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Review

Bioengineered *in Vitro* Tissue Models to Study SARS-CoV-2 Pathogenesis and Therapeutic Validation

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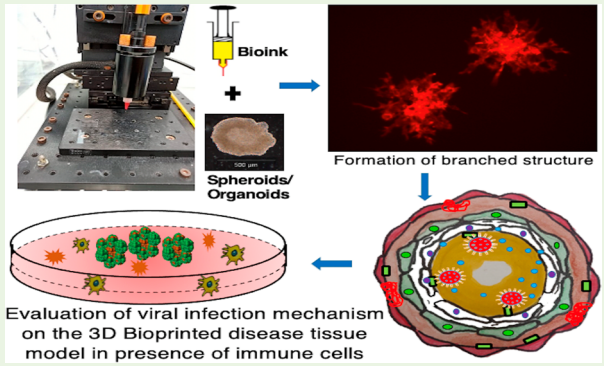
ABSTRACT: Given the various viral outbreaks in the 21st century, specifically the present pandemic situation arising from SARS-CoV-2 or the coronavirus, of unknown magnitude, there is an unmet clinical need to develop effective therapeutic and diagnostic strategies to combat this infectious disease worldwide. To develop precise anticoronavirus drugs and prophylactics, tissue engineering and biomaterial research strategies can serve as a suitable alternative to the conventional treatment options. Therefore, in this Review, we have highlighted various tissue engineering-based diagnostic systems for SARS-CoV-2 and suggested how these strategies involving organ-on-a-chip, organoids, 3D bioprinting, and advanced bioreactor models can be employed to develop *in vitro* human tissue models, for more efficient diagnosis, drug/vaccine development, and focusing on the need for patient-specific therapy. We believe that combining the basics of virology with tissue engineering techniques can help the researchers to understand the molecular mechanism underlying viral infection, which is critical for effective drug design. In addition, it can also serve to be a suitable platform for drug testing and delivery of small molecules that can lead to therapeutic tools in this dreaded pandemic situation. Additionally, we have also discussed the essential biomaterial properties which polarize the immune system, including dendritic cells and macrophages, toward their inflammatory phenotype, which can thus serve as a reference for exhibiting the role of biomaterial in influencing the adaptive immune response involving B and T lymphocytes to foster a regenerative tissue microenvironment. The situation arising from SARS-CoV-2 poses a challenge to scientists from almost all disciplines, and we feel that tissue engineers can thus provide new translational opportunities in this dreadful pandemic situation.



KEYWORDS: *SARS-CoV-2, tissue engineering, 3D bioprinting, organoids, vaccine trial, in vitro model*

1. INTRODUCTION

Throughout human history, mankind has witnessed several viral pandemics, and presently, the world is living on the edge, fighting a race against coronavirus disease 2019 (COVID-19) that has pushed the human race to a time of extreme socio-economical and health-related uncertainty. The pandemic is caused by the viral pathogen called severe acute respiratory coronavirus 2 (SARS-CoV-2, formerly known as 2019-nCoV), which was first reported in the Hubei province of China in December 2019 and has spread throughout the world at lightning speed, affecting 216 countries and territories and claiming more than 1 million lives within 10 months. With plummeting infections each day and growing concerns of a "second wave" of infection in different regions, the pandemic demands prompt action on many fronts, from finding simple and accessible testing for the virus to developing effective vaccines and antivirals. In this extraordinary crisis of the human race, medical researchers and engineers across the world are actively collaborating, sharing knowledge and expertise, to provide innovative solutions in the swift development of diagnostic tools and preventive and curative strategies and to devise new technological platforms to uncover how SARS-CoV-2 operates.

1.1. Profile of the Killer Virus. Coronaviruses (CoVs) constitute a large family of single-stranded RNA viruses with a vast difference between the members within the family. This family of viruses is the largest known of RNA viruses causing diseases in mammals and birds, and they are divided into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus.[1] Of the diverse groups of CoVs, six CoVs are recognized to infect humans, including the alpha-CoVs HCoVs-NL63 and HCoVs-229E and the beta-CoVs HCoVs-OC43, HCoVs-HKU1, Severe Acute Respiratory

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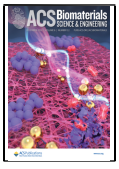

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syndrome-CoV (SARS-CoV), and Middle East Respiratory syndrome-CoV (MERS-CoV).[2] Both SARS-CoV and MERS-CoV are highly pathogenic and caused the 2003 SARS and 2012 MERS outbreaks. The third coronavirus to emerge in the human population is SARS-CoV-2, which was identified in China through a surveillance mechanism for "pneumonia of unknown etiology," originally established during the 2003 SARS outbreak for timely identification of novel pathogens.[3] Although SARS and MERS have remarkably higher case fatality rates (CFRs) than COVID-19, the latter is more infectious as the underlying SARS-CoV-2 virus spreads more easily among humans, outweighing the overall number of deaths from SARS and MERS. Upon the release of their genome into the cytosol of the host cell, coronaviruses use a distinct coding strategy: the virus translates two-thirds of its RNA into two large polypeptides that are further cleaved by proteases to give rise to nonstructural proteins, and the remaining viral genome is transcribed into a nested set of subgenomic mRNAs.[4,5] The spike (S) proteins protrude from the viral envelope and appear like the "spikes of a crown" under the electron microscope, from which the virus has derived its name.[6] On the basis of the data obtained from the structural[7,8] and biochemical studies,[9,10] the S protein of the SARS-CoV and SARS-CoV-2 binds to the human angiotensin-converting enzyme 2 (ACE2) membrane receptor protein. While the S1 subunit of the S protein facilitates viral attachment to the surface of target cells by engaging the ACE2 receptor, the viral entry additionally requires priming of the S protein by a membrane protease TMPRSS2.[11] Following attachment to the host cell surface, the S protein is cleaved at the S1/S2 site by TMPRSS2, which allows S2 subunit-driven fusion of the virus along with the cellular membranes.[11] Once inside the target cell, the virus hijacks the cellular machinery, makes myriad copies of itself, and finally leaves the cell to invade new cells. If the immune system of the infected individual does not beat back the virus during the initial phase of infection, the virus treks down the windpipe and attacks the lungs, which can turn deadly.

1.2. Emerging Perspectives to Combat COVID-19 Pandemic. The unique and unpredictable pattern of COVID-19 infection and the lack of effective vaccines and antivirals have pushed the global human population to the edge. However, the world responded to the pandemic with remarkable speed and with prompt community interventions and a rapid boost in COVID-19-related research. Without the availability of any SARS-CoV-2-specific antivirals or vaccines, several attempts are currently being made to repurpose existing drugs as potential countermeasures and develop effective vaccines using existing and novel strategies. To meet this important goal, the current healthcare strategies involve treatment by experimental antiviral drugs such as Favipiravir,[12] Remdesivir,[13] and Lopinavir−Ritonavir[14] invasive/noninvasive oxygen support.[15,16] In addition, various off-label therapies such as antiretroviral, anti-inflammatory components, anti-parasitic agents, and convalescent plasma therapy are also being explored.[14,17,18] At the same time, it underscores the need for personalized medicine, which involves treating a patient by keeping in view the individual's health in terms of distinct characteristics including age, genetic constitution, height, weight, diet, environment, already existing disease, etc. Tailoring treatment to the genetic profiles of the individuals provides an opportunity to contemplate treatment of patients with a probability of a better response, simultaneously escalating the dose density and potency without the need of uplifting the toxicity profile.

In this overwhelming pandemic situation, stem-cell-derived *in vitro* tissue models and therapeutics involving tissue engineering strategies can serve as a blessing. Conventionally, tissue engineering had focused on developing engineered constructs for organ replacement or regeneration. Despite the enormous potential, very few engineered constructs were successful so far in human clinical trials.[19] Tissue engineering strategies can provide innovative solutions and can be well applied in this vicious situation involving a constellation of unknown questions. Tissue engineering can be employed for the development of a healthy tissue model (such as human hair follicle,[20] cartilage tissue[21]) as well as a diseased tissue model (osteoarthritis model,[22] ophthalmic pathological condition[23,24]), etc. Three-dimensional bioprinting enables even more advancements, as it entails the layer-by-layer specific positioning of the living cells, biochemistry, growth factors, orientation of ECM, etc. When combined, a 3D structure can be fabricated which simulates the ultrastructure of the native tissue.[25] In this Review, an attempt has been made to propose how organ-on-a-chip, 3D bioprinting, organoids, and advanced bioreactor models composed of a coculture of cells from endodermal, mesodermal, and ectodermal origin can be used in this dreadful pandemic situation arising from SARS-CoV-2 to develop *in vitro* human tissue models that can be exploited for more efficient diagnosis, drug delivery, and customized development of drugs and vaccines and the delivery of small molecules at targeted anatomical sites.

2. PHYSIOLOGICALLY RELEVANT *IN VITRO* MODELS—THE NEED OF THE HOUR

The use of animal models and *in vitro* transformed cellular systems in viral infection studies enabled historic developments in vaccines and therapeutics against several viral pathogens. However, some pathogens have unique host specificity, because of which many human pathogens fail to infect animal models. Moreover, owing to differences in immune response upon challenge with pathogens, animal infections often fail to reproduce or poorly mimic human infection pathophysiology.[26,27] Despite the inherent limitations of animal models in studying human infections, attempts were made to identify suitable animal models for studying specific viral infections. Recently, Chandrashekar et al.[28] and Deng et al.[29] used rhesus macaques to study SARS-CoV-2 infection, where they established that the animals recapitulated human COVID-19 infection. Despite differences between SARS-CoV-2 infection in macaques and humans, the macaques infected with the virus developed an immune response that protected them during viral rechallange.[28,29] Upon administration of an adenovirus-vector-based ChAdOx1 nCoV-19 vaccine in rhesus macaques, the animals developed a robust humoral and cell-mediated immune response. The immunized animals showed significantly reduced viral titers in bronchoalveolar lavage fluid and lower respiratory tract tissue as compared to the control animals after challenging them with SARS-CoV-2, and the vaccinated animals did not develop viral pneumonia.[30] Another study showed that rhesus macaques immunized with an array of DNA vaccines against the S protein of SARS-CoV-2 developed a humoral and cell-mediated immune response upon the SARS-CoV-2 challenge. Interestingly, the titers of neutralizing antibodies in the immunized animals were

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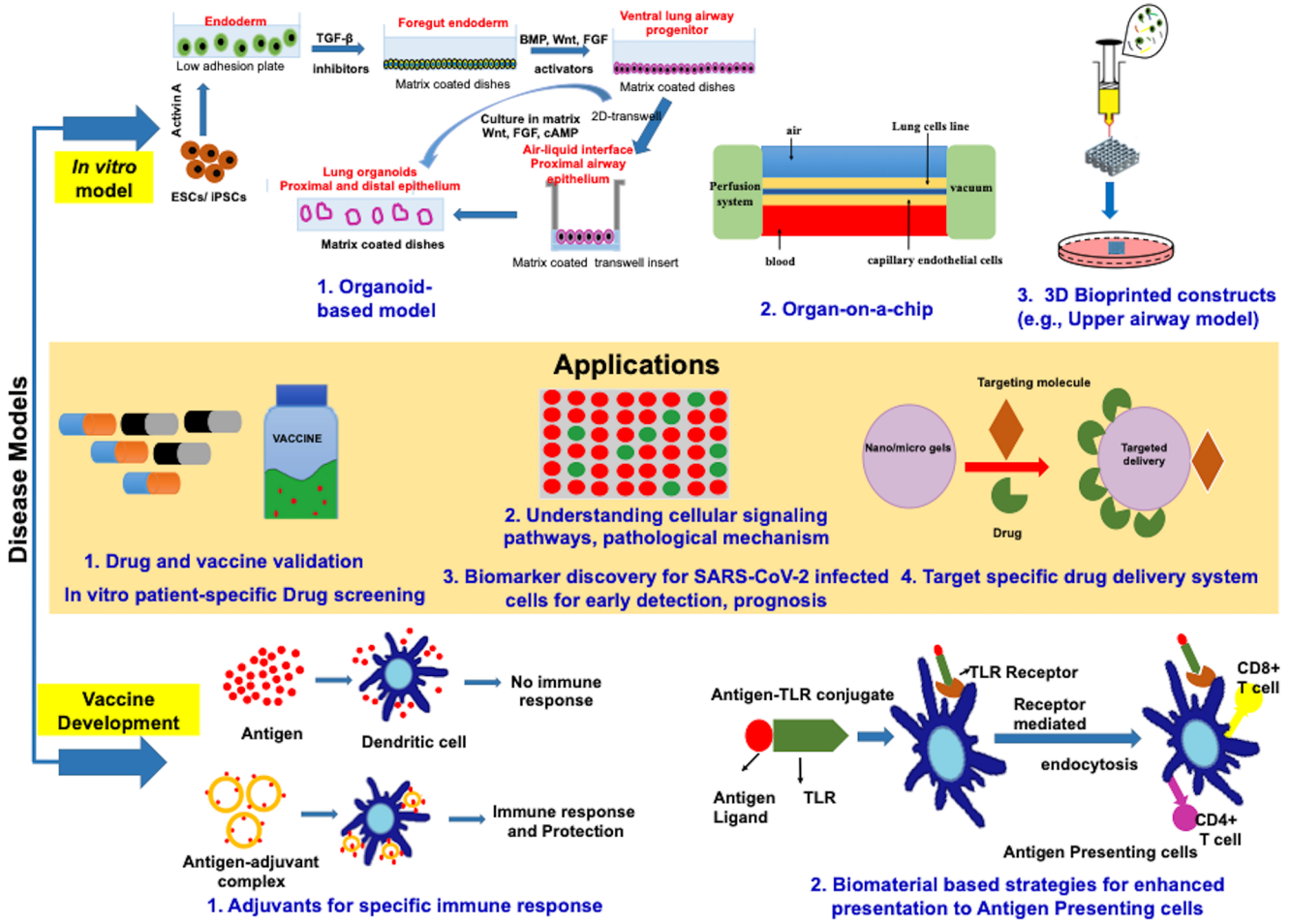

Figure 1. Application of tissue engineering techniques toward the development of disease models in a backdrop of SARS-CoV-2. Three-dimensional bioprinting, microfluidics (organ-on-a-chip), and organoids can be used for the development of an *in vitro* lung tissue model. The addition of adjuvants to the antigen and its binding to toll-like receptor ligands increase the antigen's display to the antigen-presenting cells and elicit an immune response, essential features vital for vaccine development against viruses.

comparable to that of humans who recovered from SARS-CoV-2 infection.[31]

Despite encouraging progress in modeling SARS-CoV-2 infection in animal models, especially nonhuman primates, most COVID-19-related research is restricted to *in vitro* cellular systems either due to the lack of suitable primate facilities or the heavy cost involved. The standard mouse model is not suitable as it does not adequately reproduce human infection pathophysiology, neither does it serve as a *bona fide* predictor of the human immune response. Therefore, research in most laboratory settings is conducted in classical 2D transformed cell culture systems. The caveat of using classical cell culture systems in studying viral infections is the high likelihood of failure in mimicking disease pathogenesis due to a lack of tissue architecture.[32] Therefore, there is a need to develop 2D nontransformed cellular systems and 3D human tissue-like models, not only for faithful recapitulation of infection to understand disease pathogenesis but also for testing the efficacy of potential drugs in blocking infection, their mechanism of action, and toxicity assessment. Recently, stem cell-derived human airway organoids and *ex vivo* bronchus cultures were adopted to model influenza A virus (IAV) infection. IAV tropism and replication competence in the human airway organoids were comparable to that of *ex vivo* bronchus cultures.[33,34] Since IAV and SARS-CoV-2 are both structurally similar viruses causing respiratory diseases, share many clinical symptoms, and are considered high pathogenicity or highly transmissible viruses (HPHTs),[35] it is expected that airway organoids that supported IAV infection, could also serve as a powerful, physiologically relevant model for rapid assessment of infection by SARS-CoV-2.

In the recent COVID-19 drug development endeavor, several potential therapies are being carried out in different phases of clinical trials. However, many of the drug trials yielded disappointing results: hydroxychloroquine with or without azithromycin did not improve the clinical status of the COVID-19 patients;[36] treatment with Lopinavir/Ritonavir did not benefit patients beyond standard care.[14] Remdesivir showed no clinical or virological benefits compared to the placebo groups, and this drug caused adverse effects in patients who received this treatment.[37] This failure in clinical trials could be attributed to a significant lack of critical information that was essential for the "bench to bedside" vault. When the gap between the "bench" and the "bedside" is bridged, improved biomimetic *in vitro* systems (Figure 1) can greatly contribute to our understanding of viral infection mechanisms and serve as better predictors of the efficacy of the prophylactic and therapeutic candidates than traditional cellular and animal models.

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2.1. 2D Cellular Models. One of the challenges of studying respiratory virus infection in general, and SARS-CoV-2 infection in particular, is to identify relevant *in vitro* cell culture models. A recent study evaluated the potential of several cell lines for SARS-CoV-2 infection and found that the cell lines Calu3, Huh7, Caco2, 293T, and U251 supported the replication of SARS-CoV-2.[38] In addition, another study showed that the Vero-E6 cell line expressing TMPRSS2 is highly susceptible to SARS-COV-2 infection.[39] SARS-CoV was able to replicate effectively in kidney-derived cell lines, possibly due to the high expression of angiotensin-converting enzyme (ACE-2) receptors in kidney tissue.[40] Other potential 2D cellular systems that can be used to establish model SARS-CoV-2 infection are the human airway epithelial (HAE) cells, which include tracheobronchial and alveolar cells, which are one of the first targets of human respiratory viruses. The tracheobronchial epithelium is columnar and pseudostratified, while the alveolar epithelium is comprised of a single cell layer.[41] These tracheobronchial cells need to be cultured in the air−liquid interface (Figures 1, 2), with the apical side of the cells exposed to air and the basolateral side submerged in a medium, and form a pseudostratified epithelial layer with morphological and functional resemblances to the human upper airway.[42,43] On the other hand, the air−liquid interface is equally important for alveolar epithelial cells to maintain a differentiated state.[44] Recently, alveolar epithelial cells were generated from human distal airway epithelial cells (DAECs). When primed with dibenzazepine and a cocktail of lung maturation factors in the absence of feeder cells, the DAECs differentiated to alveolar epithelial cells. Removal of feeder cells helped the DAECs to differentiate into airway club cells, while the inclusion of small molecules and growth factors after the expansion phase induced differentiation into type II pneumocytes, followed by trans-differentiation into type I pneumocytes. These differentiated pneumocytes were amenable for genetic perturbation such as RNAi and supported influenza A virus replication.[45] Very recently, the same group showed that SARS-CoV-2 can bind to and successfully infect the DAEC-derived pneumocytes. Instead of using genetically transformed cell lines, these DAEC-derived pneumocytes can develop physiologically relevant cellular systems and give an authentic reflection of treatment with antiviral compounds.

The acute respiratory distress syndrome related to SARS-CoV-2 infection is associated with virions targeting the epithelium of the distal lung, particularly the facultative progenitors of this tissue, alveolar epithelial type 2 cells (AT2s). Little is known about the initial responses of human lung alveoli to SARS-CoV-2 infection due in part to the inability to access these cells from patients, particularly at the early stages of the disease. Alveolar epithelial type 2 cells that are isolated from human lung biopsies cannot be expanded for more than three passages, without losing their phenotypic characteristics. Huang et al.[46] developed a human iPSC-derived alveolar epithelial type 2 cell line, by directed differentiation of human induced pluripotent stem cell (iPSC) lines to distal/alveolar lineage. After 1 day of SARS-CoV-2 infection, NF-κB signaling was elevated, leading to an inflammatory response, and a progressive loss of lung alveolar epithelial function could be noticed. Over time, infected cells exhibit cellular toxicity that can result in the death of these key alveolar facultative progenitors, as is observed *in vivo* in COVID-19 lung autopsies. Importantly, drug testing using these cells confirmed an antiviral dose response to remdesivir

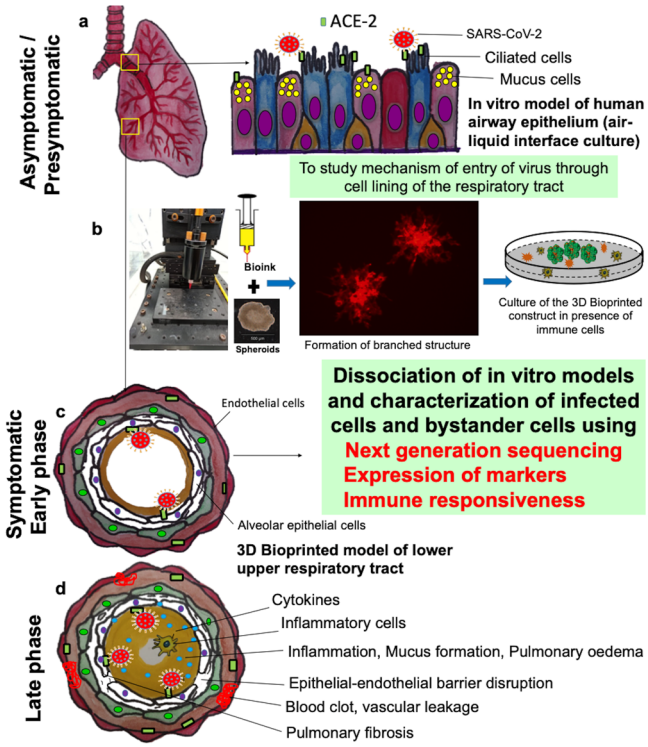

Figure 2. Development of in vitro model of the upper and lower respiratory tract by 3D bioprinting. (a) Tracheobronchial and alveolar cells cultured in the air−liquid interface can be used to study the mechanism of SARS-CoV-2 infection of the angiotensin-converting enzyme (ACE2) expressed by the epithelial cells of the upper respiratory tract. (b) Type I and II alveolar cell spheroids added to the bioink can be 3D bioprinted, followed by self-assembly within the bioprinted construct to form a branched structure. (c) Infection at the lower respiratory tract can be 3D bioprinted in a stage-wise manner depending on the infection. SARS-CoV-2 infects ACE2 expressing type II alveolar epithelial cells in the lower respiratory tract. (d) In the severe late phase, the epithelial−endothelial barrier is disrupted; there is marked infiltration of neutrophils and other immune cells, and the formation of intense blood clots can be induced. Such patient-specific models will allow studying how the intravascular deposition of viral antigen in the presence of excessive cytokines would cause inflammation and, in turn, localized blood coagulation.

and demonstrated the efficacy of TMPRSS2 protease inhibition, validating a putative mechanism used for viral entry in human alveolar cells.

2.2. 3D Organoid Models. In 2D cultures, cells are mostly composed of a homogeneous cell type, and therefore, interactions among different cell types during viral pathogenesis within the complex tissue architecture remain elusive. Viral pathogenesis in a complex tissue organization is traditionally studied in animal models such as rodents and primates. However, animal infections do not adequately reproduce human disease conditions, and many pathogens are unable to infect animals as they have a unique human host range.[27,47] Developments in research involving stem cells and the discovery of iPSC technology have allowed unprecedented opportunities to model the pathophysiology of human viral infections in complex 3D structures, mirroring human tissue architectures. Recent advances in stem cell research have enabled the development of 3D cell cultures termed organoid cultures, which closely simulate the *in vivo* organization of several organs and tissues.[20] Organoids hold great promise for

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Table 1. List of Organoid Models of SARS-CoV-2

s. no.	organoid type	method of preparation	key findings	limitations	reference
1.	a. blood vessel	a. induced pluripotent stem cells were differentiated to endothelial lineage and cultured in matrigel−collagen gel in 96 well plates to form human capillary organoids and were infected with SARS-CoV-2	a. closely simulates human vascular capillary growth with a lumen, $PDGFR^+$ pericyte coverage $CD31^+$ endothelial lining, and formation of a basal membrane	a. concentrated only on the early stages of infection and not on the later stages	52
	b. kidney	b. kidney organoids established from human embryonic stem cells into 3D suspension culture	b. (i) demonstrated prominent tubular-like structures	b. prediction about the effect of human recombinant soluble ACE2 during the later stages of disease progression cannot be done using such a model	
			(ii) expression of markers of proximal tubular identity	c. this model could not recapitulate blood clot or renin-angiotensin system, which is an important complex cascade of pathways that resulted in SARS-CoV-2 infection	
			(iii) tubular like cells manifested the solute carrier SCL3A1 together with SCL27A2 and SCL5A12		
2.	human small intestine	human primary gut epithelial cells were used to establish small intestinal organoid in 3D culture in Wnt-high expansion medium, under four different culture conditions to SARS-CoV and SARS-CoV-2	a. upon infection with SARS-CoV-2 transcriptome analysis revealed cytokines and interferon-stimulated genes due to interferon response type I and III	the model lacked relevant immune constituents such as natural killer cells, macrophages, eosinophils, etc. that may also regulate severe COVID 19	51
			b. SARS-CoV-2 produces a stronger interferon response than SARS-CoV in this human small intestinal organoid model		
3.	human liver	differentiation of human pluripotent stem cells to eight organoid types representing three specific germ layers; the definitive endoderm was induced to differentiate into liver organoids	a. transcript profiling demonstrated that the liver organoids showed upregulated chemokine expression akin to the tissue profile obtained from COVID 19 patients' autopsy	focused on the initial stages of viral entry; advanced stages in viral replication, cell lineage differentiation for release of the virus, and secondary infection have not been explored	138
			b. besides ACE2, an effector protein TMPRSS2 is also involved in viral entry		
4.	human brain	two different induced pluripotent stem cell lines, Donor 1, IMR90, and Donor 2, Crx-iPS, were differentiated into brain organoids (neuronal epithelium)	a. the human neurons were found to be a target for SARS-CoV-2	did not provide insights to dissect the mechanisms involving viral replication and the presence of, if any, ACE2 independent pathway for entry of the virus	139
			b. identification of Tau phosphorylation at T231 in SARS- CoV-2-positive neurons, which could activate a cascade of downstream effects that can initiate neuronal stress and toxicity		
5.	human colon	both colon-derived cell lines and primary nontransformed colon organoids were used	type III interferon plays a key role in controlling SARS-CoV-2 at the intestinal epithelium	a. did not give a clear view of the origin of the replicating SARS-CoV-2 in the intestinal epithelium	140
				b. characterization of the SARS-CoV-2 enteric life cycle is not provided, which makes it difficult to determine its mode of transmission in the gut	
6.	human lung	differentiation of human pluripotent stem cells first to definitive endoderm, then to anterior foregut endoderm, AFE/lung progenitor cells, and at last to lung organoids	a. transcriptomic analysis demonstrated induction of chemokines and cytokines with interferon type I or III signaling, akin to that found among human COVID-19 pulmonary infections; b. validated the use of FDA-approved drugs such as imatinib and mycophenolic acid as potential inhibitors of viral entry	a. did not study the role of AT2 cells and alveolar macrophages, essential in controlling an immune response	141
				b. the study only focused on the use of FDA-approved drugs for use as a repurpose drug for SARS-CoV-2 but did not focus on the viral replication mechanisms and the mechanism of infection particularly in the later stages	
7.	human kidney proximal tubule	normal human kidney proximal tubule epithelial cells were grown under conditional reprogramming and were then established in Matrigel organoids in 3D condition	the model expressed angiotensin-converting enzyme 2, a receptor for binding of SARS-CoV and SARS-CoV-2	did not study the interaction between SARS-CoV-2 and host epithelial cells, possibly via a normal airway cell model of the respiratory tract	142
8.	human distal lung	alveolar epithelial type II (AT2) cells were progressively expanded as feeder-free distal human lung progenitor organoids	a. human AT2 cells renewed themselves remarkably and were able to trans-differentiate to AT1 cells	signaling cascades involved or triggered during the viral replication were not investigated	143
			b. single-cell RNA sequencing of the organoid basal cells having the KRT5 marker showed two subsets of cells, named basal cells 1 and 2, essential for cell fate determination		
9.	human eye	pluripotent stem cells were used to produce a whole eye organoid model which consisted of the retina, retinal pigment epithelium, ciliary margin, iris lens, and cornea	a. interferon response type I and III is suppressed upon infection with SARS-CoV-2	mainly focused on the route of viral entry, but did not highlight the mechanisms by which it affects other organs of the body	144
			b. the limbus region of the eye was found to be at risk, due to the high expression of ACE2 and TMPRSS2		

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addressing important questions in virus−host interactions, the virus spread through tissues, and the mechanism of infection. Using stem-cell-derived organoids of different lineages, many infection models were put forward, in which infections with viruses such as Zika virus, rotavirus, adenovirus, enterovirus, and influenza A virus were studied.[33,48] Being closest to human tissue organization and function, such *in vitro* 3D organoids are now being considered "game changers" in the field of infection biology, not only because they are by far the best *ex vivo* systems to study disease mechanisms but also for their potential in predicting clinical trial outcomes in the therapeutic analysis.

On the basis of the principles of deriving organoids from stem cells or iPSCs, lung organoids were generated from human pluripotent stem cells (hPSCs) by manipulating developmental signaling pathways. The human lung organoids had structural similarity to native lungs, and global transcriptional profiles showed remarkable similarity to the human fetal lung.[49] However, these lung organoids were devoid of branching airways. Subsequently, another group generated lung bud organoids from hPSCs that contained mesoderm and pulmonary ectoderm, and after xenotransplantation and in Matrigel 3D culture, the organoids developed branching airway and early alveolar structures (Figure 2). Upon infection with the respiratory syncytial virus, the organoids exhibited swelling, detachment, and shedding of infected cells into the organoid lumens, closely resembling clinical manifestations in infants such as small airway obstruction and bronchiolitis.[50] Airway organoids simulating human airway epithelium were established as a successful infection model for influenza. The organoids supported infection by different strains of the influenza virus, extending the current armamentaria of the respiratory infection research toolbox.[33]

Recently, it was found that SARS-CoV-2 could infect human lung and small intestine organoids.[51] Both SARS-CoV and SARS-CoV-2 readily infected a 2D culture of human airway epithelium derived from lung organoids. Although the viruses targeted the ciliated cells, the goblet cells were not infected. The viruses also efficiently infected the intestinal organoids, and significant titers of the viruses were detected, indicating the organoids supported virus replication. Strong induction of a generic virus response program was detected by gene expression analysis.[51] Recent evidence also suggests that SARS-CoV-2 can directly infect human iPSC-derived kidney and blood vessel organoids.[52] These data (Table 1) strongly indicate that human organoids can be used as faithful experimental models to study SARS-CoV-2 infection and serve as a powerful tool to devise strategies against COVID-19.

2.3. Tissue-Engineered Models and Organ-on-a-Chip. At present, the hardest challenge which the research fraternity is facing is the need to speculate the course of the patients suffering from the viral infection. In such a scenario, the prime clinical requisite is the fabrication of representative 3D *in vitro* models for lungs, throat, airways, kidney, etc. that can play three critical roles during this pandemic: (a) it can provide deep mechanistic insight and characterization of the host−pathogen interaction and the process of infection; (b) it can act as a high-performance screening platform for newly discovered drugs and therapeutics; (c) it can serve for biomarker profiling in *in vitro* disease tissue models,[53] which may permit better resource assignment. The concept of personalized medicine is very much new, and it is still in the infancy stage in global research. However, precise *in vitro* modeling to apprehend the mechanism of viral replication and transmission along with the host response of an individual patient to the infection is vital at this point of the global pandemic. Therefore, distinguishing the biomarkers rigorously to carry out the person-specific diagnostics using hypothesis-based specific therapeutics is crucial at this stage (Figure 1).

Animal models and static monolayer cultures have several drawbacks and are limited in their use due to the variation between human and animal biology. Monolayer cell culture, on the other hand, fails to mimic the native extracellular matrix (ECM), 3D cell−cell interactions, and the shear force.[54] Despite several methods existing for *in vitro* testing of respiratory viruses, a more effective testing platform is a prerequisite. As a solution to these constraints, tissue-engineered *in vitro* models employing human cells can replicate the complex pathophysiology, for example, the production of pulmonary edema when exposed to inflammatory signals like interleukin-2,[55] to study pulmonary thrombosis at the organ level and determine the outcome of the use of experimental drugs in this process.[56] Another example is an exposure of the virus to a tissue-engineered model that consists of a collagen matrix supporting the growth of epithelial progenitor cells in a free medium devoid of serum along with mesenchymal stroma.[57] SARS-CoV can target stem cells, and this explains why lung regeneration after the viral infection is challenging.

It is found that around one-third of the COVID-19 patients who are in critical condition are showing the development of substantial blood clots resulting in deep vein thrombosis and pulmonary embolism.[58] Surprisingly, microclots have also been noticed in the small capillaries of the skin and lung, which impedes the flow of oxygenated blood.[59] A probable reason accounting for these hemostatic abnormalities is an enhancement of the D-dimer and biproducts of fibrin degradation along with disparity in the renin-angiotensin-aldosterone system.[60] One more reason that could be attributed to this is the attack on the endothelial cells by SARS-CoV-2. It is interesting to note that the endothelial cells possess a similar ACE2 receptor to which the virus also attaches during its entry into the lung cells (Figure 3). Moreover, many studies have confirmed that the endothelial cells have a likelihood to become infected.[61] Presently, systemic blood-thinning drugs such as heparin are injected as the standard medication, however high doses of such drugs can have potential risks.[62,63] Although the mechanism of thrombosis has been substantially studied in animal models, however, its mode of causing the pathogenesis in various blood vessels is poorly understood, and the evaluation of the drug remains uncertain. The present animal and *in vitro* models fail to simulate this organ-level complexity. However, microfluidics or the organ-on-a-chip system can efficiently simulate the interplay in the blood−endothelial−epithelial system, due to its ability to enclose cells and blood specifically from the patients, lacking in other available thrombosis models.[64]

Fabricating organs on a chip can help in getting a detailed perspective of how the SARS-CoV-2 is behaving. A lung-on-chip model[65] has been developed which can simulate the environmental, structural, and functional complexity of the native tissue. In that respect, a soft-lithographic technique was used to create the alveolar-capillary unit present in the native human lung tissue which consisted of a three-layer compartment made up of human alveolar epithelial cells that formed the upper layer; pulmonary microvascular endothelial cells fill the lower layer separated by an elastomeric microporous

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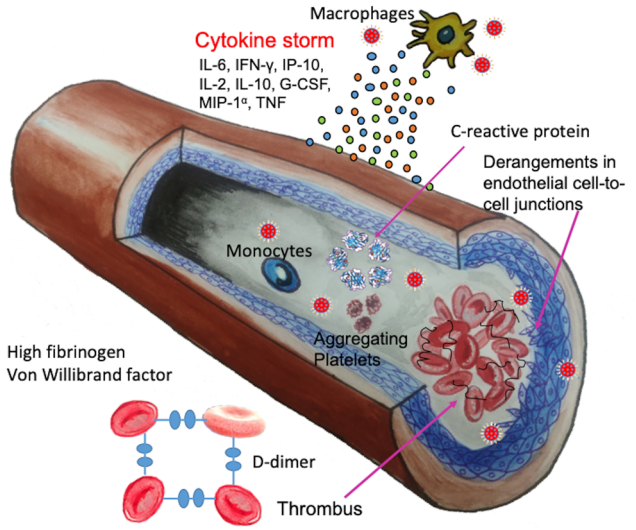

Figure 3. Mechanistic insights into the high incidence of thromboembolism is still limited. Upregulation of the D-dimer, derangement of endothelial cells, ad cytokine storms are a few factors responsible for massive blood clots. The bioengineered blood vessel can elucidate the mechanism of blood clotting due to SARS-CoV-2 infection.

membrane having a thickness of 10 μm composed of poly(dimethylsiloxane) that mimicked the alveolar-capillary interface.[66] When the cell attachment was established, culture medium was continuously perfused through the microchannel to induce cell growth. This was followed by the exposure of alveolar epithelial cells to air to allow them to differentiate; this entire culture system gave rise to the formation of a microengineered alveolar-capillary unit in close proximity. Thacker et al. tried to establish a vascularized lung-on-chip infection model by coculturing primary human alveolar epithelial cells.[67] Compared to the monolayer cultured sample, lung-on-a-chip controls at the air−liquid interface offer apical-basal polarity. This *in vitro* model could recapitulate a few features of clinical infection such as persistent infection even with low viral replication, an NF-KB inflammatory response depicted by IL-6 secretion, and a loss of barrier integrity in the endothelial layer. In a recent study, Si et al.[68] devised a microfluidic system lined by human lung airway epithelia in a differentiated manner at an air−liquid interface supported by a continuous flow of medium that could be used as a suitable model for not only SARS-CoV-2 but also viruses such as influenza etc. This airway chip created by them led to the high-level expression of ACE2, which was used for the screening of seven different drugs clinically approved for SARS-CoV-2. The advantage of such an enclosed system with continuous perfusion culture[69] is that it would enable healthcare personnel to study the problem without the need to physically touch a human and, thus, avoid the chances of any potential harm arising from it. Additionally, research can be carried out in the earlier stages of viral infection, which is not possible in humans since once the virus enters the body the incubation period is as long as 14 days. Organ-on-a-chip facilitates monitoring of the virus infection after the virus enters the organ. This system would also enable us to study the early innate response of the immune system to this pandemic-causing virus.[57] Although this lung-on-a-chip furnishes new probabilities to recapitulate the physiological functions at the organ level originating from the complex interactions between different tissue types present in the living human lung, it cannot mimic the micro-architecture of the respiratory tract, which is composed of bronchi and further divided into much smaller branches called the bronchioles, ultimately ending in microscopic air sacs.

2.4. 3D Bioprinted Models. One of the obstacles in faithfully recapitulating viral infections in organoid models is the lack of complex organ architectures despite having well-defined tissue organizations. Moreover, the inherent heterogeneity of organoids stands as an impediment in studying virus tropism and spread, compromising the reproducibility of results. To address this issue, 3D bioprinting comes to the rescue, which repurposes state-of-the-art, layered printing technology to reconstruct human organ-like structures using tissue-specific cell types laden in bioink.[70] The organomimetic systems generated by 3D bioprinting technology hold great promise in studying human viral infections as they provide the most realistic physiological environment or arrangement maintaining apical vs basolateral cell polarity as much as achievable in *in vitro* platforms. In particular, the 3D bioprinted lung-like structures could be ideal in studying respiratory infections as they provide an air−tissue interface in complex, hollow architecture composed of multiple layers and cell types, which is far-fetched by traditional tissue engineering methods. In our laboratory, we have successfully fabricated various *in vitro* healthy[71] and diseased[72] tissue models using silk fibroin-gelatin bioink laden with specific cell types. These models closely recapitulated native tissues and ECM ultrastructure. The intricate 3D architecture of the lung tissue has been fabricated by 3D bioprinting, such as the branched morphology of the respiratory tract as a bifurcated set of tubes.[73] Instead of a monolayer culture of tracheobronchial cells,[42] a strategically designed 3D bioprinted human upper airway construct would bring more anatomical relevance and would be the closest possible organ-like platform to study respiratory infections outside the human body. In the 3D bioprinted human upper airway construct, differentiation of the embedded cells can be induced, and different cell types can be incorporated to mimic airway tissue layers including basal, ciliated, and goblet cells, with an expression pattern comparable to that of the *in vivo* bronchial epithelium.[42]

A need for accelerating change has been at the core of innumerable technological revolutions, and especially in this time of uncertainty due to the COVID-19 crisis. Due to extreme customizability and robustness, the 3D bioprinted models would help in gaining deep mechanistic insight into the virus infection routes, which would otherwise remain elusive in the traditional tissue culture models. However, 3D bioprinting is associated with several challenges that include controlling mechanical stress during bioprinting, adequate supply of nutrients and growth factors, maintaining sustained release of the growth factors, and developing suitable cytocompatible materials.[74−77] Taking advantage of the 3D bioprinting technology to generate lung-like structures suitable to modeling influenza A virus (IAV) infection, a recent study developed hydrogel bioink, consisting of Matrigel, alginate, and gelatin, and embedded in the construct human alveolar cells, A549. The 3D bioprinted human lung-like model was infected with the IAV Pan/99 (H3N2) strain, and a clustered infection pattern was observed. The observed infection pattern was reminiscent of natural lung infection, which is generally not observed in 2D cell culture, and the bioprinted cells exhibited a basic immune response by secreting the antiviral factor

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interferon λ_1.[78] Another study of 3D bioprinted human HepaRG liver cells with bioink composed of alginate, gelatin, and human ECM was conducted, and the tissue models were tested for adeno-associated virus (AAV)-mediated transduction and human adenovirus 5 (hAdV5) infection. The efficient transduction of the tissue models was observed upon AAV infection in which the endogenous target was successfully silenced by shRNA encoded by the AAV expression cassette. The 3D bioprinted tissue models also efficiently supported hAdV5 infection, further underscoring the utility of such models in modeling viral infections.[79] Importantly, the 3D bioprinted tissue model allowed efficient transduction by AAVs, which is otherwise challenging in spheroids/organoids as they are too dense in the organization to be penetrated to the core by large viral vectors.[79] Considering the myriad of advantages offered by 3D bioprinted humanized *ex vivo* systems in modeling viral infections, the efforts should now be directed to generate 3D lung models that would support SARS-CoV-2 infection. In summary, efforts should be directed toward the fabrication of 3D lung, bronchiolar, or alveolar models to recapitulate SARS-CoV-2 infection in pursuit of translation from the bench to the bedside in the trying times of the COVID-19 crisis.

3. VACCINE TRIAL

Since the last century, vaccination against different pathogens has played a critical role in disease prophylaxis. The global research fraternity is racing against time to develop a safe and effective vaccine against the SARS-CoV-2 to combat the disease.

An ideal vaccine against SARS-CoV-2 should fulfill the following requirements: (i) it must evoke an effective neutralizing antibody response against this virus and its mutated types; (ii) it should provide defense against contamination and disease transmission; (iii) it should provide safety by not triggering infection intensifying antibodies or any detrimental immune or inflammatory response; (iv) it should be effective in both humoral as well as cell-mediated immunity. With the progress in the fields of immunology, biomaterials, tissue engineering, and regenerative medicine, approaches are being undertaken to invoke an immune response to the pathogen to escalate the process of vaccination.[80,81] Most of the currently available and candidate vaccines are poorly immunogenic, furnishing only short-lived protection, and are even feared of retrieving pathogenicity in some immune-compromised conditions. Lately, advances in the field of biomaterial engineering have the prospective of overcoming these pitfalls through upgraded formulation, immune signal control, and delivery.[82] The distinct physical (size, shape) and chemical (surface chemistry, etc.) properties of the biomaterial enable the cargo of interest to either conjugate or encapsulate with it. Interestingly, in many cases, they act as a vehicle for drug delivery not only for the vaccine but also for the adjuvant critical for triggering distinct immune responses.[83] This attribute is particularly advantageous compared to the traditional method of adjuvants and soluble antigen delivery in the soluble vaccine. The objective of most of these systems is to trigger the antigen-presenting cells, for example, the macrophages and dendritic cells, and to direct the Th1- or Th2-specific responses. For example, Barry et al. used PLGA nanoparticles for a vaccine against Chagas disease carried out in a murine model[84] due to its ability to direct the Th1 immune response in comparison to other biomaterials and no vaccine carriers; besides, it also has been exploited to target delivery in specific immune populations.[85] As the biomaterial implantation entails this above mechanism, it becomes essential to perceive how the material property may modify the immune interactions with regard to tissue engineering.

Several biomaterials display distinct structural characteristics, known to activate recognition as damage-associated molecular pattern molecules (DAMPs) and pathogen-associated molecular pattern molecules (PAMPs). Specifically, the immune system often responds to the polymer chains present in a repetitive pattern that can bear a resemblance to the bacterial polysaccharide, materials of the hydrophobic region, as well as the particulate characteristic of the nano- and microparticle that exhibit key features of viral and bacterial pathogens.[86−88] It has been well documented that even the most frequently used biomaterials, when devoid of immunostimulatory signals, can trigger inflammatory pathways such that the biomaterials' inherent physicochemical features can influence an immune response. Dendritic cells (DC) cultivated on thin films of natural (such as agarose, chitosan, alginate, and hyaluronic acid) or synthetic polymers (e.g., PLGA) persuade the surface activation markers to express differentially. These signals involve CD40 (DC maturation marker), the MHC II complex, and costimulatory markers such as CD 80 and CD 86. These costimulatory signals behave as a secondary signal required for activating the B and T cells when the MHC complexes present antigen.[89] Chitosan, for example, has been altered into a thermoresponsive intranasal vaccine targeted against the influenza virus H5N11.[90] In addition, silver nanoparticles have also been employed for the local transport of inactivated influenza vaccine-specific to lung immune cells that result in increased titers of IgG and a reduction in the mortality rate when experimented in a murine model.[91] Apart from the different platforms that are particle-based, tissue engineering methods have been explored to develop a scaffold-based method for vaccination improvement. With distinct physicochemical characteristics such as pore size along with the sketch of the release signals, for instance, granulocyte-colony stimulating factor, scaffolds fabricated from PLGA[92,93] along with silica rods[94,95] have been employed to engage and centralize antigen-presenting cells to the vaccine constituents. These findings have prompted new studies to investigate these types of intrinsic immune characteristics of biomaterials to drive responses of the vaccines more specifically to ameliorate therapies to different pathogen-caused diseases.[96]

Recently, bioengineered immune tissue constructs such as bone marrow, lymph nodes, thymus, and spleen were developed,[97] which holds promise to accurately study the human immune response *in vitro*. Many clinical trials of vaccines failed due to either efficacy or safety issues, which could not be predicted during the preclinical trials because of the lack of authentic models capable of mimicking the human immune response. Engineered 3D human immune tissues could provide a more accurate niche for research as compared to conventional cell culture or animal models that poorly recapitulate human immune response.[98,99] Moreover, such *in vitro* immune tissue models could be used to proliferate rare immune cells isolated from patients in a controlled niche that might reveal new insights that are otherwise difficult and risky to study in humans. Engineered immune models could also be instrumental in studying extracellular cues such as antigens, cytokines, mechanical forces, etc., which could shed light on the functions of specific immune cell subsets and their

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mechanisms of differentiation.[97] Using porous hydrogels laden with nanoparticles, germinal center-like immune organoids were recently generated that matched the stiffness of natural secondary lymph organs. Along with fibroblasts expressing CD40L and BAFF (B cell surviving and activation signals), B cells were encapsulated in these hydrogels, which developed phenotypic markers of GC.[100,101] Moreover, the 3D organoids promoted B cell proliferation by 100-fold and facilitated antibody class switching to IgG1 and IgE, which demonstrated pathogen inactivation by phagocytosis.[100,101] Following the development of secondary lymph organs, bone marrow and thymus organoids were also developed that could provide insight into the development of immune cells. Using porous 3D colloidal crystals and seeding them with stem cells and bone marrow stromal cell lines, Nichols et al. developed a bone marrow model, which promoted the stem cells to efficiently differentiate to B cells.[102] Recently, organs-on-a-chip tools have been fabricated that provide precision control over immune cell interactions and interactions with defined extracellular signals. Moreover, with the integration of organoid technology and organs-on-a-chip fabrication methods, new hybrid devices are being developed to investigate immune functions. Placing lymph node slices in a perfusion chamber with a microfluidic injector, Ross et al. developed a hybrid system that allowed modeling the effects of therapeutics in different microdomains of lymph nodes with spatiotemporal controls.[103] With the combination of realistic *in vitro* immune models with emerging tissue engineering technologies, new strategies can be devised to accelerate candidate vaccine screening, which could greatly help in the current exploration of new vaccines against SARS-CoV-2.

Although presently several studies are investigating the development of vaccines against SARS-CoV-2, few successes have been reported in the mouse model, in which nanoparticles that were produced from the SARS-CoV peptide sequence could activate the immune system to protect SARS-CoV-2. The sera were obtained from the mice immunized with the nanoparticles inhibited by SARS-CoV-2 infection in Vero cells.[104] Currently, most of the SARS-CoV-2-directed vaccines under development are targeting the spike glycoprotein (S-protein) that helps the virus to attach to the ACE-2 receptors present on the lung cells. Successful vaccination would generate antibodies directed against the S protein, which may neutralize the virus, blocking infection and spread. Genomic sequence analysis has revealed that the entire S-protein is not immunogenic, rather only a part of it is. Such parts of the protein (epitopes) can be specifically targeted by subunit vaccines. The S protein is comprised of two domains S1 and S2. While the S1 domain is responsible for receptor binding, the S2 domain is critical for mediating membrane fusion. Since the S2 domain is less susceptible to mutation than the S1 domain,[105] a vaccine targeting the S2 domain may be more effective in neutralizing the virus even when it is mutated. Such a vaccine could serve as a broad-spectrum vaccine, protecting various mutated forms of the virus. Taken together, we suggest that the particle- and scaffold-based vaccine systems with different epitopes attached to them can be a highly promising strategy in rapid vaccine development against SARS-CoV-2, and *in vitro* tissue models can expedite that process.

4. TARGET SPECIFIC DRUG DELIVERY SYSTEM

While new therapies based on small molecules emerge through the pipeline, tissue engineers can pursue designing a drug delivery system for (i) targeting drug formulations to particular organ systems to enhance the bioavailability and (ii) expanding the controlled drug release such that repeated administration is not essential.

Categories of molecules that have been proposed to serve as probable therapies comprise small molecule drugs, monoclonal antibodies, as well as an oligonucleotide.[106] For instance, in a small clinical study,[107] azithromycin and hydroxychloroquine combination has been proposed as an avenue to lessen the viral load arising from SARS-CoV-2; however, the results obtained a lack of convincing evidence of success.[108] Over the past decade, there has been a great urge among researchers for discovering small-molecule-based drug carriers against viral diseases, for example, smart materials, "nano/microgels" into which the cargos can be enclosed and allow a sustained drug release rather than the traditional drug delivery system.[109] PLGA microparticles can aid in the trafficking of azithromycin to 60 days by following the zero-order release kinetics.[110] Optimizing the composition of PLGA nanospheres would be important, as the ratio of 50:50 served as a suitable delivery vehicle for the release of peptide receptor radionuclide therapy for neuroendocrine tumors compared to the 75:25 nanospheres.[111]

Efforts were made for targeting small molecules which can impede SARS-CoV entry into the host cells.[112] Ling Yi et al. employed a two-way screening approach and selected two small molecules from 121 Chinese herbs that exhibited impeding activity against SARS-CoV. Targeting virus entry is an interesting step for developing suitable therapy since it can obstruct the multiplication of the virus at an early stage, thus reducing the probability of the virus to grow and become drug resistant. Blocking the entry for numerous viruses has already been developed. For instance, inhibitors for virus entry have already been carried out for the HIV type;[113,114] enfuvirtide (T20) is one of these, which is now used clinically.[115,114] The small molecule RFI 641[116,117] has been produced against the entry of the respiratory syncytial virus (RSV). On the other hand, entry blockers such as the FGF4 signal peptide[118,119] as well as n-docosanol[120] for the herpes simplex virus are under development. Although research is being carried out by the global scientific fraternity to find a suitable solution against the SARS-CoV-2 infection, some success has been achieved by Pant et al. where they have identified around 66 drugs already approved by the FDA using the repurposing method.[121] The study carried out by them furnishes a comprehensive analysis of the vital residues as well as the ligand−receptor interactions for producing a peptide-like structure akin to SARS-CoV-2 main protease inhibitors. Several other approaches are being undertaken to target by small molecules either the SARS-CoV-2-encoded proteins or the human host factors.[122] However, additional studies are warranted for further validation. The above-cited examples indicate that scientists in the field of biomaterial science and tissue engineering have been working for decades on the formulation of drug delivery vehicles specifically for small molecules in general.

Apart from the small molecules, another compelling class of medication is the use of monoclonal antibodies due to their success as therapeutics against the Ebola virus.[123] In general, antibodies constitute a natural part of the humoral immunity and can be modified to obstruct distinct receptors or receptors crucial for viral functioning. Monoclonal antibodies are progressively recognized as an effective class of drugs targeted for infectious diseases, specifically the viral surface proteins,

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and have also been shown to have therapeutic potency for certain viruses.[124,125] However, to become successful these therapies principally need to be given intravenously. For instance, one to three infusions were required for patients suffering from the Ebola virus infection depending on the type of antibody. Meulen et al. developed a monoclonal antibody against SARS-CoV that was found to be successful in a ferret model.[126] Additionally, monoclonal antibodies screened against the same virus have shown cross-reactivity to at least one of the antibodies developed against SARS-CoV-2.[127] In a recent study that was aimed to explore the effectiveness of the monoclonal body as a suitable biotherapy against this pandemic-causing virus, Wang et al. reported that the antibody developed by them attaches to a conserved epitope present on the receptor-binding domain of the spike, depicting its potential to cross-neutralize both SARS-CoV and SARS-CoV-2 employing a method which is not dependent on a receptor binding inhibitory mechanism. Interestingly, the developed antibody could be used for the developing serological assays and antigen detection tests specific against SARS-CoV-2.[128] The beauty of this neutralizing antibody is that they can modulate the course of the infection in the host aiding in the clearance of the virus or on the other way to protect an uninfected person upon exposure to the virus.[124] Therefore, this antibody could serve as a potential therapeutic to avert/or treat not only SARS-CoV-2 but also other future emerging diseases in humans from the viruses of the subgenus Sarbecovirus when used alone or in combination. In spite of a large number of monoclonal antibodies exhibiting potential outcomes in neutralizing the infection caused by SARS-CoV and MERS-CoV, their production on a large scale not only requires intensive labor but also is time-consuming and costly, which outweighs the clinical application of the monoclonal antibody. Instead, platforms involved in the production of the therapeutic protein ease the production costs and make it affordable. Besides this, an allylamine-based polymer coated on tissue-engineered nanoporous scaffolds was successful in releasing a monoclonal antibody named Rituximab, developed against the B-cells for 30 days.[129] Likewise, human immunoglobulin G1 (IgG1) monoclonal antibody was released from an alginate-based drug delivery system in a rat model in a single dose manner when carried out for 28 days.[130] Recently, several neutralizing monoclonal antibodies have been tested that are targeted against the S1 and S2 subunits of the S protein of SARS-CoV-2. Moreover, human monoclonal antibodies such as B5, B38, H4, and 47D11 have shown promising results in blocking SARS-CoV-2 infection.[131]

In addition, short interfering RNA (siRNA) has also been exploited both as a therapy and as prophylaxis against the infection caused by SARS-CoV-2.[132] siRNA was found to be potent against the SARS-CoV-specific sequences in an NHP model, with an ensuing reduced viral load as well as alveolar damage.[133] Four intranasal doses were given over 5 days in the treatment arms. The researchers did not use any additional vehicles for the delivery of their siRNA, for example, polyethyleneimine owing to the probability of lung inflammation arising from the carrier. As a solution, a more complex form of the vehicle has been fabricated particularly for pulmonary usage, which also includes mesoporous silica nanoparticles[134] as well as cationic liposomes.[135] Recently, in an assessment of the potential of RNAi in the management of COVID-19, several companies have started to explore the possibility of using siRNA for the prevention and treatment of SARS-CoV-2 infections.[136]

In conclusion, as small molecules, monoclonal antibodies, and siRNA technology continue to aid as suitable therapeutics against SARS-CoV-2, biomaterial scientists and tissue engineers pursue their work for creating drug vehicles for specific target areas infected with the viral load. We believe that even if these strategies do not show fruitful results against SARS-CoV-2, they may play a key role during other viral outbreaks in the future.

5. PERSPECTIVES

In over a century, the world has not witnessed a greater devastation and health crisis by any viral pandemic than the ongoing COVID-19. In such an unprecedented scenario, which has made nations across the globe grapple with serious impacts on their present and future, physicians and scientists are remarkably collaborating for speedy identification of antiviral strategies to curb SARS-CoV-2 infection and spread. The challenge in developing prophylactic and therapeutic interventions against this contagion is even compounded by the innate heterogeneity of this virus, its continuous re-emergence, and extreme contagiousness. Moreover, our inadequate understanding of the immune response elicited by the virus is greatly impeding the progress in designing a rational vaccination strategy, toughening the challenge in combating the crisis.

In such a period of great uncertainty, what would be myopic is to restrict our research efforts within the confinement of traditional tools and systems that may potentially limit breakthroughs. The need of the hour is to open significant scientific frontiers to generate improved infection models, which will demand active partnership between tissue engineers and infectious disease experts. Developing physiologically relevant, biomimetic *in vitro* tissue model systems may serve as better predictors of clinical trial outcomes for vaccines, therapeutics, and host-targeted immunotherapies. Such patient-specific *in vitro* disease tissue models provide the possibility to independently identify and regulate cellular and molecular factors that contribute to the onset of disease and progression and thus help in replicating specific diseases in significantly less time compared to the animal models. The field of biomaterial research has provided new understanding and exhilarating ideas which could be leveraged for developing advanced vaccines against infectious diseases.[137] We believe that employing a combinative approach such as 3D bioprinting, tissue engineering, amalgamated with multiomics, and single-cell genomics would help in depicting the best possible portrait of the disease. Instead of using traditional animal models for drug discovery, 3D bioprinted disease models would furnish better and more accurate results to determine the drug targets. *In vitro* models provide a higher degree of reproducibility, allowing a larger number of samples to be screened on different models at a relatively reduced time. Importantly, such bioprinted disease models would enable the researchers to determine the genes that are turned on/off or the signaling cascades involved in disease progression. Information obtained from this would not only be useful in determining a larger picture of the infection process, the host–pathogen interaction, but would also help us in understanding the disease pathogenesis. Further screening of the experimental drugs in organoids would furnish 3D and multicellular interactions. Therefore, such stepwise testing would help in

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the generation of more precise models in early drug discovery, thus avoiding any pitfalls later in clinical testing.

It has been found that the SARS-CoV-2 virus enters only those cellular surfaces having a combination of unique types of molecules, hence we suggest that it would be more rational to determine those specific types of molecules present in the cells within the human body besides focusing on the development of vaccines or suitable *in vitro* models that can help answer the two questions—(i) the reason behind the virus infecting so many organs and (ii) why different individuals are more susceptible to the infection than others. Although research is underway to help determine the answers to these questions, together we can be successful in overcoming the current pandemic crisis and make ourselves prepared to combat future viral outbreaks.

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Notes

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