The role of biomaterials and three dimensional (3D) in vitro tissue models in fighting against COVID-19

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Over the past century, viral respiratory pandemics have been a leading cause of infectious disease worldwide. A deep understanding of the underlying mechanisms of the viral interactions with host cells at the target sites is necessary for a rapid response to such pandemics. To meet this aim, various testing platforms are required to recapitulate the pathophysiological behavior of the virus within the respiratory tract. These bioengineered platforms can effectively be used for the development of different therapeutics and vaccines. This paper briefly reviews the progress in the areas of biomaterial use for pulmonary tissue regeneration and integration with current bioengineered platforms including engineered tissues, orga- noids, and organs-on-a-chip platforms for viral respiratory disease studies. Finally, a brief overview of the opportunities presented by organ-on-a-chip systems for studying COVID-19 and subsequent drug development is introduced.

1 Introduction

Viral respiratory diseases such as influenza, Severe Acute Respiratory Syndrome (SARS), and Middle East Respiratory Syndrome (MERS) target different host cells in the lower or upper respiratory tract. These viral diseases can cause acute lung injuries and acute respiratory distress syndrome (ARDS) due to lung inflammation, which can precede acute hypoxic respiratory failure. As of September 2020, the recent SARS-CoV-2 global pandemic has affected over 30 million people and has caused approximately one million deaths worldwide. While the global effort is currently focused on “flattening the curve” by practicing social distancing, performing widespread testing, and developing new therapeutics and vaccines, the management of the long-term health issues confronting recovered patients has become the next primary concern. Doctors leading clinical research projects on the long-term consequences of COVID-19 (coronavirus disease 2019) are painting a worrying picture of recovered patients with lung, heart, and/or kidney damage. ARDS-associated respiratory viruses, SARS-CoV-2 in particular, generally lead to an increased serum level of pro-inflammatory cytokines including IL-6 (interleukin 6), IL-1β (interleukin 1 beta), IL-2 (interleukin 2), IL-8 (interleukin 8), IL-17 (interleukin 17A), G-CSF (granulocyte colony-stimulating factor), and GM-CSF (granulocyte-macrophage colony-stimulating factor), followed by pathological symptoms such as fever, fibrosis, and uncontrolled lung inflammation. With such ARDS-associated respiratory viruses, medical X-ray imaging analysis of intensive care patients reveal signs of pulmonary fibrosis, a chronic lung condition stemming from lung tissue scarring, and damage. These viral infections can also cause repetitive alveoli injuries, resulting in idiopathic pulmonary fibrosis (IPF) and dysregulated wound healing. As a feature of pulmonary fibrosis, fibrogenic mediators TGF-β1 (transforming growth factor-beta 1) and PDGF (platelet-derived growth factor) cause excessive proliferation of epithelial cells and accumulation of extracellular matrix (ECM) components, increasing the stiffness of lung tissues and impeding functionality.

Presently, understanding of the underlying immunopathology and systemic inflammation of SARS-CoV-2 infection is required to create effective treatments but is a major clinical and research challenge. In the preclinical drug development stage, validating the efficiency of pre-existing and new drugs should be done in a model that accurately mimics the disease pathophysiology. Most current studies rely on either animal models or traditional monolayer culture systems. However,
given the dependence of human pulmonary function on its physiological characteristics, conventional monolayer culturing systems fail to mimic the complexities of the in vivo respiratory system conditions.\textsuperscript{13,14} In monolayer culture systems, cells are grown on a hard, flat, two-dimensional (2D) surface (e.g. polystyrene), therefore pulmonary fibrosis modeling cannot be achieved. Animal models have also been considered as a lucrative solution for modeling respiratory diseases and therapeutics development.\textsuperscript{15–17} However, none of the available models fully replicate the severe disease cases seen in humans, and the kinetics of replication is more rapid in animal models than humans. This observation could lead to an overestimation of the importance of innate immunity over adaptive immunity in the clearance of SARS-CoV-2, and the importance of antibodies in providing viral protection. Additionally, interspecies genetic variations, cost, and ethical issues remain as major barriers against the use of these models for high throughput screening of therapeutics.\textsuperscript{15,18}

Tissue engineering concepts have recently been developed to overcome the shortcomings of 2D culture methods, and in this way, functional diseased tissue models can be developed for antiviral or antibacterial drug assessment.\textsuperscript{19–21} A three-dimensional (3D) in vitro infected lung model for evaluating the efficacy of novel and existing therapeutic compounds would greatly improve upon the limitations of the conventional models. These models would allow for a better understanding of the underlying host-pathogen interaction in a microenvironment similar to that of a human organ.\textsuperscript{22} A major advantage of such tissue engineering-based models is their compatibility with primary human cells with expressed entry receptors of different viruses (e.g. angiotensin I converting enzyme 2 (ACE2) for SARS-COV-2), rather than the use of immortalized animal cell lines (e.g. Vero E6 cells).\textsuperscript{23,24} Moreover, small rodent disease models including genetically-modified mice, modified mice with adenoviruses or CRISPR, or animals infected with mouse-adapted viruses, do not mimic the proper viral disease pathogenesis and observed lung disease phenotypes seen in hospitalized patients.\textsuperscript{25} Finally, the limitation in the speed of production of these genetically modified mice in viral pandemic conditions restricts the pace of vaccine development studies, while in vitro tissue-engineered models do not suffer from similar restrictions.

Accordingly, the goal of this review (summarized in Fig. 1) is to provide an overview of the different biomaterials used in engineered 3D in vitro lung models and existing virally infected lung models. Further, a final section presents the future trajectory of SARS-CoV-2 infected model development using lung-on-a-chip concepts for evaluating the efficacy of novel viral treatments.

2 Biomaterials for in vitro lung tissue bioengineering

Regeneration of human lung tissue is limited due to its poor capacity for regeneration following the disruption of the alveolar-capillary membrane barrier. As such, there is a critical need for new biomaterials and tissue engineering techniques
to increase the overall regenerative capacity of injured lung tissues. The material source of engineered lung tissue scaffolds can be categorized into two groups; natural and synthetic/artificial biomaterials. For example, collagen, Matrigel, Gelfoam, PGA (polyglycolic acid), and Pluronic™ F-137 are widely utilized polymers in lung tissue engineering. Collagen-glycosaminoglycan, used in the initial study on lung tissue engineering reported by Chen et al., allowed for organized, ciliated, pseudostratified epithelium and presumptive alveoli, observable on the scaffolds.26 Recently, Wang et al. used biomimetic collagen with collagen-binding hepatocyte growth factor (CBD-HGF) to fabricate a 3D collagen scaffold for alveolar regeneration after acute lung injury.27 As demonstrated in Fig. 2A–C, endothelial cells and alveolar stem cells successfully entered into the surface engineered collagen scaffold in the early stages of regeneration. Moreover, inflammation and fibrosis decreased with the use of this modified hydrogel scaffold, while the functional alveolar structure was shaped in the later stage of regeneration.27 Matrigel, a gelatinous protein mixture secreted by sarcoma cells, has also been widely used in tissue engineering and lung tissue applications.28 The applicability of Matrigel in pulmonary tissue regeneration was reported by Mondrinos et al.,29 and compared to PLLA (poly-L-lactic acid) and PLGA (poly(lactic-co-glycolic acid)) 3D structures for culturing AE2 (alveolar epithelial type II) cells and FPC (murine fetal pulmonary cells). With the addition of tissue-specific growth factors, the researchers observed alveolar structure formation of FPC and epithelial branching morphogenesis of the distal lung architecture in the Matrigel 3D structures, but not in PLLA and PLGA structures.29 Gelfoam, a gelatin-based porous sponge, is another example of a natural scaffold biomaterial that holds promise for biocompatibility and proliferation of fetal lung cells for 35 days, which is mandatory for the appropriate differentiation and proliferation of alveolar epithelial cells.30 Hyaluronic acid (HA) is a molecular weight and non-sulfated glycosaminoglycan biopolymer that is an ECM component in the majority of human tissues. In this regard, a tunable methacrylated HA-based hydrogels (MA-HA) was introduced by Spearman et al. to be used in the in vitro regeneration of lung tissue,31 known to be a soft tissue with the lowest modulus (0.31 ± 0.04 kPa) among the muscle, nerve, kidney, spleen, heart, brain and liver. Considering the mechanical properties of host lung tissue, MA-HA hydrogels can therefore be used to design appropriate scaffold for in vitro regeneration and modeling.

Synthetic polymers have also attracted research attention in the area of tissue engineering, and specifically lung tissue engineering. For example, PDLLA (poly(D, L-lactic acid)) was utilized to culture human lung epithelial cells, which could provide a biocompatible environment for pneumocytes and be used for lung epithelial cell growth.32 In another study, substantial vascularization and alveolar regeneration of pulmonary tissues were reported using PGA scaffolds.33 However, the surface properties of the fabricated scaffolds demonstrated considerable effects on the function and phenotype of cultured cells including adhesion, proliferation, differentiation, migration. Lacking the appropriate surface properties for cell seeding, the synthetic PGA polymer failed to drive cell function. Subsequent surface modification of the material with natural ECM proteins showed promising results with enhanced cell adhesion and proliferation.34,35

The in vitro regeneration of the vascular structures of the airway is essential for lung function; in this respect, many strategies have been developed to target this specific regeneration. Although synthetic materials provide beneficial features such as suitable mechanical properties and greater cell adhesion and proliferation, these materials are less suited to the construction of branching networks or the generation of in vivo cell microenvironment conditions, which acts as a bridge between epithelial and endothelial cells. Additionally, native ECM is composed of different proteins (e.g. collagen and fibronectin) that create important structural and biochemical supports for cells, which are not found in synthetic scaffolds. To this end, decellularized lung scaffolds have been introduced to overcome the weaknesses of synthetic scaffolds in lung tissue engineering. To obtain a decellularized scaffold, cellular components of the lung tissue ECM are removed, while other essential components are preserved. Using this method, Lwebuga-Mukasa et al. investigated decellularized lung scaffolds of various animal species and the functional performance of implanted lung tissues after grafting. The biological performance of these scaffolds was confirmed by nutrient transference with the aid of respiration and ventilation, which has a vital effect on the differentiation of pneumocytes. In a study by O’Neill et al., decellularized porcine lung tissue demonstrated suitability for lung tissue engineering applications.36 However, species-dependent cell–matrix interactions and species-specificity of decellularization in different models of lung regeneration use these types of matrices for tissue engineering applications challenging. Balestrini et al. assessed decellularized scaffolds produced from rats, pigs, primates, and humans.37 They observed that the collagen level after decellularization was similar between animals, however, the human and primate lungs were stiffer, contained more elastin, and retained fewer glycosaminoglycans than pig or rat lung scaffolds.37 Moreover, decellularized matrices can provide suitable micro-mechanical properties through which cell–matrix interactions such as distribution, differentiation, and function of lung cells could be regulated by mechanical cues.38

Over the last decade, nanotechnology has made a large impact on regenerative medicine approaches. Angiogenesis is one of the most prominent mechanisms in healthy or diseased tissue formation, and hereby making the proper stimulation or inhibition of angiogenesis a vital step in the regeneration and modeling of in vitro functional tissues. The role of nanotechnology in the manipulation of angiogenesis was reviewed by Kargozar et al.,39 Moreover, angiogenesis phenomena were investigated in viral associated lung diseases like COPD (chronic obstructive pulmonary disease) or SARS-COV-2, in which angiogenesis is dramatically increased or reduced, respectively.40,41 In this regard, nanoparticles can potentially be used in adjustment and manipulation of the angiogenesis.
in *in vitro* tissue models. As an example, the physicochemical and mechanical properties, as well as the angiogenesis behavior of bioactive glasses (BGs) and their composites with different polymers were studied and demonstrated promising potential for regenerating injured pulmonary tissues. The surface of sol–gel 58S glass scaffolds was coated by an amine, laminin, and mercaptan groups, which increased the biocompatibility of the BGs with murine lung epithelial cells (MLE-12).42 In the other study, ZnO (zinc oxide) nanoparticles were incorporated into poly (vinylidene fluoride-co-trifluoroethylene) (PVDF-TrFE) electrospun nanofibers, for alveolar tissue regeneration applications with significant features such as angiogenesis, anti-inflammation, and high mechanical properties into lung tissue engineering.

### 3 Three dimensional *in vitro* respiratory infectious disease models for drug testing

Towards the aim of studying pulmonary infectious diseases, the *in vitro* generation of the respiratory tract with biomimicry of the physiology and function of human lung tissue is an essential need. Along with the important role of biomaterials, the impact of bioengineered tissue platforms becomes evident. Generally, 3D *in vitro* infected airway models are used for two main types of studies, including (1) infectivity research to understand how viruses infect epithelium cells in the respiratory tract and (2) screening of antiviral therapies to understand their functional efficacy in the human body environment.
3D bioprinting is a well-known and powerful tool used for fabricating bioengineered tissues. This technology has been widely used for creating in vitro disease models relevant to lung tissue. For example, seasonal influenza A virus (IAV) infection has been successfully modeled using 3D-printable cell-laden hydrogels. In one study, the spatial distribution of alveolar epithelial cells (A549) was achieved by blending different compositions of alginate, gelatin, and Matrigel hydrogels. The nonhomogeneous clustered infection pattern, efficient replication of the virus (H3N2, Pan99), and anti-inflammatory response of the epithelial cells with IL-29 (interleukin 29) secretion in the bioprinted lung tissue model closely mimicked the occurrences in human lungs with IAV infection (Fig. 2D–F). In another study focused on investigating IAV pathogenesis, primary human small airway epithelial cells were cultured on chitosan/collagen scaffolds at an air–liquid interface and infected with IAV subtypes H1N1 and H3N2 strains. The primary epithelial cells in the developed 3D model expressed significant AQ5 (aquaporin-5) and CK14 (cytokeratin-14) when compared to 2D models of the lung tissue. Following viral infection, the researchers showed that, along with cell morphological changes such as enlarged nuclei and structures resembling inclusion bodies, pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) were released and the immune responses were distinctly increased.

Airway organoids (AOs) derived from stem cells (pluripotent stem cells (PSCs) and organ-specific adult stem cells) are 3D structures in which the specific physiological functions of respiratory systems can be accurately emulated. Various viral infectious respiratory diseases including influenza, RSV (respiratory syncytial virus), enterovirus, and cryptosporidium have been successfully modeled using different airway organoid cultures, which are suitable for medium- to high-throughput drug screening studies. As an example, the specific interaction between RSV and host epithelial cells was modeled using AOs with iPSC (induced pluripotent stem cells) – derived human airway epithelium. Using this model, the researchers could monitor the secretion of IP-10 (interferon gamma-induced protein 10) and RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) from RSV-infected AOs and the substantial chemo-attraction of the neutrophils to the infected cells. Moreover, following treatment with palivizumab (an antibody against the RSV F-glycoprotein that prevents RSV-cell fusion) the viral replication stopped in the developed infected organoid model.

Other influenza virus series (e.g. avian (H7N9), pandemic H1N1, and parainfluenza) that cause human infections have been modeled using lung organoids. For example, a transwell multicellular culture of AOs including four types of airway epithelial cells (ciliated, goblet, club, and basal cells) was conducted to evaluate the infectivity of the H7N9 and H1N1 pathogenesis on adult stem cell-derived organoids. The AOs morphologically and functionally simulated the human airway epithelium and may be applied for the rapid assessment of respiratory virus infectivity. AO models have the potential to overcome the limitations of reproducibility and availability of the current in vitro modeling systems, to study newly developed antiviral drug efficacy and repurpose current antiviral drugs. Moreover, these multicellular in vitro AOs can discriminate the target of the infection through different host-virus interactions and be used to model infection within the different areas of respiratory tissues through either basolateral or apical epithelial cell infection. To screen COVID-19 antiviral inhibitors, researchers have recently generated SARS-COV-2 organoid models using different cell types expressing the specific virus receptor (ACE2) with the capability of hosting the virus. They used kidney organoids derived from human embryonic stem cells and showed that human recombinant soluble ACE2 (hrsACE2) protein inhibited the growth of SARS-COV-2. While the PSC and ASC (adipose-derived stem cells) - derived AOs may be used to imitate lung architecture that plays a key role in various physiological functions, these models lack lung tissue microenvironmentstomal cells, including immune cells and endothelial cells. In this regard, AOs encapsulated in suitable hydrogel-based biomaterials like Matrigel, laminin, or collagen-family hydrogels with incorporated immune components (e.g. macrophages and natural killer cells that modulate severe COVID-19) may better recapitulate the function of the respiratory system in different studies.

Moreover, AOs can be considered as the primary component of the bioengineered in vitro lung-on-a-chip models to precisely mimic the microphysiology and function of lung tissues.

Microfluidic-based culture platforms have become widely utilized as an alternative to traditional animal models in virology studies. This technology is capable of replicating organ-level physiology, physiological, and pathological responses of the tissue microenvironment. It provides a promising strategy to study virus-induced diseases, therapy-resistance progress, and innovative antiviral therapeutics for human respiratory diseases. As an example of biomimetic microfluidic cell-culture devices, Benam et al. developed a human lung small airway-on-a-chip, which supported differentiation of pseudosтратified columnar epithelium with cells isolated from both healthy individuals and patients with COPD and can replicate clinical exacerbation by exposure to viral/bacterial infections. Given that pathogenic infections are the main source of COPD exacerbation, the researchers simulated viral and bacterial infections in vitro with viral mimics poly(I : C) and lipopolysaccharide endotoxin (LPS), respectively. Moreover, the secretion of different cytokines such as IL-8 and M-CSF (macrophage colony-stimulating factor) in the COPD chips was analyzed. M-CSF levels were found to rise only in poly(I : C) treated small airway chips, suggesting that M-CSF could serve as a new biomarker for COPD exacerbations stimulated by respiratory viruses.

Organ-level responses to bacteria and subsequent secretion of inflammatory cytokines have been further studied using a human breathing lung-on-a-chip microdevice. This device integrates the biological, mechanical, and chemical functions of living lungs, allowing for quantitative analysis and extensive visualization of various cellular responses. To demonstrate the ability of the microdevice to replicate the immune response to...
bacterial infection, *Escherichia coli* bacteria were added to the alveolar microchannel of the device. Following microbial infection on the micaalveolar surface, the migration and phagocytic activity of the neutrophils were initiated until the infection was cleared. In the other study, a human airway lung-on-a-chip device developed by Nawroth et al. was designed with differentiated mucociliary bronchiolar airway epithelium underlined by a microvascular endothelium. A pro-inflammatory response was evoked by exposing the airway chip to human Rhinovirus (HRV), the source of asthma exacerbation, which led to the recruitment of neutrophils flowing through the endothelium. The researchers also compared the HRV-infected IL-13 (interleukin 13) – treated airway chips with HRV stimulation alone, which showed an increase in neutrophil recruitment as well as a dramatic change in the secretion of the inflammatory markers such as IL-6, IFN-γ, and CXCL10.

IAV, another cause of respiratory infection disease, was studied via similar human organ-on-a-chip microfluidic platforms. Generally, the mutation of seasonal influenza is caused by rapid evolution among human populations, making previously existing vaccinations ineffective. Therefore, the development of a new culture technology is necessary to better predict the evolution of the virus and the therapeutic approach. An IAV chip developed by Si et al. consisted of two parallel microchannels and a membrane coated with an ECM scaffold. Primary human lung airway epithelial cells (HLEACs) and human pulmonary microvascular endothelial cells (HPMVECs) were cultured on one side exposed to an air-liquid interface and on the other side in the presence of the medium, respectively. The mutation in IAV evolution through gene reassortment by the transmission of the virus from patient-to-patient was simulated by transmitting the virus between chips with various antiviral drug pressures. It was shown that 8 human chip-to-chip passages led to the formation of an amantadine-resistant virus population. To study the crosstalk of co-infection with *Staphylococcus aureus* and IAV, monocyte-derived macrophages and alveolar epithelial cells (NCI-H441 cells) were cultured in a chamber in contact with the air phase, within an organ-on-a-chip based platform. In this device, human umbilical vein endothelial cells (HUVECs) were cultured in the lower chamber and were provided with a medium. As shown in Fig. 3A–C, with the integration of macrophages as the largest population of resident cells in the respiratory tract, a better air-blood barrier function of the model was acquired through which the interaction of the immune system with the host–pathogen was monitored. As a result, high levels of IL-1β, IL-6, MCP-1 (monocyte chemoattractant protein-1), and IFN-γ (interferon-gamma) secretion, as well as greater epithelial barrier function disruption, was observed in the co-infection condition, when compared to single infection conditions.

4 Conclusion and the perspective of organs-on-a-chip for COVID-19 studies

Considerable effort has been put towards the development of functional airway tissue models. These models aim to recapitulate the critical functions of the respiratory system and allow for the concurrent analysis of the organ-level pathophysiology of the lung with molecular-scale resolution. However, to develop a comprehensive model for studying the pathogenesis of viral associated respiratory diseases, three prominent criteria must be considered, namely biomaterial type, cell type as the virus target, and assembly method. It should be noted that the lung microenvironment can be controlled by tuning the cellular architecture, composition materials, and blood perfusion conditions. The combination of an appropriate biomaterial (as a key component of the extracellular matrix of the airway system) with disease-relevant cell types in the form of airway organoids constitutes a tissue-like structure with which promising functional and morphological properties can be generated. Moreover, to regenerate and model the critical conditions of the lung tissue microenvironment, improvements upon and optimization of lung-on-a-chip systems are unmet needs and may pave the way toward discovering new
drugs and treatments for complicated pulmonary diseases such as novel SARS-CoV-2. Furthermore, integrating lung-on-a-chip with other organs-on-a-chip systems including heart, liver, and kidney, is a promising approach toward discovering potential side effects of newly formulated drugs and vaccines for SARS-CoV-2. Combined organ-on-a-chip systems could additionally provide a rapid and cost-effective multi-organ response to newly formulated therapeutic molecules.

Similarly to other respiratory viral infectious diseases, elucidating the pathophysiological processes of COVID-19 is a crucial step for developing drugs and vaccines. By gaining insight into the interactions of the virus with the respiratory tract host cells and residential immune cells, access to bioengineering platforms, such as organ-on-a-chip models, is an essential need. Using these models, the virus-involved cellular compartments at the onset of the virus entry and replication within the body environment can be mimicked, and consequently, the mechanism of action of a drug or vaccine, absorption, distribution, toxicity, and genotoxicity can be evaluated. As SARS-CoV-2 targets the respiratory tract and leads to complications such as pneumonia, lung-on-a-chip technologies are necessary for evaluating the efficacy of new drugs and vaccines. The organ-on-a-chip technology may also expedite the repurposing of previously FDA-approved drugs for COVID-19 treatment. In this regard, to simulate infection, Si et al. developed a microfluidic air channel containing human lung epithelial cells with exposure to SARS-CoV-2 pseudo-particles. A therapeutic study showed that toremifene and amodiaquine may be potential entry inhibitors of SARS-CoV-2. Microfluidic-based technology has shown an indisputable potential for providing effective in vitro models for bacterial and viral infections causing human respiratory diseases by integrating epithelial, endothelial, and immune cells, however, there is still a sensible lack in models with incorporated human lung cartilage and other vascular and immune components. Therefore, more realistic models comprised of biomaterials mimicking the complexities of native lung ECM are needed to further investigate new therapeutic approaches to novel viral respiratory diseases.

Conflicts of interest
There are no conflicts to declare.

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