



Airway and Alveoli Organoids as Valuable Research Tools in COVID-19

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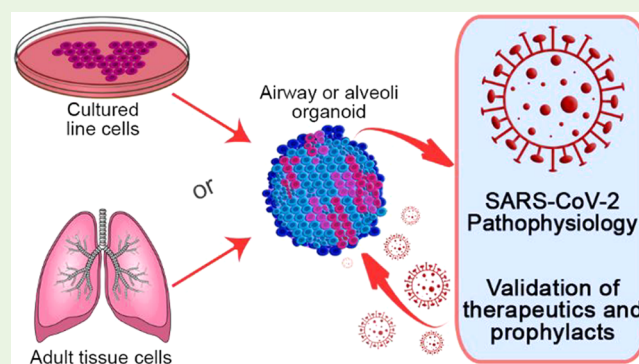
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ABSTRACT: The coronavirus disease 2019 (COVID-19), caused by the novel coronavirus, SARS-CoV-2, affects tissues from different body systems but mostly the respiratory system, and the damage evoked in the lungs may occasionally result in severe respiratory complications and eventually lead to death. Studies of human respiratory infections have been limited by the scarcity of functional models that mimic in vivo physiology and pathophysiology. In the last decades, organoid models have emerged as potential research tools due to the possibility of reproducing in vivo tissue in culture. Despite being studied for over one year, there is still no effective treatment against COVID-19, and investigations using pulmonary tissue and possible therapeutics are still very limited. Thus, human lung organoids can provide robust support to simulate SARS-CoV-2 infection and replication and aid in a better understanding of their effects in human tissue. The present review describes methodological aspects of different protocols to develop airway and alveoli organoids, which have a promising perspective to further investigate COVID-19.

KEYWORDS: lung, virions, SARS-CoV-2, respiratory disorders, coculture, 3D cell models



INTRODUCTION

For the past year, the world has been facing the new coronavirus pandemic, caused by the Severe Acute Respiratory Syndrome-CoronaVirus-2 (SARS-CoV-2), which leads to the Coronavirus Disease 2019 (COVID-19) in approximately 40–45% of infected patients.^{1,2} Ever since its outbreak in December 2019, in Wuhan, China, SARS-CoV-2 has rapidly spread throughout the country, reaching the first epicenter in the city of Hubei and, shortly thereafter, the globe.^{3–6} The clinical outcomes observed in patients infected with SARS-CoV-2 are variable, ranging from asymptomatic cases to acute respiratory discomfort and multiorgan failure.⁷ Among the most common COVID-19 symptoms are cough, fatigue, headache, loss of taste and smell, myalgia and sputum, and diarrhea (mild outcome); and cyanosis, dyspnea, thoracic pain, shortness of breath, hypoxemia, severe pneumonia, pulmonary edema, and multiple organ failure (severe outcome).^{7–9} One prominent feature once patients undergo thorax tomography is the ground-glass opacity observed in the lungs, affecting both lungs in the periphery of inferior lobes; this occurs even in asymptomatic patients.^{7,10} In addition to the commitment of lungs, other systems may be severely affected, in particular, digestive, cardiovascular, epithelial, renal, and central nervous system.⁶ Nevertheless, pulmonary tissue involvement is of greatest concern since it is the one involved in the

development of acute respiratory distress syndrome (ARDS) and the risk of death.^{11,12}

The main structural viral proteins that constitute SARS-CoV-2 are small envelope glycoprotein (E), membrane glycoprotein (M), nucleocapsid protein (N), and spike glycoprotein (S), along with some accessory proteins.¹³ The S glycoprotein is particularly important for the development of COVID-19, since it is a transmembrane protein located in the external part of the virus that directly binds to angiotensin-converting enzyme 2 (ACE2), expressed in human host cells, in particular, pulmonary cells,¹⁴ including epithelium alveolar cells type 2 (AT2).¹⁵ Additionally, other host factors enable and/or facilitate viral entry, such as transmembrane protease serine 2 (TMPRSS2), which acts on protein S priming; furin paired basic amino acid cleaving enzyme (FURIN), which activates protein substrates and pathogenic agents; and neuropilin-1 (NRP1), involved in angiogenesis and highly expressed on vascular cells and epithelia facing external

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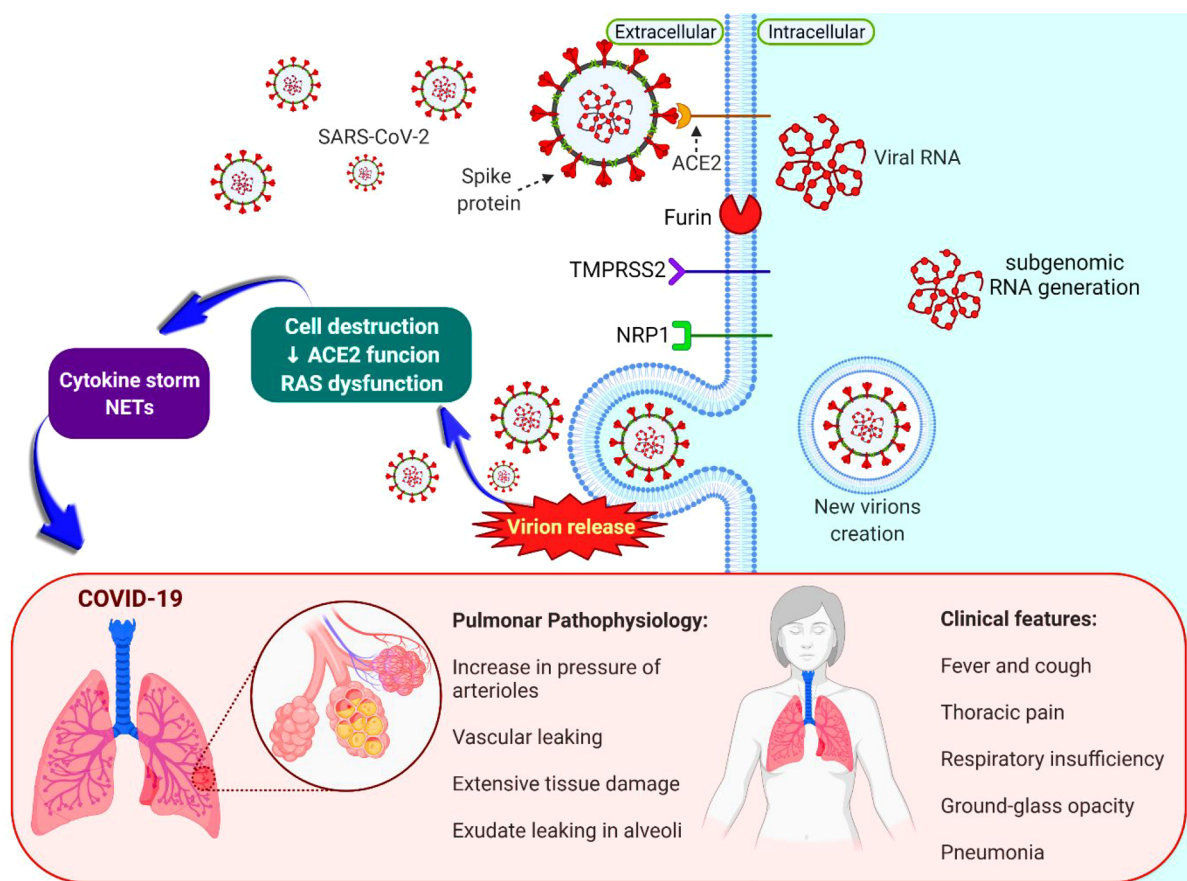


Figure 1. Pathophysiology of SARS-CoV-2 in human alveolar cells. SARS-CoV-2 infects human respiratory cells through the S protein linkage to host cell entry mediators; after cleavage, the virus delivers mRNA into the cell and the viral replication begins. Once the new virions are released, inflammatory response takes place, and cytokine storm and NETs release start, leading to clinical features. Abbreviations: SARS-CoV-2: Severe Acute Respiratory Syndrome-CoronaVirus-2; ACE2: angiotensin-I-converting enzyme-2; NRP1: neuropilin-1; Furin: furin paired basic amino acid cleaving enzyme; TMPRSS2: transmembrane serine protease 2; NETs: neutrophil extracellular traps; COVID-19: CoronaVirus Disease 2019.

environment, and which participates in tissue infiltration.^{16–20} Although most research on virus entry focuses on ACE2, it is understandable that the availability of virus receptors and the interaction among cofactors help determine infectivity; in the case of SARS-CoV-2, cells with low ACE2 expression are also infected, which could be explained by the involvement of cofactors.^{16,17} In addition, diverse genes have been reported to be upregulated in patients that develop severe COVID-19, possibly due to a direct correlation with the viral cycle in the human organism; these include ADAM metallopeptidase domain 10 (ADAM10), Toll-like receptor 3 (TLR3), histone acetyltransferase 1 (HAT1), histone deacetylase 2 (HDAC2), lysine demethylase 5B (KDM5B), sirtuin 1 (SIRT1), member renin-angiotensin system (RAS) oncogene family (RAB1A), and FURIN.^{18,21} Importantly, there is a link between upregulation of these genes and ACE2 expression, and consequently virus entry.²¹ After this cleavage, the virus manages to infect the host cell and releases mRNA into the cells, which is then translated into protein and generates new viruses.²² Ever since the first genomic sequencing to this date, a considerable number of mutations of the SARS-CoV-2 have been observed throughout the world, mainly concerning S proteins, which can miscarry the effectiveness of vaccines, in addition to favoring the rise of new pathogenicities.^{23,24} In the past months, several new strains have emerged and raised concern, since they usually present a significantly higher

transmission rate possibly due to improved host–cell receptor interaction, and what results in a more effective infection establishment and propagation; these events make it more challenging to contain the virus spread.²⁵

At the initial infectious stages, the viral replication occurs rapidly, leading to death of pulmonary cells, especially AT2 cells; as the virus keeps on infesting more cells, there is subsequent vascular leaking, which in turn triggers an exacerbated inflammatory response and leads to an event called storm of cytokines and chemokines.^{26,27} The cytokines are possibly related to an elevated concentration of hyperactive neutrophils in the affected pulmonary areas; acting together, they trigger neutrophil extracellular traps (NETs), which lead to extensive tissue damage.²⁸ NETs, extracellular webs of protein, are released by neutrophils in the aim of fighting ongoing infections; nevertheless, when not properly regulated, they originate thromboinflammatory states, including the ones underlying respiratory failure.^{29,30} Moreover, blood plasma from deceased and hospitalized patients indicates a significant correlation between NET levels and clinical outcomes; remarkably, there are significant differences in NET levels among intubated and non-intubated patients, suggesting that this parameter could be considered for evaluating the chance of survival and clinical outcome prognosis.²⁹ Post-mortem investigations indicate the presence of microvascular thrombi and active neutrophils, with NET release, together with

platelets, in particular, in patients with rapid disease progression.^{29,31} Cytokine storm has been demonstrated to be followed by infection in sera from patients;³² thus, increases in cytokine and NETs levels, one related to the other, associated with vascular disorders, are important to predict prognosis and possible treatment interventions. Besides, it has been suggested that the negative regulation of ACE2 evoked by cell death can impair the anti-inflammatory function of RAS. This effect increases the vascular permeability of pulmonary arterioles and further aggravates the inflammatory response.^{26,33} Such events can result in exudate leaking in the alveolar aerial spaces and end in pulmonary edema and consolidation,³⁴ alveolar cell desquamation, and hyaline membrane development.³⁵ On top of that, the healing process from the pulmonary inflammation can evolve to fibrosis, which worsen the patient's prognosis.³⁴ A scheme containing the respiratory cell infection and the clinical outcomes is described in Figure 1.

Because of the viral infection through ACE2, SARS-CoV-2 transmission depends mostly on respiratory droplets, and the primary viral replication is considered to occur in the human respiratory tract, with the distal airway, lung included, being the most vulnerable target.³⁶ Due to the complicated and not yet completely understood characteristics of COVID-19, reliable models that allow a substantial evaluation of SARS-CoV-2 infection in the pulmonary tissue are required, along with consistent methods in the constant search for possible therapeutic agents, effective antiviral drugs, and vaccines.

RESEARCH MODELS FOR RESPIRATORY DISEASE

The first models used to investigate human pathology were animal-based.³⁷ Such investigations enabled incredible advances in life sciences and are of obvious importance to this date, but present some downsides and considerations in regard to translatability, since some human aspects and disease characteristics are not correspondent in laboratory animals.³⁸ Under the respiratory spectrum, for instance, the presence of excessive mucus production, observed in some chronic pulmonary diseases as a key symptom, cannot be replicated in rodents due to the anatomical structures of the bronchial glands of mice and rats and their difference from the human respiratory anatomy.³⁹

Monolayer cell cultures, consisting mostly of one cell type, present uniformity and allow patterned and consistent investigations that rely on morphological, genetic, and physiological aspects. This traditional cell culture method is easily replicated, low on financial demand, usually of fast development and data collection, easily interpreted, and sustainable for long-term cultures, which leads bidimensional (2D) cultures to be, to date, the most commonly used lab method.⁴⁰ They have aided in the discovery of many biological and disease processes; however, they are unable to realistically simulate complicated microenvironment cells experience *in vivo*.^{40,41}

In humans, responses to infection, inflammation, cell recruitment, tissue remodeling, and regulation of homeostasis, including in the respiratory system, are complex events involving different types of cells.⁴² Overall, studies of human respiratory infections have been limited by the scarcity of functional models that reproduce physiology and pathophysiology *in vivo*. In addition, drug testing from both animal and *in vitro* models yields low success rates for new medicines; less than half of the new drugs under testing fail due to lack of

efficacy or concerns on safety before hitting the market.^{43,44} In this aspect, tridimensional (3D) culture models represent a more consistent alternative method, since they allow the integration of diverse cell types that spontaneously organize, and cell–cell and cell–matrix interactions; therefore, they replicate key histological and functional aspects of the target *in vivo* organ.⁴⁵ Although the majority of these 3D cultures are organoids, more recent models, such as organs-on-a-chip, are rapidly gaining popularity, and so far, there have been reports of 3D cell culture models for almost every human tissue and organ, including of the respiratory system.^{15,46} In particular, while both spheroids and organoids usually spontaneously organize themselves once primary or stem cells are allocated on a nonadherent surface or on top of scaffold,⁴⁷ for 3D tissue models, usually 3D-printed, the scaffolding structure is designed and engineered separately, and then the cell cultures are developed on the scaffold to form the final model.⁴⁸ While the former can usually “freely” grow in resemblance to the natural development, manipulated by the growth medium, the latter are artificially orchestrated into growing in an architecture determined by the researchers using physical templates.^{49,50} As an example, organs-on-a-chip combine 3D cell culture and microfluidic workflow, resulting in a dynamic biomimetic device, in contrast to the more static cultures.⁴⁷ The greatest advantage of using spheroids and organoids over organs-on-a-chip relies on the level of complexity of the culture; although more complex in comparison to 2D cell cultures, organoids are still much less complex to establish than organs-on-a-chip; and for institutions to obtain a patterning on organ-on-a-chip technology, they usually first go through spheroid and organoid experimentation. This can be observed by a recent publication investigating metrics on organoid and organ-on-a-chip publications: while over 2000 institutions were working and publishing with organoids, only 811 were researching organs-on-a-chip.⁵¹ As of a comparison between spheroids and organoids, spheroids are usually more suitable for replicating tissues and simplified versions of organs, while organoids offer the possibility to mimic multiple tissues within an organ, recapitulating the morphogenesis *in vitro*,⁴⁷ making it plausible to understand the quantity of research using organoids as a model in comparison to other 3D tissue models.

Each of the previously mentioned approaches presents indications of use, as well as advantages and limitations; a brief summary with the most prominent characteristics are shown in Table 1. Taking all these aspects into consideration, models that mimic human physiology and pathophysiology, such as organoid models, could be explored and are of great value due to the urge evoked by zoonotic infections, such as the ones caused by Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), influenza A virus (IAV) pandemics,⁵² as well as the new SARS-CoV-2.

SARS-CoV-2 may affect diverse human body systems, such as digestive, cardiovascular, and urinary; however, so far data indicate that the most drastically affected system is the respiratory.¹¹ Zhao and collaborators¹⁵ showed through the use of human liver ductal organoids that the *ex vivo* virus infection of liver tissue evokes severe damage, demonstrating that there is a need for human organoids to deeply investigate SARS-CoV-2-related tropism and pathogenesis in other tissues, which could also be valuable to treat COVID-19 patients. Besides, Monteil and colleagues¹¹ developed human blood vessel and kidney organoids, and showed that SARS-CoV-2 successfully infected the *in vitro* culture. In these models, the

Table 1. Evolution of Research Models in Life Sciences

time of method introduction	characteristics		
	type	contribution/possibilities of use	advantages
500 B.C.	animal model Mainly rodent models (murine + rat)	Provided much of what is now known in life sciences. Pharmacology, behavior, toxicology	Low cost; easy access
XX (1950)	Bidimensional (2D)	Enabled uncountable advances ever since the first cell lineage isolation, HeLa cells. Understanding molecular, chemical and physiological processes	cell culture Uniformity, patterned, consistent. Easily replicable, low cost, fast
XX and XXI	Tridimensional (3D); spheroids and organoids	Represents in vivo more consistently. Investigating normal function, disease, infection, etc.	Allows cell–cell and cell–matrix interactions; controlled environment, structure and aspects; more accurate representation of histological and functional aspects of tissue
XXI	Tridimensional (3D); organs-on-a-chip	Allows microfluidic circulation, intersystem interactions; intrasystem interaction is possible (i.e., trachea + bronchus)	Similar to 3D stated above; in additions, allows continuous fluid circulation; representation of several different structures combined is possible
			limitations Some aspects cannot be studied due to significant differences to human Unable to mimic microenvironment of in vivo High cost; sometimes difficult to replicate; usually represents isolated tissue or organ High cost; and can be difficult to pattern

authors proved that a higher expression of a recombinant form of ACE2, the protein used as a receptor for the virus in mammalian cells, significantly reduced the cell infection rate by the virus. Yet, this was only achieved if delivered during the earlier stages of infection. Since both studies model other tissues than the ones in the respiratory system, and considering that the lung is the most affected organ during a SARS-CoV-2 infection, it is of utmost importance to evaluate the virus behavior in the lung tissue, preferably in in vitro models that are able to mimic in vivo conditions, such as organoids.

This review investigated and summarized the most recent publications that present airway and alveoli organoid protocols, in order to present different methodologies that might be of great help in better understanding and eliciting critical aspects related to SARS-CoV-2 infection and the resulting COVID-19.

■ GENERATING LUNG ORGANIDS

The starting point to create an organoid can vary considerably. In the models used to date, the tissue structure is mainly obtained from embryonic stem cells, adult cells/stem cells/progenitors collected from human biopsy of the target organ, or adult tissue-derived induced stem cells. Therefore, the cell types that can be used to initiate an organoid are (1) pluripotent stem (PS) cells, cells with the capacity to differentiate into every cell type present in the organism, and that include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs); (2) multipotent stem (MS) cells, which are able to differentiate into every cell type within a germ layer, and include cells obtained from fetal tissue (9 weeks of development and on) and cells collected and isolated from some specific tissues, such as adipose tissue; and (3) adult tissue (AT) cells, which possess restricted differentiation capacity, oligo or unipotent, usually of one or two cell lineages present in the organ in which they are found, and can be isolated from adult tissue, mostly through biopsy of the target organ. Despite the cell lineage used as a starting point, all the mentioned cells are suitable for organoid generation because of their apparent infinite expansion potential in culture.⁵³

With respect to lung organoids, several research groups have already shown the successful generation of 3D models from pluripotent stem cells or primary respiratory cells,^{37,54–56} and most studies are based on the protocol by Miller and colleagues, published in 2019.⁵⁷ The resulting structure presents a similar cell organization to what is observed in a living lung, consisting of diverse cell layers and cell types, making lung organoids a highly sophisticated in vitro model to study developmental, homeostatic, and pathological processes.³⁷

These lung organoids that present multiple lung cell lineages are very attractive as research models due to their potential use in developmental and regeneration investigations, as well as more complex studies involving inflammatory and toxicological effects to particles and inhalants. Furthermore, they support the seductive possibility of developing transplantable material for patients suffering from pulmonary diseases that are, so far, incurable, such as fibrosis and degenerative lung diseases in general,^{58–60} in addition to viral infections, such as the one evoked by SARS-CoV-2¹¹ that could leave substantial sequelae in the recovered patient.⁶¹ Thus, regenerative medicine approaches, aimed at repairing, regenerating, and restoring missing function or tissue through isolated cells or lab-constructed 3D cell models, could aid in the treatment of COVID-19.¹²

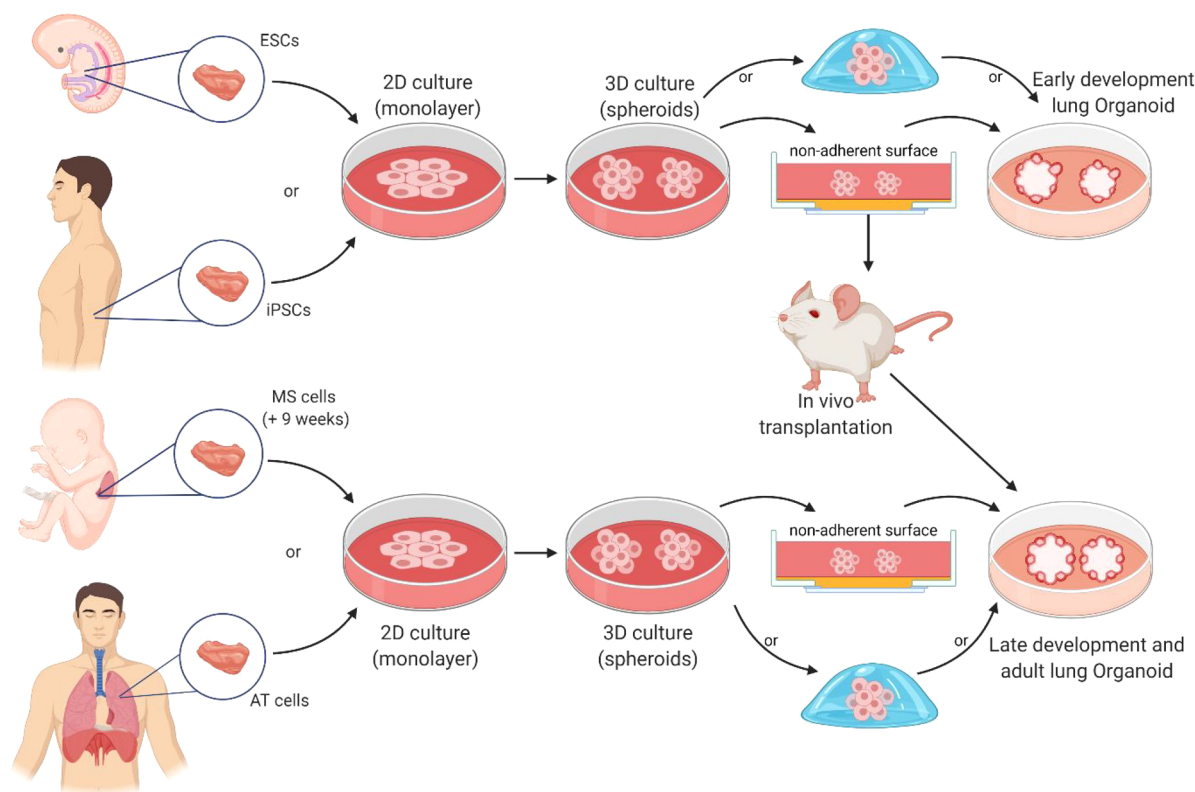


Figure 2. Organoid starting cell types and cultivation development. Human distal airway and alveoli organoids can be developed from different origin cells, and cultivation include particular steps. Abbreviations: ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; MS: multipotent stem; AT cells: adult tissue cells.

In order to replicate the extracellular matrix, most organoids are placed into a scaffold to which they interact and get support to form the cell agglomeration, which induces the 3-dimensionality. The first scaffolds used were microporous filters applied on metal, collagen, or sponge grid; they were gradually preferably replaced by porous membrane inserts.⁶² There are naturally derived scaffolds, such as collagen, gelatin, elastin, fibrin, and fibrinogen; synthetic polymers; or hybrids containing biomaterials and synthetics.⁶³ Any selected scaffold aims to reconstruct the physical properties of the extracellular matrix⁶⁴ and elicit cultivated cells to exhibit biofunctionalized characteristics of the *in vivo* cells.⁶³

The micro architecture resulting from porosity, permeability, and mechanical stability enables biophysical and biochemical interaction among cells. Thus, the addition of a scaffold into the organoid culture allows the regulation of the spatial configuration, as well as cell migration, differentiation, and proliferation. There are two main configurations using a scaffold: the organoid may grow immersed in the scaffold, or it may grow on top of scaffold; in the latter case, culture media is added to help cultivate and nurture the cells.⁶⁵ The choice of scaffold depends on the cell type cultivated and the intent of the study, and diverse growth as well as inhibitory factors might be added to the culture in order to regulate and control the organoid development.^{63,64} In the past decade, because of the increased research with organoid and 3D cultures in general, advances of cultivation techniques led to the mass commercialization of scaffold input, such as Matrigel, one of the most used scaffolds observed in the latest published research.⁶⁶

The exposure of organoids to the external environment is still limited, and recent approaches have proposed the contact of lung organoids to air, which enables the complete differentiation of the adult airflow cells, and an even more realistic investigation of the effects of interaction with pollutants such as toxic gases or toxic micro- and nanoparticles,⁶⁷ including viral particles. This method is known as the air–liquid interface (ALI), in which the organoids are exposed to air by removing or partially removing the culture media, so that at least a part of the organoid is exposed to air. It is also possible to convert organoids into a monolayer by dissociating and seeding the cells on transwells in order to obtain 2D ALIs.⁶⁸ The advantage of such a technique is the rapid generation of multiple homogeneous monolayers, consisting of the same diverse cell types previously present in the 3D cell culture, but with easier access to apical and basal surfaces, and regular exposure to medium.⁶⁹ Airway cells maintained under these conditions self-organize in a more natural manner, replicating more consistently the *in vivo* respiratory epithelium, with the development of ciliated pseudostratified cylindrical epithelium and goblet cells.^{70,71} A simplified visual description of the main steps to generate an organoid considering the starting point, cultivation methods, and 3D induction is in Figure 2.

To date, there are diverse organoid models to represent the respiratory system, including cultures to mimic proximal and distal airway, and alveoli. The most recent organoid methods used in the pneumological field and based on human tissue are described below, according to the origin cell lineage used to start the culture (PS, MS, or adult stem cells/progenitors).

■ AIRWAY AND LUNG ORGANOID MODELS

1. PS Cell-Derived Organoids. Pluripotent stem cell-derived organoids include organoids developed from both ESCs and iPSCs. Either way, lung organoids derived from human PS cells (hPSCs) possess complex tissue structure *in vitro*, with epithelium and supporting tissue (cartilage, smooth muscle, fibroblasts). Yet, they reflect the fetal airway, and adult airway-like structures only appear after *in vivo* transplantation.⁷² This has been shown for several other organoids and hPSC-based systems.^{37,73}

Considering organoids originating from ESCs, Zhao and colleagues⁷⁴ used human lung bud organoids derived from human embryonic stem cells (hESCs) to test viral infection. Bud tip progenitor cells generate all murine lung epithelial lineages and are found in the developing human lung, and although their certain role in development of human tissue is not completely clear, they might be involved.⁷⁵ The steps used to achieve the organoid passed through definitive endoderm differentiation with activin A, then induction of the anterior foregut until the formation of a lung bud organoid. The resulting lung bud organoid was embedded in a scaffold (Matrigel) in transwell inserts in order to induce an air–liquid interface (ALI). The authors demonstrated with this research that lung organoid derived from hESCs is able to simulate the infection of influenza virus to human, suggesting the clinical perspective of these organoid model,⁷⁴ suggesting that this model most probably is replicable for investigating other viral infections. In late October 2020, Han and colleagues⁷⁶ successfully showed that lung—and colonic—organoids, derived from hESCs, were permissive to SARS-CoV-2, and also performed a drug screening of FDA-approved medicine, proving that organoids are valuable models to investigate SARS-CoV-2 infection and COVID-19.⁷⁶

In another recent research study, Dye and collaborators⁷⁷ demonstrated that organoids derived from ESCs mimic early stages of fetal development but are able to mature at the molecular and structural levels when transplanted into immunocompromised mice. They transplanted hPSCs-derived lung organoids allocated in different biomaterial scaffolds to immunodeficient mice and observed that after some weeks *in vivo*, organoids presented improved tissue structure and cellular differentiation. Their aim was to define the physicochemical biomaterial properties that maximally enhance transplant efficacy and concluded that, while microporous scaffolds of poly(ethylene glycol) (PEG) hydrogel inhibited growth and maturation (they report that organoids remained consisting mostly of immature lung progenitors), scaffolds of polylactide coglycolide (PLG) or polycaprolactone (PCL) allowed maturation to tube-like structures that resembled the structure and cellular diversity of adult airways. Since PS cell-derived organoids usually derive from fetal-like structures, it is important to understand the best scaffold to be used in case a transplantation *in vivo* of lung organoids is required, in order to induce further maturation.

Other researchers working with ESCs proposed replication of genetically related lung diseases, such as fibrosis, in organoids. To date, there are organoid models with the introduction of mutation in diverse genes with the help of lentivirus or plasmid inserts, proving a resulting organoid that replicates alterations observed *in vivo*.^{46,74,78} The model presented by Strikoudis and colleagues⁷⁸ replicated the human airway and alveoli, composed of epithelial and

mesenchymal cells, in particular, a large fraction of AT2 cells, and importantly presented a branching organization, with a configuration that resembles the second trimester of human development. AT2 cells synthesize, secrete, and recycle all components of the surfactant; dysfunctions in its metabolism result in diseases (including distress syndrome and interstitial lung disease⁷⁹). Such models allow genetic manipulation in order to replicate specific lung diseases, with the possibility of investigating potential drug targets and medical treatments for specific diseases.

Still, in the case of ESCs-derived organoids, Porotto and coworkers⁸⁰ developed a model containing pulmonary mesoderm and endoderm, and branching airway and early alveolar structures after plating in scaffold, such as Matrigel.⁴⁶ The authors investigated viral infections common to infants that affect the distal airway. This method enables investigations related with viral pathogenesis in the developing or infant lung.⁸⁰

Another possibility to work with PS cells besides using embryonic cells is to induce pluripotency from adult cells, resulting in iPSCs.⁸¹ Organoids derived from these cells are very similar to those developed from ESCs, representing early stages (pseudo glandular and canalicular) of lung development. In a publication from 2019, Leibel and colleagues⁷⁹ also studied lung diseases with a genetic background, related to surfactant protein B deficiency. This deficiency leads to a fatal disease due to problems in surfactant production and depends on AT2 cells. The authors developed lung organoids from patient biopsy material that presented epithelial and mesenchymal cell populations of the proximal and distal airways, including AT2 cells. These, however, due to the genetic patient-specific configuration, presented alterations related to surfactant metabolism. Later, the researchers inserted a wild-type, normal gene using a lentivirus. The “corrected” organoids presented normal lamellar bodies and secretion of surfactant, similar to a healthy lung. This publication corroborates the idea that it is possible to mimic genetically related lung diseases *in vitro*, and in addition, it is possible to alter the genetic background of organoids. It is important to mention that the previously described PS cell-derived organoids are induced to 3-dimensionality at the anterior foregut stage; this work postulates that, by using the lung progenitor stage as the starting point, the organoid differentiation was shorter, and the culture presented cells in different stages of lung development.⁷⁹

Huang and colleagues⁸² used commercially available iPSCs to generate AT2 cells (referred to as iAT2), which presented the ability to endlessly propagate inside a 3D culture. The iAT2 cells were first cultivated as alveolospheres; then, cells were dissociated and further maintained in 2D ALI, with help of transwells. These 2D ALI cultures were permissive to viral infection and, thus, able to simulate apical viral infection by SARS-CoV-2. Although models using ESCs usually replicate immature and fetal stages of development, gene expression analyses demonstrated the presence of maturation genes, including surfactant proteins. In addition, air exposure seems to be important to induce further cell maturation, since iAT2 cells in 2D ALI presented maturation markers. After incubating the organoid with SARS-CoV-2, it was proven that the lamellar bodies, responsible for extracellular surfactant release and present inside iAT2 cells, were infected by virions; this result might further contribute to the current knowledge of the pathophysiology of COVID-19 and proves that this model is

representative for investigations involving SARS-CoV-2 and a reliable platform for drug screening.

2. MS Cell-Derived Organoids. Multilineage Cells. Skardal and co-workers⁸³ developed lung organoids modeled on the structure and cellular organization present in the airways by mounting them in three layers: in the lower layer, they added lung microvasculature endothelial cells, commercially acquired; in the middle layer, there were airway stromal mesenchymal cells, donated; and the upper layer was made up of bronchial epithelial cells. Thus, although they used multipotent isolated cells, they combined them in order to create a multigerm line-resulting 3D culture, which resembles PS cell-derived organoids but mimicks adult tissue. The purpose of the study was to perform a drug screening from commercial drugs removed from the market by the Food and Drug Administration (FDA) due to toxicity in humans. In addition to the lung organoids, they developed six other bioengineered models and articulated them in a microfluidic methodology (further mentioned in [Future Perspectives in COVID-19](#)).

Fetal Bud Progenitors. Organoids can also be derived from fetal tissue (as already mentioned, 9+ weeks of development) or fated cells originated from PS cells. In this case, the cells isolated are no longer pluripotent but multipotent. Miller and colleagues^{57,75} successfully generated lung organoids from PS cells induced as bud tip progenitors, that presented in vivo similarities, including airway expansion, only observed after maturation inducement with transcription and growth factors. Although not all organoids survived the induction, the remaining ones presented multiepithelial cell lineages, such as secretory, multiciliated, club, goblet, and neuroendocrine cells, along with the remaining progenitor cells, and thus represented the developing prenatal lung epithelium.⁷⁵ This model is very valuable to study human lung development in vitro. By the end of 2020, researchers from the same laboratory managed to expose the human lung organoids to SARS-CoV-2 infection.⁸⁴ Samuel and colleagues investigated whether anti-androgenic drugs could have an effect on the viral infection; the results indicated that there was a decrease in ACE2 levels, and a consequent reduction of the number of cells infected by the virus, showing that the anti-androgenic property presents a protective effect against SARS-CoV-2.

Another study, also working with fetal bud progenitors, was able to show that, with a specific combination of signal ligands and inhibitors, a fetal-like organoid possessing AT2 cells can be generated.⁸⁵ In addition, besides working with AT2 populations, the authors showed that alveolar epithelial type I cells, that constitute a thin wall for gas exchange in lung, can also be cultured in vitro. In order to do this, differently from Miller et al.,^{57,75} they used cocultures from primary alveolar cells and magnetically activated cell sorting, and then further sorted into wells with a scaffold. It is important to mention that fibroblasts were removed from all cultures, and by doing so, the resulting organoid presented alveolar cells (AT2, mainly) and not only epithelial cells; in addition, there were organoids with and without empty cavities, resembling the airway. When fibroblasts were present, tracheospheres were generated. The authors also succeeded in inducing retroviral genetic modification to evoke fibrosis-associated alterations in organoids, suggesting that this method can also be used to induce diverse genetic-associated lung diseases. This indicates the possibility of use of this model for genetic alterations.

Lamers and colleagues⁸⁶ adapted a protocol from Miller et al.,⁸⁷ based on lung bud tip organoids, and started at fetal tissue (15–20 weeks) to derive bronchioalveolar organoids. The resulting model presented diverse cell types, including club, goblet, and ciliated cells, with more than 90% of alveolar-like cluster cells of which 46% were AT2 cells. After the organoid was established, cells were dissociated and seeded on transwells for ALI-monolayer cultivation, as previously explained. Then, the model was exposed to SARS-CoV-2 and proven to be susceptible to viral infection. An important point shown in this article is that AT2 cells seem to present low ACE2 expression, and in accordance with studies using human tissues, SARS-CoV-2 infection might start by other cell populations, such as ciliated cells, indicating a systematic infection by the virus.⁸⁸ The authors also used the model to perform drug screenings and presented results that indicate that interferon treatment with low doses was sufficient to reduce viral replication and tissue infection. Thus, alveolar-like and airway models present the potential to be used for further investigations on SARS-CoV-2 infection and COVID-19 therapeutics.

Mesenchymal Stem Cells. A final possibility when working with MS stem cells for lung organoids is to use mesenchymal stem cells as the starting point. Wang and collaborators⁸⁹ developed an alveolar organoid with epithelial stem/progenitor cells from mesenchymal cells obtained and isolated from the postnatal human lung, obtained from pediatric patients undergoing elective surgery for some airway abnormalities. This method may be used to study congenital lung lesions and alterations, and although this publication used pediatric-derived tissue, the use of adult isolated cells might replicate adult in vivo tissue.

3. AT Cell-Derived Organoids. Finally, lung organoids can be developed from stem and progenitor cells isolated from adult tissue (AT). The convenience of this method is that adult cell-organoids present the physiological dynamic consistent with adult in vivo tissue, which was not possible in stem cell-derived models.⁷⁶ In research from 2019, Kim and colleagues⁹⁰ successfully reconstituted the lung cancer morphology and histological features of original tissues in an organoid model starting from primary lung cancer tissues. In order to do so, cells isolated from patient biopsies were paired with non-neoplastic airway tissues, creating a biobank of 80 lung cancer organoid lines from five subtypes of lung cancer and five normal bronchial organoids. The aim of the study was to perform an anticancer drug screening, but the model can also be used for predicting individual patient responses to different drugs. As organoids developed, tubule-like structures were observed, and the morphology was maintained for subsequent passages (over 10 passages). The model presented a pseudostratified epithelium composed of basal cells, a type of progenitor cell, and luminal cells including secretory and ciliated cells. The authors confirm that normal bronchial generated organoids maintain the histological and genetic characteristics of their respective parental tissues, and thus have potential for use in patient-specific drug trials and proof-of-concept studies on targeted therapy and resistance mechanisms.

Also starting from biopsy material, Bui and colleagues⁹¹ generated organoids from differentiated primary human bronchial epithelial cells and type-I-like alveolar epithelial cells from nontumoral residual tissues of patients undergoing surgical resection. The resulting airway organoid was positive for a variety of lung epithelial cells, such as goblet, basal, and

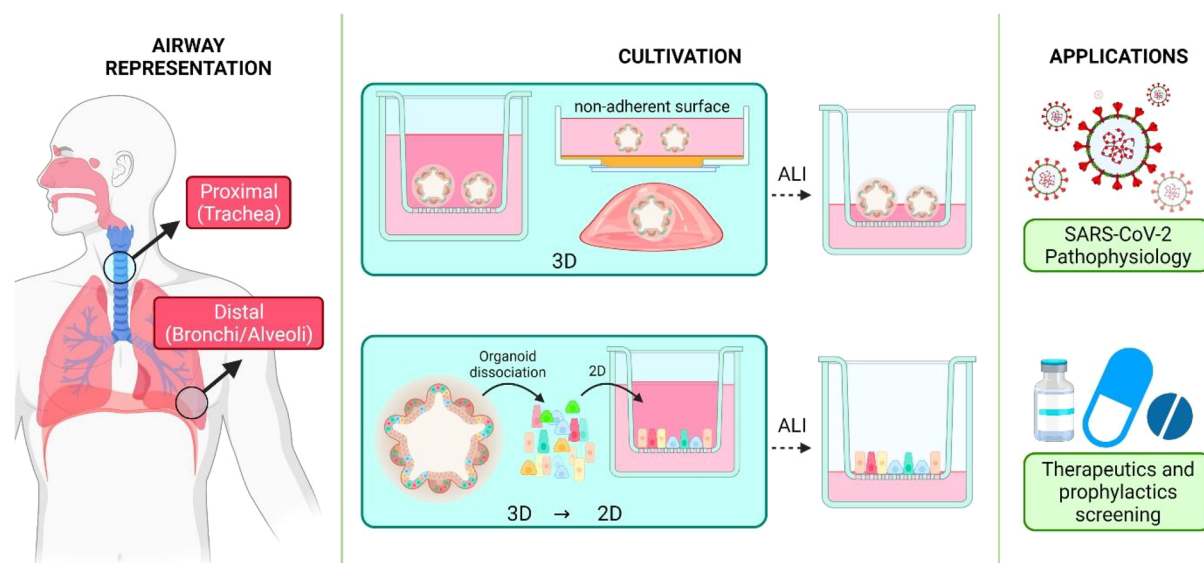


Figure 3. Airway organoid model possibilities. Airway organoid models can be derived to represent either proximal or distal airway. For cultivation methods, they can be maintained in 3D configuration using scaffold, nonadherent surface, or transwell, or dissociated and kept as 2D monolayer for easier pathogen exposure, for example; both methods can also be exposed to air using ALI. Organoids can be used to further explore the pathophysiology of a SARS-CoV-2 lung infection, and to screen for therapeutics and prophylactics. Abbreviations: ALI: air liquid interface.

ciliated cells, along with the presence of mucus within the lumen and active ciliary beating. The model was used to study viral infection, and the authors show that the virus infected the organoid cells, demonstrating a similar tropism to what is observed in the in vivo human airway. This indicated the possible use of this method to study viral infections in general.

Considering studies that already managed to investigate the SARS-CoV-2 infection in organoids, Salahudeen and colleagues⁷¹ used biopsy-derived human lung distal pulmonary tissue in order to generate AT2 and basal organoids, which were later exposed to SARS-CoV-2 (and H1N1) in order to better investigate the viral tropism in the pulmonary tissue. The authors discovered that, in relation to SARS-CoV-2, the virus targets in particular club cells, while ciliated cells seem not to be infected.

Wang and colleagues used club and AT2 cells isolated from adult mice to generate lung organoids, which were tested to investigate drug efficacy in SARS-CoV-2 infections. Although the effect of drugs on organoids cannot be fully translated to clinic expectations, this first glimpse using organoids exposed to SARS-CoV-2 is very useful in the search for new therapeutics.⁹²

Similarly, Youk and colleagues⁹³ also used AT2 cells, but isolated from biopsy material from human lung tissue. The results indicate that AT2 cells serve as stem cells in the model, generating a feeder-free human 3D alveoli-like organoid, suitable for self-maintenance for at least 6 months. Interestingly, authors also managed to infect the model with SARS-CoV-2 and showed that alongside AT2-infected cells, viral transcripts were also found in the supernatant, suggesting secretion of viral particles by infected cells. These findings prove that the model successfully replicated key aspects of SARS-CoV-2 infection and simulate the viral propagation within the organ and to outer structures similarly to the circulatory viral spread observed in human patients. In addition, after transcriptome investigation, it was shown that both genes that regulate interferons and proinflammatory genes were overexpressed, indicating that the model is able to

show an endogenous innate immune response after viral infection. Likewise, Katsura and collaborators⁹⁴ isolated AT2 cells from human lung biopsies and generated alveolospheres for subsequent SARS-CoV-2 infection. These authors also developed mouse-derived alveolospheres for comparison, as well as clinical analyses from human patients. The results indicate that 3D-cultured AT2 cells expressed ACE2 and were permissive to SARS-CoV-2 infection, and the culture presented an inflammatory state post-infection, indicating a delayed innate immune response, consistent with the investigation by Youk et al.⁹³ In addition, there was a significant down-regulation of surfactant proteins release in spheroids, similarly to what is observed in human patients and that underlies the alveolar collapse. In addition, the model was used to investigate preinfection treatment with interferons, which was shown to present prophylactic effects against SARS-CoV-2 infection, and should be further investigated with regard to its potential against the disease. Overall, the model is representative of diverse responses observed in human in vivo lungs and also in drug and therapeutic screenings for COVID-19.

More recently, Mulay and colleagues⁹⁵ also developed alveolospheres, but in addition, they developed proximal airway cultures, which are important for representing the initial site of viral infection. The starting points were tracheal and upper bronchial human tissue collected from deceased organ donors.⁹⁵ For both types of culture, isolated cells were cultivated with fibroblasts, and proximal airway culture was maintained in ALI. After establishment, 3D cultures were exposed to SARS-CoV-2, and the infection was heterogeneous: at first stages, the virus predominantly targeted ciliated cells, in accordance with previous investigations using in vitro models and patient biopsies;^{88,96} besides, proximal cultures were more easily infected by the virus than distal cultures, which had to be gently “opened” to be permissive. This step had to be performed so that the apical cellular membrane, usually facing inward, was exposed and allowed viral infection and replication. Moreover, after AT2 cells were infected, a proinflammatory response was observed, along with interferon

Table 2. Recent Human Lung Organoid Models^a

differentiation potential	cell line	origin	initial 2D culture	3D induction	cell types in organoid	representation	possible uses	limitations	refs
PS cells	ESCs	Standardized commercial cell lines (NIH)	PSCs induced to endoderm, induced to anterior foregut, induced to spheroids	Transferred into scaffold + Transwell insert (ALI) + in vivo transplantation for maturation	Endoderm cells Airway epithelial cells + support tissue	Distal airway (fetal + adult) airway	Viral Infection, due to air exposure Adult-like lung model	Fetal-like lung model Necessity of vivo transplantation (use of animals); only airway representation	Zhao et al., 2020 ⁴ Dye et al., 2020 ⁷⁷
MS cells	iPSCs	Skin biopsy (fibroblast)	iPSCs induced to endoderm, induced to anterior foregut, induced to lung progenitors	Transferred into scaffold	Epithelial + mesenchymal cells; large fraction of AT2 cells Mesoderm + endoderm cells Epithelial + mesenchymal cells	Airway + alveoli Distal airway + alveoli Proximal and distal airway + alveoli	Mimic lung diseases (fibrosis) Respiratory viral pathogenesis in infant lung Disease-specific targeting; Surface metabolism	Fetal-like lung model Fetal-like lung model; not vascularized	Strikoudis et al., 2019 ⁷⁸ Porotto et al., 2019 ⁸⁰ Leibel et al., 2019 ⁷⁹
MS cells	Mesenchymal Endothelial Epithelial	Stroma donation; Standardized commercial cell lines	Separated cell lineages cultivated	Transfer all cultures transferred into microfluidic semipermeable membrane	Stromal, endothelial, epithelial cells	Airway	Effects of drugs; interaction with other tissues (6 tissues)	Necessity of a chip technology; only airway representation	Skandal et al., 2020 ⁸³
PS cells - Fetal bud tip progenitors	Human lung fetal tissue (+12 weeks)	Human lung fetal tissue (+12 weeks)	Bud tip progenitors isolated and cultivated	Transferred into scaffold	Endoderm, mesoderm, epithelial cells AT2 cells mainly	(fetal + adult) airway Alveoli	Study human lung; regenerative medicine, tissue engineering, and pharmaceutical safety and efficacy testing Induce and test genetic manipulation	Need for vivo transplantation (use of animals); only airway representation Fetal-like lung model	Miller et al., 2019 ⁵⁷ and 2020 ⁷⁵ Shiraishi et al., 2019 ⁸⁵
Mesenchymal	AT2 cells isolated and cultivated AT2 and fibroblasts cocultured	Adult tissue (biopsy) (pediatric patients)	Tissue dissociation; mesenchymal cells isolated; cultivated in monolayer	Transferred into scaffold	AT2 + fibroblasts Mesenchymal cells	Trachea Alveoli	Study congenital lung lesions and COPD	Fetal-like lung model; only airway representation Postnatal lung model; not yet known if adult-derived tissue evokes adult-like tissue	Wang et al., 2020 ⁸⁹
AT cells	Bronchial progenitor cells Bronchial and type-I-like alveolar cells	Adult tissue (biopsy)	Tissue dissociation; cell isolation; monolayer cultivation	Transferred into scaffold	Epithelial cells Bronchial progenitor cells + AT1-like cells	Distal airway + alveoli Airway	Develop patient-specific drug trials Viral infection (Influenza B)	Absence of stromal and immune cells Absence of stromal and immune cells; only airway representation	Kim et al., 2019 ⁹⁰ Bui et al., 2019 ⁹¹

^aPluripotent stem (PS) cells; ESCs (embryonic stem cells); induced pluripotent stem cells (iPSCs); multipotent stem (MS) cells; adult tissue (AT) cells; AT1 (alveolar type 1) cells; AT2 (alveolar type 2) cells.

Table 3. Recent Human Lung Organoids Models Used to Study SARS-CoV-2^a

differentiation potential	cell line	origin	initial 2D culture	3D induction	cell types in organoid representation	applications in study	limitations	refs
PS cells	PS cells - Fetal bud tip progenitors	Standardized commercial cell lines (NIH)	PSCs induced to endoderm, induced to anterior foregut, induced to spheroids	Transferred into scaffold + in vivo transplantation for maturation	Airway + alveoli	Exposure to SARS-CoV-2 and drug screening	Necessity of vivo transplantation (use of animals); only airway representation	Han et al., 2021 ⁷⁶
	iPSCs	Standardized commercial cell lines (SPC2)	iPSCs induced to iAT2	iAT2 cells cultivated as alveolospheres; then, cells were dissociated and further maintained in 2D ALI	Alveoli	Exposure to SARS-CoV-2 and drug screening	Lack of AT1, mesenchymal and immune cells	Huang et al., 2020 ⁸⁵
MS cells	PS cells - Fetal bud tip progenitors	Standardized commercial cell lines (NIH)	PSCs induced to endoderm, induced to anterior foregut, induced to spheroids	Transferred into scaffold + Transwell insert (ALI)	Distal airway	Exposure to SARS-CoV-2 and drug screening	Fetal-like lung model. Lack mesenchymal and immune cells	Samuel et al., 2020 ⁸⁴
	Fetal bud tip progenitors	Human lung fetal tissue (+12weeks)	Mostly alveolar cells	AT2 cells cultivated as organoids; then, cells were dissociated and further maintained in 2D ALI	Alveoli	Exposure to SARS-CoV-2 and drug screening	Lack of mesenchymal and immune cells	Lamers et al., 2021 ⁸⁶
AT cells	Lung cells	Adult tissue (biopsy)	Tissue dissociation; cell isolation; monolayer cultivation	AT2 cells cultivated as organoids; then, cells were dissociated and further maintained in 2D	Alveoli	Exposure to SARS-CoV-2	Absence airway and single type cellular representation	Youk et al., 2020 ⁹³
	Airway tissue		Tissue dissociation and monolayer cultivation	AT2 cells cultivated as alveolospheres; then, cells were dissociated and further maintained in 2D	Alveoli	Exposure to SARS-CoV-2 and treatment	Absence airway and single type cellular representation	Katsura et al., 2020 ⁹⁴
				Transferred into scaffold	Club cells, ciliated cells and AT2 cells	H1N1 or SARS-CoV-2	Absence of stromal and immune cells	Salahudeen et al., 2020 ⁷¹
				Cell cultivates with fibroblast, proximal airway maintained in ALI	Proximal airway cells; distal airway cells	Exposure to SARS-CoV-2	Lack immune cells	Mulay et al., 2020 ⁹⁵
				Cells cultivated as organoids; then, cells were dissociated and further maintained in 2D ALI	Airway	Exposure to SARS-CoV-2 and drug screening	Lack of mesenchymal and immune cells	Lamers et al., 2021 ⁸⁶

^aPluripotent stem (PS) cells; ESCs (embryonic stem cells); induced pluripotent stem (iPSCs); multipotent stem (MS) cells; adult stem (AS) cells; AT1 (alveolar type 1) cells; AT2 (alveolar type 2) cells; iAT2 (induced alveolar type 2) cells; ALI (air-liquid interface).

pathway upregulation, in accordance with other authors investigating lung organoid SARS-CoV-2 infection;⁹³ this response was followed by an increase in proteins associated with cell-autonomous and non-cell-autonomous apoptosis, which contributes in the long run to alveolar injury.

In addition to the human fetal bud tip lung organoids, Lamers and colleagues⁸⁶ also derived airway organoids from adult human donors. Following a similar protocol, after organoid generation, cells were dissociated and seeded on transwells to induce 2D ALI; these cultures were compared to the fetal-derived culture, already described. The model represents the small airway epithelium, also very valuable to investigate viral infection.

A systematic example of organoid cultivations mentioned in the articles present in this review and used in COVID-19 investigations are listed in Figure 3. A summary of the mentioned models suitable for infectious diseases can be seen in Table 2, and airway models specifically for COVID-19 investigations are listed in Table 3.

Future Perspectives in COVID-19. Due to the possibility of reproducing the *in vivo* tissue in culture, human lung organoids can provide robust support to simulate SARS-CoV-2 infection and replication in humans. The virus tropism in the organism tissues is not completely understood, due to the lack of suitable research models that allow this investigation; thus, mechanisms of SARS-CoV-2 pathogenesis mainly depend on clinical characteristics and autopsy reports and bioinformatics analysis.³⁵

Although it seems that alveoli are the most affected structures during severe COVID-19, it has already been demonstrated that the distal airway as a whole is more drastically affected, because SARS-CoV-2 can significantly infect a higher number of cells, due to an increased expression of ACE2 proteins in cells of the inferior respiratory tract; in addition, ciliated cells from the superior respiratory tract tend to direct the viral load to the esophagus (digestive system), while viruses that reach the larynx (inferior respiratory tract) continue in the descendant path toward the alveoli, indicating that once SARS-CoV-2 manages to infect the inferior tract, it can replicate and continue to infect respiratory cells.^{8,97} Previous studies indicate a high prevalence of type II pneumocytes, bronchial epithelial cells, and AT2 cells as major targets of SARS-CoV-2 infection.^{98,99} In the present review, the most recent protocols to develop human airway organoids are listed and briefly described, and they include models that replicate alveoli in specific and distal airways, and also the proximal airway; in addition, these models can be developed from diverse origin cells, from PS such as ESCs and iPSCs, MS such as mesenchymal but also fetal cells, to AS cells. Thus, such organoid models can be adapted and employed to help better understand COVID-19 and SARS-CoV-2 behavior in the lung and, as a consequence, provide insights on possible interventions such as treatments and vaccines.

In addition, according to Elbadawi and Efferth,¹⁰⁰ as recently reviewed, the coculture of alveolar cells with cells from different tissues would be ideal for COVID-19 investigations, such as immune cells, which would deliver an overview of the immunological response to the virus and effects of immunomodulatory drugs. Adding vascular cells such as endothelial lineages to the organoid model could also represent an interesting approach, since the virus reaches the circulatory flow through intimate contact with the pulmonary capillary bed, as also reported for SARS-CoV¹⁰¹ as well as SARS-CoV-

2.^{102–104} This could be more easily modeled using a transwell or by adding the ALI system, inducing mucus production, formation of stratified epithelium, and functionality of pulmonary cells for diverse pathogen-induced pulmonary diseases such as tuberculosis.^{105,106} These particularities will allow further investigation of viral effects on human pulmonary tissue, virus tropism, and cytokine release and permit investigation of therapeutic drugs, with the help of complementary methods, such as polymerase chain reaction (PCR), Western blot, and immunohistochemistry.¹⁰⁰ Thus, lung organoids are very helpful for high-throughput assays for host–pathogen interaction and outcome characterization, such as proteomics, phosphoproteomics, and global transcriptome, among others.¹⁰⁷ These approaches enable the achievement of tissue-specific results, without the interference of systemic factors, and provide insight on the physiology and pathophysiology of the target organ, such as the lungs.

In addition to the pulmonary tissues, several researchers have shown that COVID-19 patients showed multiorgan damage and dysfunction, possibly also leading to multiorgan failure.^{5,7,96,108} In view of the need to understand the tropism of SARS-CoV-2 in the human organism, other 3D approaches than lung organoids, including microfluidic on-a-chip technologies, that mimic *in vitro* the interaction of different organs, must be explored. As an example, Zhao and colleagues¹⁵ (already described under **PS Cell-Derived Organoids**) developed an integrated system with six different organoids and simulated a circulatory system among them. Considering what is now known for SARS-CoV-2 infection and how the virus spreads throughout the body to induce COVID-19-related alterations, a more detailed—and integrated—in *vitro* replication such as these could give a more complete perspective on how we could overcome COVID-19 and the SARS-CoV-2 pandemic.

Considering all the possible respiratory tract organoid models to investigate COVID-19, either representing proximal or distal airway, it is important to consider that in spite of the advantages, there are some important limitations. A major downside is the absence of vasculature and immune cell components in the majority of the mentioned protocols, crucial for the systemic understanding of a viral infection. In a physiologically relevant *in vitro* model of study, in such cases, it is important to ensure that all prominent cellular components are present in the cultivation.¹⁰⁹ In the will to overcome this barrier, some researchers have developed 3D cell culture models using commercially available cell lines and artificially combining AT2, endothelial, macrophages, and mast cells, cultivated in ALI.¹¹⁰ Such tetra-culture can also be very valuable for COVID-19 investigation, since it represents mature alveolar tissue. Similarly, there is increasing investigation using artificially assembled microfluidic approaches that enable the cocultivation with capillary-like structures, as well as exposure to immune cells, with the advantage of also presenting exposure to “blood” flow.^{111,112} To date, there are reports of airway microfluidic cultures, cocultivated with vascular and immune cells, for posterior exposure to pathogens, including SARS-CoV-2.^{38,102–104} The microfluidic upside is that the blood-like tissue is pumped throughout the culture system, using a fluid flow, simulating the *in vivo* situation.^{111,112} There are reports of the establishment of distal airway microfluidic models, or specifically alveolar cultures, cocultivated with microvasculature^{102,104} and immune cells^{102–104} on a chip, for further SARS-CoV-2 exposure and

investigation. The results indicate the important involvement of immune responses through the alveolar barrier, showing that the microfluidic system resembles in vivo situations and could be valid for research that requires interaction to blood and immune cells.

A combination of organoid and chip technology was proposed in 2010,¹¹³ and since then further developed. Organoids-on-a-chip improve the efficiency and reproducibility of organoid cultures.¹¹⁴ In this approach, diverse cultivation parameters can be precisely controlled by the chip technology, with the cell development and spontaneous arrangement typical from organoid culture.¹¹⁴ Considering that microfluidic devices provide long-term culture system, they are able to deliver nutrients, metabolites, and gas exchanges to the organoids via laminar flow.¹¹⁵ Furthermore, it is possible to induce vascularization in the organoids by the microfluidic chip and, with this, increase representability and organ functionality.¹¹⁶ Though promising, this type of model presents some limitations, in particular in relation to the necessity of external pumps and connectors to correctly operate, which reduces the patterning and elevates the costs.¹¹⁴ Still, it is expected that, in the future, such models could be more easily replicated and accessible to a higher number of researchers.

CONCLUSION

In the current scenario, where humankind is confronted with a new and highly infectious virus, with so many aspects yet to be discovered, the access to models that consistently represent the human organs on a dish is undoubtedly urgent. Organoids and other 3D models are valuable complementary tools to investigate COVID-19 pathophysiology and the effects of SARS-CoV-2 in human tissue and further enable the development and validation of therapeutics and prophylactics.

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Author Contributions

All authors performed the literature search, drafted sections of the manuscript, and prepared figures and tables. M.E.S. supervised the manuscript and reviewed the language. All authors subsequently revised the manuscript.

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