requiring financial investment to develop antiviral drugs and vaccines to better understand the viral mechanism and their efficiencies on host–virus interactions.[90] As such, recent biotechnological attempts to uncover host–virus interactions along with related immune reactions vary from genome editing tools (i.e., CRISPR/Cas) to metagenomic analysis.[89]

The most proactive approach to dealing with a virus is immunizing the host to prevent infection and/or minimize the disease severity. There are many success stories where vaccines have achieved these and perhaps most remarkably caused the eradication of diseases such as smallpox and rinderpest.[91] Vaccines work by exposing the immune system to inactivated whole or part of a pathogen that has the required immunogenicity but unable to cause disease. The immune response instigated by the vaccine triggers the development of specific memory B and T-cells that can respond by quickly proliferating and differentiating into effector cells clearing a pathogen before it has the chance to cause disease.[92] Vaccines can be separated into four classical groups: Live-attenuated vaccines;[93] inactivated vaccines;[94] subunit, recombinant, polysaccharide, and conjugate vaccines;[95–97] and toxoid vaccines.[98] These groups use weakened pathogen, dead pathogen, a small part of the pathogen, or a product of the pathogen, respectively. A good example of using different strategies in developing new vaccines is COVID-19 where the current vaccines in clinical trials[99,100] broadly cover the above vaccine groups: Whole virus, protein subunit,[101] nucleic acid,[102] and viral vector.[103] The whole virus uses live-attenuated or inactivated viral particles. Protein subunit and nucleic acid use their namesake, and viral vector vaccines use a different virus to transfer the genetic information to make cells produce the COVID-19 antigen. One of the bottlenecks in vaccine development is poor physiological relevance of animal models for testing vaccine safety and efficacy.[104] This was further highlighted in the recent search for COVID-19 vaccines,[105] which can be a potential application of humanized microphysiological platforms. In the absence of previous immunity and effective vaccines, antiviral drugs targeting virus entry to host cells and/or replication can provide some level of protection. They can either be specific to a virus or broad spectrum to treat a variety of viruses. Antiviral drugs such as Tamiflu (oseltamivir) have been widely used for treatment of influenza,[106] while many others targeting respiratory viruses, such as lopinavir, ritanivir, interferon, and remdesivir, are at different stages of development.[107–109] Antivirals work by inhibiting viral progression at various stages depending on the drug. Main mechanisms of action for common antiviral drugs include inhibition of viral attachment, viral endocytosis inhibition, viral replication inhibitor, signal transduction inhibitor, and viral assembly, budding, and release inhibitor.[110] Due to the limited number of physiologically relevant animal models that can replicate viral infection and non-predictive conventional *in vitro* models, testing the efficacy and side effects of anti-viral drugs could be quite challenging. This was clearly highlighted in the recent COVID-19 pandemic where the lack of suitable animal models continues to hamper search for effective therapies. Indeed, an airway lung-chip lined with epithelial cells expressing high levels of ACE-2 was utilized to evaluate clinically approved drugs. Among seven drugs,

amodiaquine and toremifene were elicited as potential entry inhibitors of SARS-CoV-2.[74]

One of the major consequences of infection with respiratory viruses, seen in both avian flu and COVID-19 patients, is the cytokine storm caused by overactivation of the innate immune system that eventually leads to an increase in vascular permeability causing a massive influx of fluid and blood cells into the alveoli and respiratory failure.[111] The cytokine storm is, therefore, the target of many anti-inflammatory and repurposed antiviral drugs through regulating signaling pathways in the immune cells to modulate cytokine release.[111] This is why drugs such as tocilizumab, a recombinant humanized anti-human IL-6 receptor, have gained significant attraction in the treatment of COVID-19 patients.[112] Broad spectrum anti-viral drugs such as remdesivir, a nucleotide analog that perturbs viral replication by inhibiting the RNA-dependent RNA polymerase, could indirectly suppress the cytokine storm by reducing dsRNA related induction of the NF-κB pathway.[113]

Apart from antiviral drugs, a cell-based therapy is an alternative approach, which is primarily centered around the introduction of phenotype-controlled cells to the body to replace or modulate certain biological processes. Recent advances in cell engineering and cell-delivery systems mean that cell-based therapies may also provide a novel therapeutic opportunity for different diseases such as combating viral infections, including COVID-19, by providing viral immunity and repairing damage caused by the virus.[114] Current cell-based therapies (Fig. 4) for COVID-19 undergoing research include mesenchymal stem cells (MSCs), natural killer cells, dendritic cells, T-cells, and using exosomes.[115]

In ARDs and acute lung injury patients, viral infection is the main cause of mortality.[116] Although the primary approach is to use antiviral drugs for treatments, damages on tissues remain a critical issue as antivirals have no effect on repairments. Therefore, MSCs promoting endogenous repair and having multi-differentiation capabilities count as another attractive approach for cellular therapy. Preventing the overactivation of the immune system (i.e., cytokine storms) through their immunomodulatory ability as well as regenerative potential, MSCs have been widely used in the management of both infectious and non-infectious etiologies.[116] As such, the study

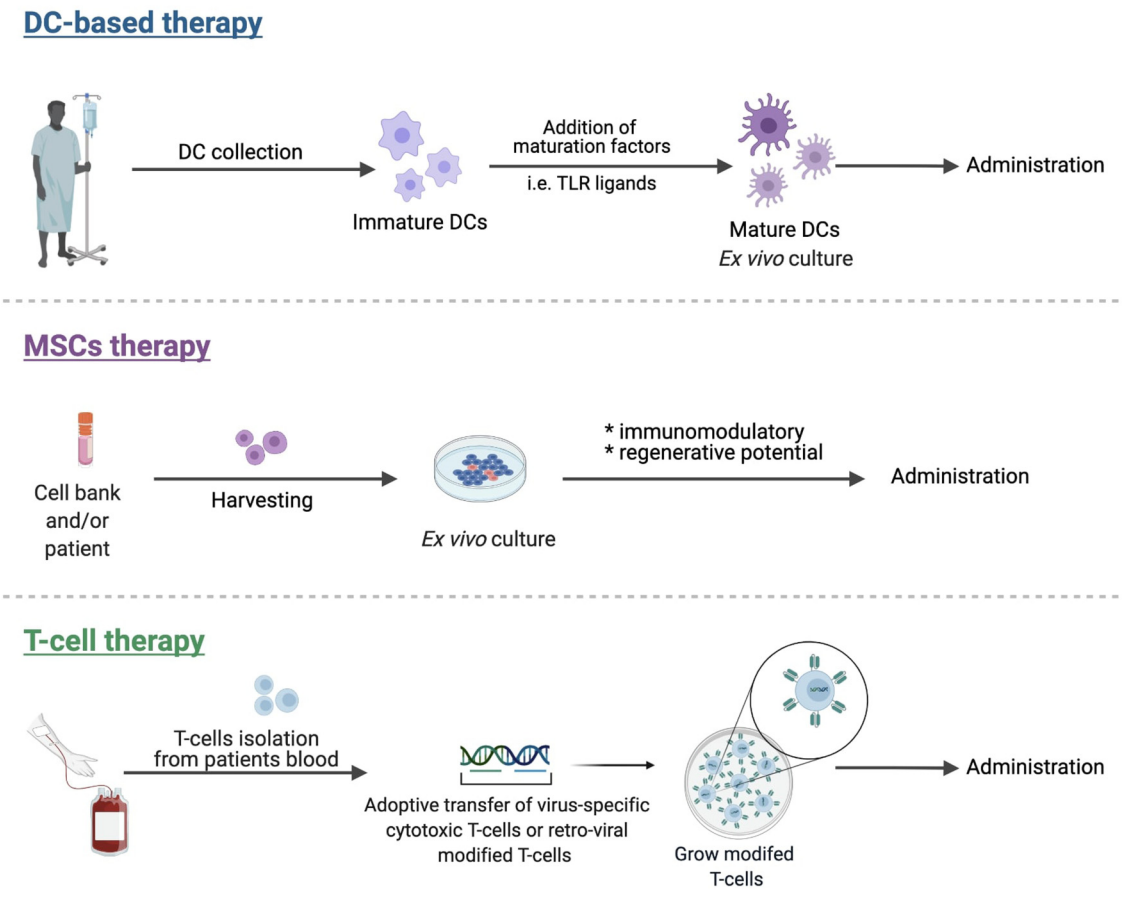

FIG. 4. An illustrative explanation of DCs, MSCs, and T-cell therapies to combat infection or prevent virus-induced lung injury. Adapted from "Cell and Gene Therapy," BioRender.com (2021); see https://app.biorender.com/biorender-templates.

of Chan *et al.*[117] presented the significance of MSCs with reduced inflammation, which was induced by A/H5N1 infection as well as improved survival from viral pneumonia and subsequent lung injury. Similar beneficial effects of MSCs in the treatment of an H9N2 AIV-induced acute lung injury were also reported through mice models in the literature.[118] Moreover, T-cell therapies can also be used for viral infections, which is based on the transfer of donor derived virus specific cytotoxic T-cells to a patient to prevent viral infection or restore virus specific immunity in an immunologically compromised patient.[119] This method uses retroviral-mediated gene transfer to modify antigen specific T-cell clones, which improves the efficacy of previous trials of this method.[120] However, this method has been hindered by limited compatibility of phase III designs with cellular therapy and regulatory restrictions.[121] The dendritic cell (DC) therapy has been widely used for developing DC based vaccination in cancer; however, there are also examples of its use in viral infection. DC based therapies rely on isolating immature DCs from host blood and their *ex vivo* stimulation with viral products and adjuvants so that when these primed DCs return to the hosts, they are presenting viral antigens to stimulate a strong immune response.[122] Given the fundamental differences between the immune systems of most experimental animal models (particularly rodents) and human preclinical evaluation of efficacy and safety of these, potentially transformative therapies have been rather slow with a high rate of attrition in treatments with promising *in vitro* results.[123,124]

D. Limitations and advantages

Lung-on-chip devices have been developed to reflect the exposure of lung to threatening factors (e.g., nanoparticles,[125] drugs,[126] air pollution[127]) and provide an insight on natural or pathogen (virus and/or bacteria) related lung disorders,[62] leading to a burden in socioeconomic life worldwide. Although there are existing animal models, which enable one to build hypothesis on diagnosis and relevant treatments, it is reported that in many diseases, promising medications have failed in human clinical trials due to toxicity in spite of successful pre-clinical results with animal models.[128] Therefore, researchers have focused on developing humanized *in vitro* models on chips. Emulating the human lung pathophysiology *in vitro,* lung-on-chips enable mimicking multicellular structures as well as ALI.[61] Among these advantages, lung-on-chips also proved to have potential to cover virus-associated cell culture models to increase knowledge regarding the underlying mechanisms of viral infections and constituted a basis for future studies. However, cellular therapies using MSCs, natural killer cells, dendritic cells, T-cells, and exosomes have not been introduced to lung chips so far, which may provide new insights into host responses to infection and expedite the development of new therapeutics.

V. CONCLUSION

The knowledge and expertise accumulated through the development of physiologically relevant lung-on-chip models have paved the way for purposing of these models to study the interaction of a number of human respiratory viruses with the airway epithelium and alveolus at an organ relevant setting. Considering the current pandemic, the very few existing animal models[129,130] do not mimic the morbidity or mortality seen in many human patients infected with SARS-CoV-2.[6] Thus, from a broader perspective, the role of human lung-on-chips has become imperative to study the pathogenesis of viral respiratory diseases and investigate host response to infections by deadly pathogens. With the advances in stem cell technology, particularly, human induced pluripotent stem cells (hiPSCs) and lung organoids are being utilized as alternatives to primary cells and cell lines to capture the complexity of the lung tissue. We envision the incorporation of cell-derived matrices[131] and decellularized lung tissues[132] to these platforms along with advanced biomaterials for modeling microvasculature and airway barrier functions as alternatives to existing polytetrafluoroethylene and polyester membranes.[133] Moreover, we anticipate that modeling host–pathogen interactions and understanding of infectious disease mechanisms will be expedited by employing 3D bioprinting technologies,[134] which enable fabrication of 3D *in vitro* models (e.g., cell-laden scaffolds), for the development of therapeutic and vaccination solutions for viral respiratory diseases as the patterned 3D network provides physiologically relevant models[135] and increases the probability of success in clinical studies. Finally, recent advances in the iPSC field[136] provide opportunities for developing patient specific tissue models. Combining such tissue models with organ-on-chip technologies will allow patient stratification, which will be highly valuable in disease modeling for both drug development and in-depth understanding of pathophysiology.

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DATA AVAILABILITY

The data that support the findings of this study are available within the article.

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