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REVIEW

Harnessing three-dimensional (3D) cell culture models for pulmonary infections: State of the art and future directions

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Abstract

Pulmonary infections have been a leading etiology of morbidity and mortality worldwide. Upper and lower respiratory tract infections have multifactorial causes, which include bacterial, viral, and rarely, fungal infections. Moreover, the recent emergence of SARS-CoV-2 has created havoc and imposes a huge healthcare burden. Drug and vaccine development against these pulmonary pathogens like respiratory syncytial virus, SARS-CoV-2, *Mycobacteria*, etc., requires a systematic set of tools for research and investigation. Currently, in vitro 2D cell culture models are widely used to emulate the in vivo physiologic environment. Although this approach holds a reasonable promise over pre-clinical animal models, it lacks the much-needed correlation to the in vivo tissue architecture, cellular organization, cell-to-cell interactions, downstream processes, and the biomechanical milieu. In view of these inadequacies, 3D cell culture models have recently acquired interest. Mammalian embryonic and induced pluripotent stem cells may display their remarkable self-organizing abilities in 3D culture, and the resulting organoids replicate important structural and functional characteristics of organs such the kidney, lung, gut, brain, and retina. 3D models range from scaffold-free systems to scaffold-based and hybrid models as well. Upsurge in organs-on-chip models for pulmonary conditions has anticipated encouraging results. Complexity and dexterity of developing 3D culture models and the lack of standardized working procedures are a few of the setbacks, which are expected to be overcome in the coming times. Herein, we have elaborated the significance and types of 3D cell culture models for scrutinizing pulmonary infections, along with the in vitro techniques, their applications, and additional systems under investigation.

Keywords 2D cell culture · 3D cell culture · Pulmonary infections · SARS-CoV-2 · Organoids · Spheroids · Respiratory viruses

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Introduction

Cell culture has evolved into an indispensable tool for understanding the basic biophysical and biomolecular processes by which cells form tissues and organs, how these tissues work, and how their functioning is affected by disease. In-vitro cell cultures hold a crucial position as research tools for simulating human development and a wide range of diseases. Cell culture assays offer a way to quantitatively assess the existence, quantity, or functional activity of a cell or tissue. Cell culture models have largely been adopted for biochemical studies (Duval et al. 2017). Although 2D cell cultures lack the required tissue architecture, it has some major advantages against animal models. Genetically engineered mouse models have a significant role in developmental cancer research, but they cannot mimic the diversity, physiology, and genetics of human conditions satisfactorily. The low cost, low maintenance time with a high reproducibility

associated with cell culture models provide major benefits when compared to animal experimentation. Simpler scalability with easy functional analysis extends the utility of cell culture models. These high-throughput assays provide physiologically relevant models, as compared to expensive, labor- and time-consuming animal models (Corrò et al. 2020). Since their initial approval in 1980, biotherapeutic proteins have been widely studied for their applications in a range of conditions. Recently, the trends in biotherapeutic protein development have shifted from mammalian Chinese hamster ovary and murine myeloma to using human cell lines, owing to the greater likelihood of post-translational modifications consistent with endogenous human proteins (Dumont et al. 2016). From a historical perspective, first cell culture was successfully cultivated from amphibious spinal cord by Harrison in 1907 and Carrel in 1912, which demonstrated that these cultivated cells can be kept alive for longer periods of time with nutrient supply in an aseptic environment. Finally, the first human cell line was cultured from cervical cancer cells, by George Otto Gey in 1951. The first effective isolation of embryonic stem cells was reported in 1981 using mouse embryos. Further, Thomson and colleagues achieved the first isolation of the human cell lineage in 1998 (Thomson et al. 1998). The establishment of induced pluripotent stem cells (iPSCs) was witnessed in 2006, followed by description of small intestinal organoids in 2009. Eventually in 2012, the first induced neural stem cells (iNSCs) were established (Sakalem et al. 2021). Flat-support two-dimensional (2D) monocultures are the most exploited in-vitro assay techniques to understanding the underlying cell behavior and uncover a range of biomedical and pharmaceutical applications, owing to their ease of handling, high-throughput, reproducibility, and cost-effectiveness. However, 2D cultures lack the required cellular cross-talking networks, which are efficiently harnessed in three-dimensional (3D) cultures with a better mimicking of in-vivo conditions (Prasad et al. 2021). Several techniques have been utilized to develop 2D and 3D cell culture models, with their respective benefits and limitations. A few commonly used techniques for establishing 2D culture models include the sandwich culture method, micro-pattering, and altering substrate stiffness. For 3D models, spheroid cultures, biopolymer scaffolds, prefabricated scaffolds, hydrogels, cell sheets, and microfluidics are commonly exercised. Despite the long-standing dominance of 2D cultures, recent developments have demonstrated a shift toward 3D culture models, due to the more accurate biochemical and biomechanical microenvironments they provide (Duval et al. 2017). A universal platform for applying 3D culture techniques does not currently exist, since many processes can be satisfactorily simulated using 2D culture models (Habanjar et al. 2021). The recent introduction of 3D cell culture has aimed at a more wholesome modeling of the in-vivo physiology and biomolecular interactions with a better biocompatibility. 3D models have opened up novel avenues for studying the basic translational machinery, cell–cell interactions, cell–matrix interactions, and other cellular behaviors (Duval et al. 2017). In the 1970s, the earliest 3D cell culture was made in a soft agar solution, by Hamburg and Salmon (Kapałczyńska et al. 2018). With further optimization of existing technologies and development of efficient scaffold, 3D culture systems are prone to become more promising to benefit patient outcomes by their integration with regenerative medicine (Su et al. 2021). These models offer significant potential for analyzing drug disposition and pharmacokinetics that affect medication safety and efficacy from an early stage of drug development since they present physiologically appropriate cellular milieu (Mittal et al. 2019). These systems come in a variety of forms, from simple spheroids to more intricate organoids and organs-on-chips. Single-cell type static 3D models, cell co-culture models with microfluidic control, and hybrid 3D systems are other subcategories (Wang 2018). Given the limited utilities and extrapolation of data with 2D cell cultures, 3D models have provided to be better platforms for cell- and organ-based assays. They enable a thorough assessment of disease models from patient-derived cells as well as the medication safety evaluation of low clearance drugs and multiple dosing studies. These organ-specific 3D models are incorporated into a variety of micro-physiological systems to evaluate the effects of various drug administration methods on pharmacokinetics and to improve drug safety and efficacy (Dame and Ribeiro 2021; Wang et al. 2021). The lack of a standardised method for 3D cultures is of significant concern since it is challenging to establish a uniform technique for various cell types. However, this flexibility and fluidity which lets 3D systems thrive and advance routinely. Moreover, the establishment of these 3D cultures is a difficult task, given the complexity and dexterity required in identifying an appropriately viable cell type, and scaffold materials that enable satisfactory development of ECM (extracellular matrix) and vascularization. With some progress and better guidance in this area, more accuracy can be obtained in replicating in vivo tissue conditions using 3D cell cultures (Duval et al. 2017; Sirenko et al. 2015). This review summarizes various 3D cell culture models in the context of providing a platform as drug development tools in pulmonary infections, which are globally the leading causes of morbidity and mortality. Recent upsurge in the emergence of pulmonary infections, including SARS-CoV-2 (COVID-19), has underscored the need to optimize strategies to tackle such infections. COVID-19 has impacted the human socialization and economy in innumerable ways. An efficient evaluation of these infections and establishment of interventions is hence vital to human health and wellbeing (de Dios-Figueroa et al. 2021; Harb et al. 2021). Figure 1 depicts some of the common pulmonary infections

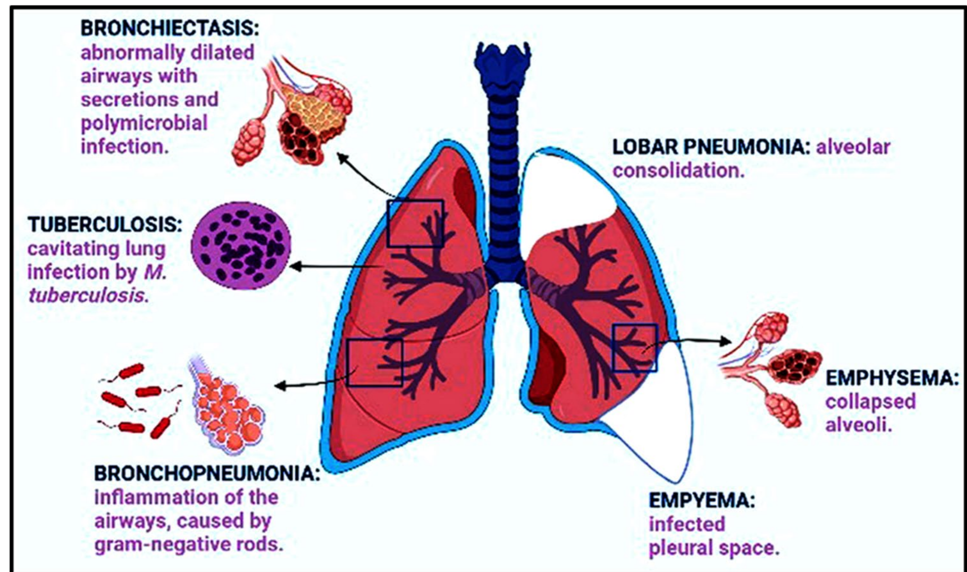

Fig. 1 Schematic representation of some of the common pulmonary infections

and Fig. 2 demonstrates a comparative representation of 2D versus 3D cell culture models. Different categories of 3D culture models, along with the in-vitro techniques for their design and development, have also been discussed. Moreover, the applications and clinical implications of these systems in different pulmonary infections and recent advancements with the ongoing clinical analyses have been communicated in this review.

Two-dimensional models allow the diffusion of soluble factors into the media, without the development of a gradient. On the contrary, three-dimensional models allows the generation of a concentration gradient of soluble molecules like growth factors, as shown in several studies, particularly in the microfluidic systems (Amadi et al. 2010; Kamei et al. 2015; Zervantonakis et al. 2010). 3D cell culture models also exhibit a better cell–cell interaction profile, compared to 2D models, which have monolayers of cells (Bonnier et al. 2015; M. B. Chen et al. 2013; Longati et al. 2013). Also, there is an insufficient expansion of cells in 2D models since they lack the complexity required for cell growth. On the contrary, 3D models have shown to demonstrate better ASC proliferation (Birgersdotter et al. 2005; Edmondson et al. 2014; Gilbert

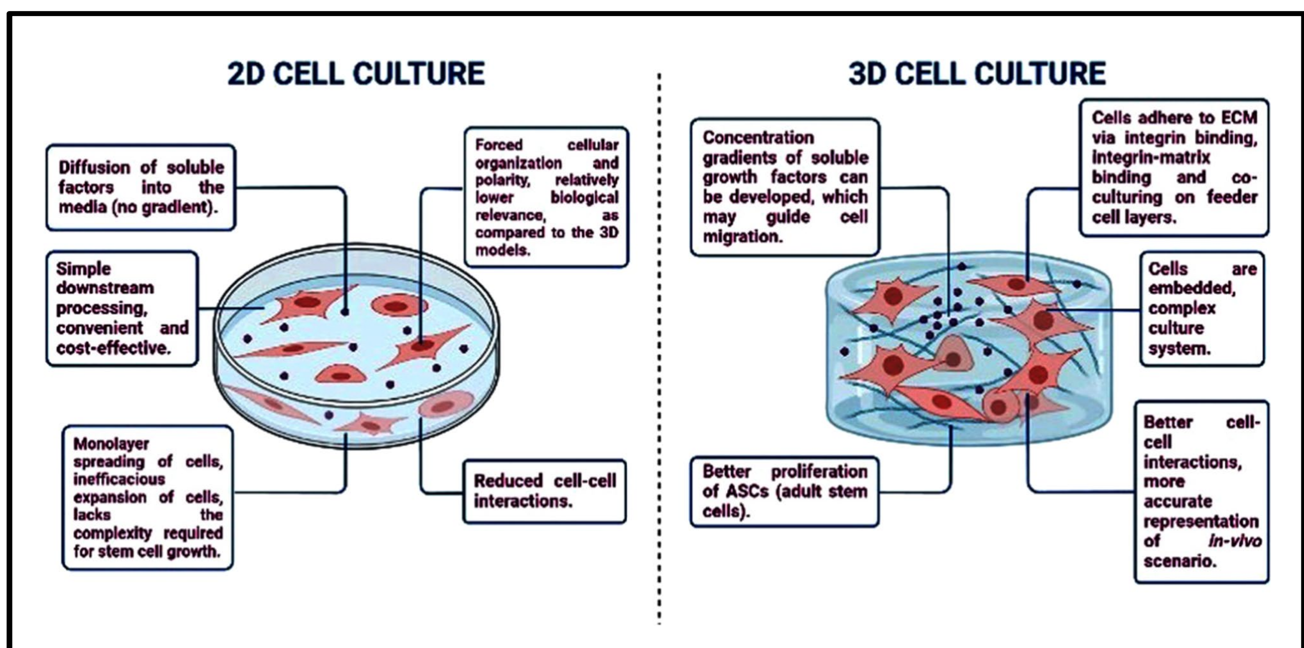

Fig. 2 A schematic overview of major differences between 2 and 3D cell culture models

et al. 2010). Integrin-matrix binding, integrin binding, and co-culturing on feeder cell layers are a few methods for the cell adherence to ECM in 3D cell culture models (Tan et al. 2021).

Three-dimensionsal cell culture methods

In-vitro models

2D cell culture has been extensively and routinely used over the past four decades. The use of immortalized cell lines and animal models has been a benchmark in understanding the human lung diseases. However, over the last few years, it has been pointed out that these models suffer from lack of complexity, reckoning that studies on cell cultures on 2D monolayers often tend to overestimate the response of therapeutic agents which is simply not reproduced, when studied in vivo. On the other hand, 3D cell culture models enable spatial cell–cell interactions and cell-ECM interactions in monoculture to study cell behaviors that imitate in vivo conditions; 3D culture techniques also give researchers a chance to co-culture several cell types to resemble in vivo conditions more nearly (Edmondson et al. 2014; Miller et al. 2019; Thippabhotla et al. 2019). These procedures must be effective, rapid, consistent, and convenient; maintain homogeneity of spheroid sizes; prevent cell damage and cytotoxicity; and promote cell development, shape, and physiology. 3D cell culture models are broadly categorized into spheroids and organoids. Using the natural characteristics of self-aggregation and adhesion, spheroids are usually scaffold-free approaches that consist of aggregates of single or types of immortalized or primary cell lines or fragments of human tissues (Giacomelli et al. 2020). Table 1 enlists some of the recently developed pulmonary 3D models for studying respiratory viruses and Table 2 represents the major advantages and disadvantages of the commonly studied 3D cell culture models.

Spheroids are spherical arrangements of cell due to self-assembling aggregates that are formed due to integrins and extracellular matrix proteins. They can be formulated using scaffold based or scaffold free approach, although latter is commonly used. The mechanical integrity of a spheroid largely depends upon the cytoskeleton of the cells. Architecturally speaking, spheroids are cell aggregates of single of multiple cells, mainly derived from primary cells of a tissue or an organ; however, they do not structurally represent the considered organ or tissue as seen in organoids (Dinh et al. 2017). With the help of microfluidics technology, organ on a chip system captures the cellular microenvironment that enables better re-creation of vital and complex organs in vitro (D. Huh et al. 2010; D. D. Huh 2015). Spheroids can

Table 1 An overview of various recently utilized epithelial cell-based 3D pulmonary models for investigating respiratory viruses

Source of cells	Pathogen studied	Findings
H358 lung epithelial cells (Derakhshani et al. 2019)	Measles virus	Amoeboid migration of MV by SphK/S1P pathway facilitates viral migration toward the epithelial cells
Human bronchial epithelial cell line (Sundström et al. 2016)	Andes virus (ANDV)	ANDV exerts long-term effects on the local lung cytokines, initiating a pro-inflammatory response which eventually results in a transition from asymptomatic phase to hantavirus pulmonary syndrome (HPS), a severe clinical entity
Human alveolar A549 cells (Berg et al. 2018)	Influenza A virus	The 3D model generated using bioprinting indicated a bio-ink-supported viral replication and proinflammatory interferon release
A549 and HepG2 cells (J. Liu et al. 2011)	Lipopolysaccharides (LPS)	3D spheroids are efficient at mimicking the in vivo cellular responses to LPS
Human airway epithelial cells (Y. X. Chen et al. 2018)	Human rhinovirus C (HRV-C) and human bocavirus (HBoV)	The growth of cilium-like protuberances resembling those found in the human respiratory system, as well as the expression of the biomarkers CK5, ZO-1, and PCK, were noted
Calu-3 cells (Rajan et al. 2013)	Human rhinovirus (HRV)	Results show a distinct difference between HRV 14 and HRV 16, as well as the origin of PBMCs, in the up- or down-regulation of a number of cytokines, including those connected to airway inflammation
Primary human small airway epithelial cells (HSAEpCs) (Bhowmick et al. 2018)	Influenza A virus	Studying the infection of HSAEpCs with the two main IAV strains, H1N1 and H3N2, was done using the 3D culture technique. The expression of marker proteins, both at the mRNA and protein levels, and the production of pro-inflammatory cytokines, both changed noticeably in the HSAEpCs

Table 2 Comparative analysis of various 3D cell culture models, based on their respective advantages and disadvantages

Model/System	Advantages	Disadvantages
Spheroids	• Simple systems which allow co-culturing with a variety of cell types, under scaffold-free conditions (Keller et al. 2020) • Reproducible and high-throughput screening, inexpensive technique (W. Y. Ho et al. 2012) • Compact, not too loose, can easily be fragmented (Halldorsson et al. 2015; Lao et al. 2015; Ziółkowska et al. 2012)	• Tedious, time-consuming, difficult to generate spheroids of uniform sizes (W. Y. Ho et al. 2012) • High shear force required and difficult to maintain the culture for longer periods (Fukuda et al. 2006)
Organoids	• Reflects the rudimentary structure of any organ or a part of an organ (Venkata et al. 2022; Y. Wang et al. 2018)	• Requires extracellular matrix and supplementation of growth factors (Gjorevski et al. 2016) • Utilization of Matrigel or other hydrogels require the use of animals (Hushka et al. 2020; Mishra et al. 2023; van der Vaart and Clevers 2021) • Given the animal-based origin of Matrigel, a risk of antigenic response stands with the use of these scaffolds (Benton et al. 2011; Mishra et al. 2023; Vukicevic et al. 1992)
3D Bioprinting	• Precise and relatively quick with well-defined scaffolds • High throughput • Improved cell viability	• Bio-inks with optimal viscosity and biocompatibility • Rapid prototyping capability and cytocompatibility • Emission of toxic degradation products may trigger immune responses • Expensive (Elalouf 2021; F. Yu et al. 2018; Y. Zhou et al. 2021)
Microfluidics and organ-on-chip models	• Sophisticated and controlled system with optimum sheer pressure and minimal consumption of reagents • Able to recapitulate vascularized models with multiorgan interactions • Simulates physiological dynamics • Better cell viability than static models (Bordeleau et al. 2017; D. Huh et al. 2010; Nam et al. 2015)	• Complex systems that require tissue engineering • Presence of a surface effect due to small size of the model • Relatively expensive

be generated through various methods, including pellet culture methods, hanging drop method, and bioreactors. Pellet formation is usually achieved through centrifugation of cells grown in a monolayer culture. The loosely formed agglomerates are cultured overnight and eventually transformed into well-defined pellets. In this model, the size of the spheroids is kept limited to avoid hypoxia at the center of the pellet. Damage due to shear force during centrifugation limits its use (Jähn et al. 2010). On the other hand, hanging drop assay was one of the first techniques and was used to generate embryonic bodies. It is a simple culture method based on the principles of gravity and surface tension, that generates 3D spheroids where drops containing cells are placed hanging on an inverted lid onto PBS-filled bottom hydration chamber and incubated to form sheets or aggregates. Finally, they are transferred to shaker flasks and incubated until 3D spheroids are formed. In addition to co-cultures of several cell types producing heterotypic spheroids, hanging drops can be used to cultivate both primary cells and cell lines. While traditional hanging drop cultures require very basic laboratory equipment and can be easily monitored microscopically, they produce relatively small number of spheroids. Essentially, automated, commercialized 96- and 384-well hanging drop arrays are available that assist in managing spheroid size and render the hanging drop approach convenient in terms of time, effort, cost, and the strength for high-throughput screening (Foty 2011). Micro-bioreactors share the same principles as large bioreactors used in biotechnological and biomedical research and were developed as a small-scale system, serving as an important tool in 3D culture. It used spinner flasks and stirred tank systems to expand and generate in vitro spheroids. The mechanical forces used modulate cell behavior and viability and are important to generate quality cultures. Bioreactors have been used and have successfully generated organoids of various primary cell lines, including bronchi-epithelial cells. Though micro-bioreactors bestow an environment with low cell damage, the spheroids generated are inconsistent in size and lack homogeneity. Use of round bottom ultra-low attachment 96-well plates has been popularized since it is an easy, affordable method that allows generation of consistent spheroids and can be visually monitored during the process. Other methods that involve use of external forces like electric and magnetic fields and ultrasound are gaining popularity since they are associated with minimal cellular damage (Grün et al. 2020). Figure 3 represents some of the techniques used for the generation of spheroids.

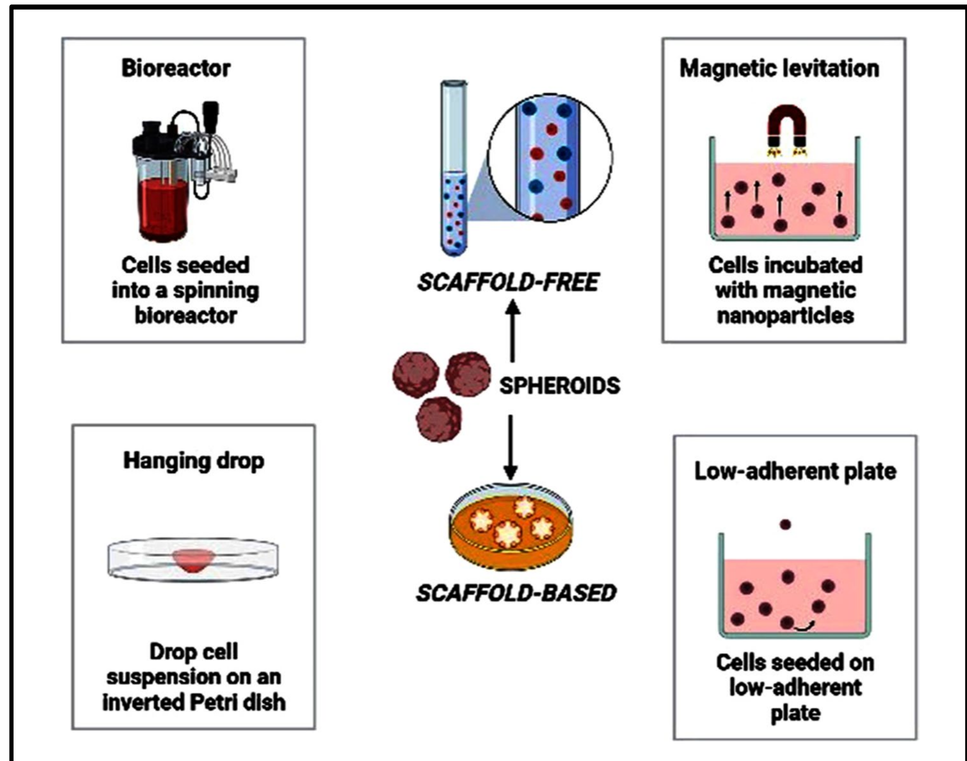

Fig. 3 Brief representation of the various techniques applied for the generation of spheroids

Air–liquid interface (ALI) is a functional and feasible physiologically relevant 3D cell culture model where the stem cells are grown with the basal surface in direct contact with media, while the surface or apical cellular layer is exposed to the air. Such organotypic models represent the human respiratory system more closely, thus, can be proven beneficial in the study of allergens, pollutants, viruses, or other pathogenic inhalants (Bluhmki et al. 2021). It allows co-propagation of epithelial and stromal cells and retains the cellular microenvironment for longer periods of time. It is believed that stromal cells are responsible for production of niche factors required for the growth of organoids, thus external supplementation is generally not required, which is not the case in adult epithelial cell cultures. ALI can be proven as a promising model for inhalation toxicity since it can extrapolate the results to in vivo models. The development of trans wells has led to the generation of ALI cultures that resemble the airway epithelium and enables the study of infectious pathophysiology and immune responses (Plebani et al. 2022). Muller et al. developed in vitro nasal epithelial model from primary cultures of non-invasive nasal scrape biopsies and outlined a protocol for reproducible cultures using ALI (Müller et al. 2013).

An organoid is basically a miniaturized organ developed in-vitro, to mimic the bio-functional features of the particular organ (W. Yang et al. 2022). Lung organoids are another type of 3D culture scaffold-based models that are derived from either pluripotent stem cells or adult stem cells undergoing spontaneous differentiation and self-organization. Pluripotent stem cells (PSCs) were derived from embryos during the blastocyst phase which raised ethical concerns. However, these were subsided after the introduction of induced pluripotent stem cells (iPSC) technology. Fibroblasts from adult humans can be extracted and converted into pluripotent stem cells by inducing the expression of transcription factors associated with pluripotency. Organoids, thus generated from iPSCs, undergo a self-autonomous organ development process. The cells are guided to differentiate into the primitively structured organs that resemble histo-physiologically (Bose 2021). Adult stem cells (ASCs) derived organoids have a much simpler and sustainable process of development. Unlike iPSCs, ASCs do not require reprogramming; instead, the cells from the desired organ are derived and isolated from the subject and culture to develop a 3D architecture model containing epithelial layers resembling the organ of interest (Barker et al. 2008). Tindle et al. developed proximal and distal airway epithelial lung organoids from isolated adult stem cells to study the pathogenic effects of SARS CoV-2 (Tindle et al. 2021). Extracellular matrix (ECM) provides cell anchorage and mechanism signaling to the tissues. Lung ECM assists in gaseous exchange and barrier protection, and collagen, laminin, and elastin were found to be the major components that provided tensile strength, stability, and elasticity (Doryab and Schmid 2022; Jeon et al. 2022). Through tissue engineering, scaffolds have been generated that mimic the ECM characteristics that are

vital in modulation of organogenesis (Lu et al. 2014; Luque et al. 2013; Williams et al. 2015). Solid ECM scaffolds are frequently used due to ease of handling and have been found relevant to natural organ environment. However, it is also prone to slow tissue-formation, and is vulnerable to results that are non-reproducible. On the contrary, scaffold-free techniques generate consistent and reproducible tissues at a faster rate through the properties of self-assembly but fail to generate organotypic structures. An approach to improve the scaffold free 3D culture organotypic models has been proposed using soluble ECM in the development of human lung alveolar organoids from suspension-based 2D cell lines (Valdoz et al. 2022). Salahudeen et al. have developed an infectious disease model for SARS-CoV-2 with human distal lung organoids that was used to characterize the role of progenitor cells (Salahudeen et al. 2020). Miller et al. generated human lung organoids and bud tip progenitor organoids from human pluripotent stem cells that possess cells of various developmental stages of lung in humans, which is suitable for studying developmental biology. According to this experiment, hPSCs were grown in a monolayer and undergo through several stages of differentiation, and through self-organization, the cells form spheroids that are subjected to scaffolds. Based on the growth factors in the matrix, human lung organoids or bud tip progenitor organoids are generated (Miller et al. 2019). Campos et al. demonstrated a three-dimensional human cell lung spheroids model for *Leptospira* infection that was efficiently able to infiltrate at the core of cell aggregates and proposed a standardization of inoculation for infection. The study also evaluated virulence and motility through assessment of gene expression and production of chemokines (Campos et al. 2022).

Organ-on-chip models is a breakthrough approach that recapitulates the in vivo tissue morphogenesis more closely, by artificially engineering the geometrical, mechanical, and biochemical microenvironments that mimic the physiological properties of any organ (Benam et al. 2016; Thacker et al. 2020). An organ-on-a-chip is a three-dimensional microfluidic cell culture model, with multichannel integrated circuit. It simulates the physiological responses of a complete organ (Kundu et al. 2021). Using 3D microthermoforming and ion track technology, Baptista et al. developed biomimetically micro-curved porous culture membranes, and proposed a 3D culture of human epithelial cells on microfluidic chips as promising futuristic model for drug delivery via inhalation. The device was also found to be capable of sustaining a coculture of epithelial and microvascular cells for 11 days (Baptista et al. 2022). Benam et al. outlined a protocol for small airway on a chip model of a human lung that includes a differentiated, mucociliary bronchiolar epithelium layer that is exposed to air, underneath which lies a microvascular endothelium that experiences fluid flow. Using microengineering, a multilayer microfluidic device is constructed, which is seeding with primary human bronchiolar epithelial cells and lung microvascular endothelium cells, each on the opposite side of the membrane to create an interface (ALI). This model has been proposed as a novel tool for disease and inflammatory response profiling of lungs in vitro (Benam et al. 2017). Van Riet et al. cultured microfluidic lung chip model from alveolar type 2 cells isolated from resected lung tissues of emphysema patients who underwent surgery. AEC2 cells processed were cultured into organoids using alveolar culture medium and seeded on both, inserts and chips for comparison, and it was found that lung-chip could be a more reliable model than conventional cell culture inserts (van Riet et al. 2022).

3D bioprinting has emerged as an excellent automated tool that allows fabrication of complex organs and 3D tissue structures in a reliable and scalable fashion. Its efficiency and reproducibility deem it as a potential technology for the development of suitable and realistic disease models (Ng et al. 2021). It involves the application of 3D-printing to replicate the natural tissues, by harvesting biomaterials like cells, growth factors, etc. (Neufurth et al. 2017). Bio-ink is composed of cells, additives, and scaffolds; however, microtissue and autonomous self-assembly based bioprinting enable a scaffold-free approach. It is important to optimize the concentration, viscosity, and thermal conductivity of a given bio-ink in order to assist cell growth and avoid mechanical damage to the cells as well as instruments. Cellular components and additives depend upon the target tissue, whereas scaffolds must be selected accordingly, depending upon the type of process being used. Hydrogel is the most widely used scaffold as it provides structural fidelity and resembles the tissue microenvironment. Their hydrophilic nature enables gaseous and nutrient exchange (Seliktar 2012). They can be produced from a variety of sources, including natural and synthetic materials. Gelatin, collagen, alginate, fibrin, chitosan, hyaluronic acid, and others fall under naturally derived polymers that can be used as bio-inks. Natural scaffolds have high biocompatibility and variable viscosity; however, they lack printing resolution and structural consistency. It has been argued that there are limited modifications possible when dealing with natural polymers (Panwar and Tan 2016). Mondal et al. developed a combination of sodium alginate and gelatin scaffold that can be optimized according to the required viscoelasticity. The prototype was also able to achieve stiffness similar to that of physiological stiffness (Arindam Mondal et al. 2019; A. Mondal et al. 2020). Synthetic bio-inks can be modified according to the target tissue. They can be purely synthetic or derived naturally; some of the examples include polyethylene glycol, polyacrylamide, polyvinylpyrrolidone (PVP) among others. These scaffolds have limited use since there is a limited opportunity for cellular interactions and low biocompatibility (Skardal and Atala 2015). Out of inkjet-based,

pressure-assisted, and laser-assisted bioprinting, inkjet-based bioprinting (also known as drop-on-demand bioprinting), with piezoelectric actuator remains the most accepted technology for it can reconstitute thin and spatially complex tissues. The local temperature in the nozzle is very high (up to 300 °C), which can harm cell viability of various tissues; however, it has been argued that it does not have a major effect on human cells. It is a challenge to fabricate microarchitecture with intact barriers and surfactant secretion. Inkjet-based bioprinting allows spatial arrangements and deposits multiple cells later by layer in an accurate and controlled fashion (Bishop et al. 2017; Kang et al. 2021). Kim et al. applied 3D bioprinting to develop a three-layered alveolar lung-on-chip microfluidics model that was shown to be physiologically relevant and could potentially be used to study pulmonary infections due to its versatility and customizable models (W. Kim et al. 2023).

The first description of human airway epithelium 3D culture was provided by Benali et al., (1993) and Wong et al. (2012) first elaborated a technique to generate human induced pluripotent stem cell-derived lung organoids. Sachs et al. elaborated a technique to develop airway organoids, with primary lung tissue from non-small cell lung cancer as the basic component. This was followed by an isolation of the epithelial cells, which were subjected to optimal conditions to generate 3D organoids, which yielded basal, multiciliated, and secretory cells on long-term differentiation. These airway organoids (AO) were characterized and the results reported the presence of functional secretory cells and cilia. The researchers also evaluated the potential of these organoids to serve as models for various pulmonary conditions. Of significance, they were able to successfully replicate the respiratory syncytial virus (RSV) in the AO. This action was reversed on a reaction with palivizumab, which is an anti-RSV cell fusion antibody. This was further analyzed to study the epithelial changes, cytoskeletal rearrangements, and syncytia formation (Sachs et al. 2019). One recent study by Lamers et al. (2020), assessed the replication of SARS-CoV-2 in human small intestine organoids (hSIOs) and concluded these organoids to be promising models for studying SARS-CoV-2 infectivity, replication, molecular processes, and host-antigen interactions (Lamers et al. 2020). In 2014, Huang et al., (2014) reported the generation of efficient airway and lung epithelial organoids from human pluripotent stem cells (hPSCs). In vivo and in vitro differentiation yielded alveolar epithelial cells, goblet cells, Clara, basal, and ciliated cells. The corresponding preclinical analysis with gene knockout mice also validated the findings, in terms of the effects of inhibiting the signaling pathway agonists (Huang et al. 2014). Another group of researchers generated lung bud organoids (LBO) from hPSCs. These organoids comprised pulmonary endoderm and mesoderm, which eventually developed into a branching airway, which reached second trimester. Alveoli also developed after xenotransplantation and in Matrigel 3D culture. An HPS1 mutation was also induced to early-onset pulmonary fibrosis (Y. W. Chen et al. 2017). Recently, a research group reported the generation of human lung organoid, from adult stem cells. The organoid was satisfactorily differentiated into proximal and distal epithelia. The research team further infected the ALO-derived monolayers, primary airway cells, and alveolar type II pneumocytes, derived from hiPSC with SARS-CoV-2. This in vitro model was validated and used for exploring the molecular patterns and biology of the coronavirus (Tindle et al. 2021). Several lung cancer organoid models have been developed from primary patient tissues and patient-derived xenografts (PDX) (M. Kim et al. 2019; Li et al. 2020; Shi et al. 2020). Development of mouse lung organoids has been reported by Hai et al. (2020).

3D cell culture models for pulmonary infections

Exposure of lung epithelia to contaminated air has been the most common cause of pulmonary infections. *Pseudomonas aeruginosa* is the most frequently encountered opportunistic bacterial pathogen in the nosocomial setting. It accounts for the second-most prevalent gram-negative pathogen and causes excruciating infections in immunocompromised patients (D. K. Ho et al. 2019). Pulmonary infections may be bacterial, viral, or fungal and may be acute or chronic, depending upon the onset and duration. Based on the anatomical location of infections, these are categorized as upper and lower respiratory tract infections. One of the most common pulmonary infections, tuberculosis, is a cavitating lung infection caused by an acid-fast bacillus, *Mycobacterium tuberculosis*. Lobar pneumonia is commonly caused by a gram-positive coccus, *Streptococcus pneumoniae* and causes alveolar consolidation, whereas bronchopneumonia is an airway and parenchymal inflammation caused by a gram-negative rod, *Haemophilus influenzae.* Empyema is characterized by a pleural space infected by *S. pneumoniae* and *M. tuberculosis*. Moreover, secondary infections in COPD and asthma may be caused by chronic dominance of some pathogens like *Haemophilus* spp., *Neisseria* spp., and *Moraxella* spp. Bronchiectasis is the presence of secretions and polymicrobial infections in an abnormally dilated airway (Cookson et al. 2018). Apart from these, the recent COVID-19 outbreak has significantly affected human health and the world economy. From time to time, novel strains have emerged with differing molecular processes affecting the virus' transmissibility and pathogenicity. The constantly appearing molecular evolutions and diversifications of SARS-Cov-2 have made the development of effective treatment and vaccines a painstaking task (Amoutzias

et al. 2022). Another major challenge that has attracted attention is pneumonia and its sequelae, acute lung injury. Translational investigation of acute lung injury has posed some major obstacles, given its heterogeneity (Narasaraju et al. 2011). Pneumonia is a lower respiratory tract infection and is a common cause of acute respiratory distress syndrome and acute lung injury. It is caused by gram-positive or gram-negative respiratory bacteria or viruses, and rarely because of fungal or parasitic infections. The common subtype, bacterial pneumonia may be community-acquired or hospital-acquired. Although other viruses such as parainfluenza virus, adenovirus, respiratory syncytial virus, human metapneumovirus, and coronaviruses may cause disease, RNA viruses, such as influenza and rhinovirus, are usually linked to pneumonia (Bochud et al. 2001). Infection with the gram-positive *S. pneumoniae* is the most frequent cause of bacterial pneumonia. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species are some of the other pathogens of concern (Coburn et al. 1954). The innate and adaptive immune responses to infection frequently cause pathology through excessive inflammation and inability to resolve the inflammatory response to infection, in addition to damage caused by pathogens (Narasaraju et al. 2011). There are no host-response targeted medicines that can treat disease since harmful host responses to infection are frequently the cause of severe disease. Particularly with the rise of bacterial strains with increased antibiotic resistance, new strategies to combat viral and bacterial co-infections are required. Cellular models that include mechanical ventilation in addition to infectious agents will probably provide more understanding of the pathogenesis of disease than those that do not (Sánchez-Guijo et al. 2020). Tuberculosis is another significant healthcare burden under the class of pulmonary infections and is caused by a bacterial pathogen, *Mycobacterium tuberculosis* (Mtb). Massive proliferation of Mtb in the lungs is achieved by causing cavitations in the lungs, from where these bacteria are released to disseminate infection (Nedeltchev et al. 2009). These cavities have recently been understood to arise from the erosion of granulomas into bronchi. The information that is now available indicates that the majority, if not all, of the frequently used animal models develop lesions that have characteristics of both primary and post-primary TB. For a better understanding of the exact molecular pathology, advanced cell culture models replicating the in-vivo conditions may warrant definite answers (Brown and Hunter 2021). Pulmonary aspergillosis is a debilitating fungal infection caused by *Aspergillus* spores and occurs mostly in immunocompromised patients. Further, it is divided into allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA). ABPA is an inflammatory condition caused by a hypersensitivity to *A. fumigatus*, and is exclusively seen in patients with asthma or cystic fibrosis (Hemmann et al. 1998). CPA is mainly seen in patients with a pre-existing pulmonary condition, in immunocompetent hosts (Yu et al. 2016). Invasion of the lung parenchyma by *A*. hyphae exposes immunocompromised patients to a serious IPA (Stergiopoulou et al. 2007). Epstein-Barr virus and cytomegalovirus have often been found in the airways of patients with COPD. By stimulating ICAM-1 expression in the airway epithelial cells via the NF-kB pathway, EBV-latent membrane protein-1 enhances inflammation, by persisting in the airway epithelium. Another clinical entity of interest is the asthma-COPD overlap, characterized by multiple different phenotypes. It presents with both, airway inflammation and obstruction. Lung development, especially from the fetal stage to the age of five, as well as the environment during early life has a significant impact on the pathophysiology of asthma and COPD. Secondly, *HHIP* and *SOX5* are the genes associated with lung growth and make the patient susceptible to epigenetic changes leading to asthma and COPD (Hikichi et al. 2018). Defects in the mucociliary clearance system, changes in the structure and function of the submucosal glands, and anomalies in the composition, volume, and acidity of the airway surface liquid are responsible for placing a high risk of developing cystic fibrosis (CF), along with the measure and function of cystic fibrosis transmembrane conductance regulator CFTR. Improved awareness and an updated knowledge are a need of the hour to better understand the underlying mechanisms responsible for these pulmonary infections and diseases. Being a leading cause of global mortality and morbidity, timely diagnosis and an efficient treatment strategy are both essential to contain these grappling conditions. For uplifting personalized medicine and progress pulmonary health, a systematic and proficient research system is required (Pino-Argumedo et al. 2022). This can be achieved by upgrading tools to overcome the current deficiencies faced in molecular and translational research setting. A swing from 2 to 3D culture systems looks like an assuring trend to advance pulmonary research studies.

The spread of COVID-19 has driven extensive research on the development of in-vitro models such as 2D cultures of primary cells and immortalized lines as well as organ-derived 3D cultures (Marhuenda et al. 2022). Given the reduction in usage of animals with in-vitro culture models, applicability of 2D cultures has advanced in the recent times. Despite of being an inexpensive and fast option for studying cellular targets, 2D models come with their set of disadvantages owing to complexity of the macroscopic system (Zhang et al. 2009). Coming to 3D models which include explants and organoids, primary cells from human airway epithelium (HAE) have also been isolated and cultivated. These cells are then cultured and differentiated on an air–liquid interface (ALI), with the apical side in contact with air.

They have high resemblance to human physiology (X. Liu et al. 2020). Recently, a 3D cell culture model was developed to study respiratory syncytial virus (RSV) infections, especially since no vaccine has been developed for their prevention. A549 cells were cultured to generate spheroids of 25×10^3 cells. These A549 spheroids were reported to possess the characteristics of RSV infections, mainly syncytia formation and mucin overexpression. The study concluded the application of A549 spheroids in investigations for RSV infections and several other pulmonary infections (X. Liu et al. 2020). Type II epithelial cell spheroids have recently been developed by Saleh et al. to study RSV infections. Unlike the alveolar epithelial cells cultivated on monolayer culture, this model presents the syncytia formation. Spheroids also represented the cortical layer-like architecture (Hasan et al. 2019).

Commonly, the hanging-drop method, spinning the cells in a bioreactor, magnetically levitating the cells, and seeding on low-adherent plates it utilized. Spheroids may be designed to mimic the extracellular matrix (ECM), i.e., scaffold-based models, or using a soluble ECM, i.e., scaffold-free models.

Applications of pulmonary 3D culture models

Human lung diseases including lower respiratory tract infections, chronic pulmonary obstructive disease (COPD), lung cancer, and tuberculosis have been the most common causes of morbidity and mortality. The high global burden of these diseases deems it necessary to understand their pathophysiology and identify novel therapeutic targets. Zoonotic pulmonary infections like severe acute respiratory syndrome (SARS), middle east respiratory syndrome (Espinosa et al.), and influenza A virus among others do not naturally affect animals to the extent that it does to humans, thus, failed to be entirely explained through these models. Over the last few decades, in vitro and ex vivo models of all the segments of the respiratory system have made remarkable advancements and have gained popularity for the study of pathophysiologic processes and therapeutic aspects of pulmonary diseases and inhalation toxicity (Kaye et al. 2022).

Pulmonary infections are caused by a variety of different organisms, which can be spread through the air, on surfaces or in contaminated water. The most common respiratory infection is the common cold, which is caused by a virus. Viral infections can range from mild to severe and can last from a few days up to several weeks. There are many different types of viruses that cause respiratory infections and each one has its own unique symptoms and treatment methods.

Respiratory syncytial virus (RSV) is a member of the Paramyxoviridae family. It is an enveloped, double-stranded RNA virus that contains a linear genome of approximately 7.5 kilobases in length. RSV has an icosahedral nucleocapsid composed of 12 T-shaped capsomeres, which are surrounded by a host protein coat and surrounded by lipid bilayer membranes. The viral genome encodes structural proteins necessary for viral replication and functions as well as several non-structural proteins to facilitate infection and spread within the body. Recently, a number of 3D models have been developed to better understand the virulence of RSV and drug screening against it. Geiser et al. developed 3D tissue cultures, both in vitro (from A549 cell lines) and ex vivo in order to better understand the pathogenicity of RSV and human metapneumovirus (HMPV) as single and co-infection models. It was found that RSV was more pathogenic than HMPV and that it dominated the host response in the case of co-infection, causing suppression of HMPV replication. (Geiser et al. 2021).

Influenza virus is a small, double-stranded RNA virus which causes the common cold and flu. Influenza viruses are categorized into three types based on their hemagglutination (HA) antibody titres, where there are two types of influenza A viruses and one of influenza B virus (Yoshida et al. 2009). The different strains of the same type can cause different symptoms in an individual person but can also cause similar symptoms in different people at the same time. There are many subtypes of influenza A viruses that can infect humans, pigs, birds, and other animals (Ma et al. 2008). In vitro models have been able to replicate results obtained through explants obtained from patients. Mockel et al. attempted to develop a biologically relevant 3D airway tissue model from Calu-3 cells and human fibroblasts in order to study the virulence of IAV using real-time imaging and microscopy. They co-cultured Calu-3 cells and human primary fibroblasts and utilized an influenza A virus replicating and carrying a fluorescent gene for real-time imaging. Basement membrane, tight junctions, and mucus production in ALI were satisfactorily expressed in the developed model. The study provided novel understanding and a direction for analyzing the spread of pulmonary viruses, including influenza A virus (Möckel et al. 2022). Bhowmick and co-researchers created 3D cultures of primary human small airway epithelial cells by depositing the cells on an air–liquid interface matrix in to investigate the immunopathology of influenza. They developed a 3D-Human Tissue-Engineered Lung Model (3D-HTLM) and studied the immunophenotypes in the context of influenza A virus infections. Chitosan-collagen scaffolds were created, which were further cultured with the above-mentioned epithelial cells at the ALI. The model provided viable cells and imitated the in vivo environment

sufficiently. When compared to 2D models, higher expressions of aquaporin-5 and cytokeratin-14 were found in the 3D culture model. The researchers studied H1N1 and H3N2 using the developed model. Changes in the marker protein levels and proinflammatory cytokines were also exhibited, emphasizing the applicability of such models to study pulmonary infections and propel drug development for the same. (Rudra Bhowmick et al. 2018).

SARS-CoV-2

COVID-19 belongs to the coronavirus family and is an enveloped, single-stranded RNA virus. Four generic subtypes of COVID-19 have been identified, vis-à-vis, alpha, beta, gamma, and delta. SARS-CoV-2 circulating in India has the G614 genotype as the dominant subtype, making its effective management challenging (Kumar et al. 2020). Consequently, several iPSC-derived spheroids have shown promising results for assessing anti-SARS-CoV-2 activity (Higashi-Kuwata et al. 2022). Scientists around the world have been working on developing strategies against the novel coronavirus outbreak in 2019. In-depth understanding of the virus morphology, virus-host interactions, tropism, receptor interactions, and testing novel vaccines and therapies has been of significance to combat the pandemic. For the same, novel three-dimensional models have merged as promising research tools (Harb et al. 2021). A number of cell lines are suited for SARS-CoV-2 viral investigations since they have been utilized in 3D cultures to acquire crucial data on the cell biology of viral infections and medication evaluations (de Dios-Figueroa et al. 2021). SARS-CoV-2 genome consists of spike glycoprotein, membrane glycoprotein, small envelope glycoprotein, and nucleocapsid protein. Of particular interest is the angiotensin-converting enzyme 2 (ACE-2) receptor, which is essential for viral infectivity via spike glycoprotein binding, followed by an endocytic viral entry (Choudhury and Mukherjee 2020; Kehinde et al. 2022). The current therapeutic effort is concentrated on utilizing antibodies or soluble proteins to prevent the viral protein S interaction with receptor ACE-2 (Poduri et al. 2020). Scalable in vitro culture models are indispensable for studying respiratory viruses, including SARS-CoV-2 and their interactions with the airway cells. Most of these models developed till date are based on immortalized cell lines; however, these unicellular and monomorphic systems fail to satisfactorily represent the respiratory viruses. To better understand the complicated interactions at the tissue and organ level, human airway organoids have proved to be more reproducible and efficient systems (McCauley et al. 2017). More sophisticated models are required to better study the infection dynamics and how they are connected to innate immune responses, and microbiota. Even the intrinsic cellular resistance mechanisms are not adequately explained with 2D cell cultures and hence, novel in vitro models have been explored since a while. Culturing primary human airway epithelial cells from nose/throat swabs or surgical matters, along with airway organoids developed from stem cells, are some of the recently developed tools to study respiratory viruses (Ferreira Lopes et al. 2017). Of the 3D culture models used for COVID-19, pulmonary models derived from iPSCs and ESCs have commonly been utilized. These include scaffold-based, organoids, organotypic raft cultures, and organ-on-a-chip. The rest include extrapulmonary models, derived from different organs like kidney, gut, liver, pancreas from which spheroids and organoids are developed. The gut organ-on-a-chip model has been used for evaluation of drugs like remdesivir. Han and colleagues designed and developed organoids which have been of immense use to study SARS-Cov-2 cellular metabolism (Han et al. 2021). Two types of alveolar models have also been developed, which include proximal–distal and proximal respiratory tract models. The former ones were designed to be organotypic raft cultures, which helped determine the process of infection from proximal respiratory tract cells, mainly targeting ciliated and goblet cells. Alveolar type 2 cells constitute a part of the distal axis model and activate proinflammatory factors like IFN-β (interferon-β) and IFN-3 (interferon-3). As a result of elevated cytokine expression (IL-6, IL-8), this model reported immune cells to play a significant part in alveolar barrier dysfunction (Mulay et al. 2021). The primary purpose of utilizing extrapulmonary models is to study the neuropathogenesis associated with COVID-19 and also to decipher a novel viral entry point, facilitated by cathepsin-L in cardiomyocytes. SARS-CoV-2 has been described to target neurons, according to certain organoid-based studies (Ramani et al. 2020). Another pioneering research by Buzhdygan et al. suggested that SARS-CoV-2 spike protein destabilizes the blood–brain barrier, and promotes inflammation, without affecting viability of the cells. Additionally, it has also been anticipated to promote micro-clot formation in central nervous system and peripheral vasculature (Buzhdygan et al. 2020). Studies on certain organoids and organs-on-a-chip have shown that certain gastrointestinal cells may also be targeted by SARS-CoV-2, indicating application of gastric organoids in studying the virus. Mainly, intestinal cells are affected, eventually disrupting the absorption, hormonal secretion, metabolism, and defensive immune mechanisms (Zhou et al. 2020). Lastly, cardiac complications of COVID-19 have garnered immense attention, and 3D cardiomyocyte models have also reinforced similar findings. The dependence of viral entry on ACE2 and an endosomal cysteine protease activity raises the prospect that additional mechanisms may make SARS-CoV-2 entry into the cardiac tissue. An endolysosomal pathway has also been anticipated

to play a role in SARS-CoV-2 infecting the cardiomyocytes (Bailey et al. 2021; Pérez-Bermejo et al. 2020; Yiangou et al. 2021).

Given the primary role of COVID-19 in affecting the respiratory epithelia, the applications of lung airway, alveolar, and bronchial organoids hold an important place for scrutinizing the viral features (Pozzi et al. 2021). Table 3 describes some of the recently developed 3D cell culture models for investigating SARS-CoV-2.

Bacterial infections

Tuberculosis is a major threat to public health especially in third world countries. With emerging resistance, there is a need for advancements in therapy and drug discovery. Monolayer cell cultures lack the additional dimensionality, which led to the need of development of in vitro 3D model of the disease to better understand the disease mechanism. Braian et al. established a collagen matrix embedded air exposure model of lung epithelial cells and fibroblasts and introduced macrophages infected with *M. tuberculosis.* The structure resembled granuloma generally seen in the infected patients. The model comprised a culture of tissue-specific epithelial cells and fibroblasts in a collagen matrix. Human primary macrophages infected with *Mtb* were infected into the model to develop tuberculosis granulomas. It has been pointed out that the model lacks neutrophils and lymphocytes and their contribution to the pathophysiology yet to be explored. It has been proposed that this model can be broadened by addition of different cell types that are usually affected by the pathogen (Braian et al. 2015). Microfluidic models have not been extensively explored to study the dynamics of host–pathogen interactions. Using lung on chip model of early tuberculosis infection, Thacker et al. recreated the tissue level complexity and studied the effect of surfactants on Mycobacteria, and cells affected, including macrophages and alveolar cells. The host pathogen interactions were studied using time-lapse imaging at the ALI. The surfactant deficiency aided an uncontrolled bacterial growth in the cells. They reported that the exogenously supplied surfactant replacement formulations reverse the surfactant-deficiency, but without affecting bacterial viability in the absence of host cells. Surfactant partially removes lipids and proteins from the bacterial cell surface, which are necessary for virulence. The attenuation of bacteria missing the ESX-1 secretion system is independent of surfactant levels, which is consistent with this process. The higher risk of getting active tuberculosis in smokers and the elderly with impaired surfactant function has also been explained by this principle (Thacker et al. 2020).

Pseudomonas aeruginosa is an opportunistic pathogen that is one of the leading causes of acute and chronic bacterial respiratory infections. It commonly affects the patients with cystic fibrosis where it develops a biofilm on the tract, leading to antibiotic resistance due to chronic exposure. This calls for novel antimicrobial options that would address the global problem of antibiotic resistance. 3D models allow a relevant infection profile that reflects the host pathogen interactions better than monolayer cell cultures and would be a promising approach for drug screening. Crabbe et al. established a three-dimensional lung epithelial model using fetal bovine serum (FBS) and bioreactor method and assessed the antimicrobial efficacy of aminoglycosides, colistin, and antibiofilm peptide-DJK-5. *P. aeruginosa* develops antibiotic-resistant biofilms on 3D cells. However, it does not alter the cell viability. The presence of FBS had an impact on the cultures, in that it helped in the biofilm formation and the lung epithelial cells primarily influence the activity of antimicrobials against *P. aeruginosa* (Crabbé et al. 2017).

Limitations and future directions

During the COVID-19 pandemic times, transportation and storage of cells posed serious hurdles, given the limited access to laboratories. Alallam and colleagues developed an alginate hydrogel bead-based technique for emergency cell storage of cancer cells. The beads enabled cell survival for up to 4 weeks, with less frequent subculturing or change of culture media required (Alallam et al. 2022). This strategy is anticipated to overcome these limitations, pertaining to pulmonary infection models as well. Another persistent pandemic of serious concern is tuberculosis, which is treated with standard regimen since years. But owing to the evolutionary changes in the virus, novel therapeutic targets are warranted to be identified. For the same, 3D collagen granuloma model has been shown to reflect tuberculosis in humans very closely (Reichmann et al. 2021). Several researchers have recently leveraged microfabrication technologies and have exploited microfluidics in order to simulate human conditions through cell-culture microenvironments. Complex microfluidic devices are recent advances in the field, and benefit the long-term differentiation of cultured cells (Hung et al. 2005). These 3D models have greatly favored the clinical outcomes in pulmonary infections, eventually assisting the development of novel therapies. 3D models also facilitate studying the pattern of drug resistance in various pathogens and hence, redesigning the currently used antibiotics. Clinically, 3D models enable the investigators to analyze host–pathogen interactions and study various repurposed drugs for their applications in pulmonary infections.

Despite the currently available 2D and 3D cell cultures used in regenerative medicine, growing concerns have emerged relating to their limited resemblance to the in vivo bioenvironment (Miao et al. 2020). Conventional cell spheroids are produced through a laborious culturing method, which has drawn criticism for being susceptible

Table 3 Recently developed 3D cell culture models for evaluating various facets of SARS-CoV-2

Model	Source	Application(s)	Primary findings
Lung-on-a-chip (Si et al. 2021)	Human lung airway epithelial cells	Screening of repurposed drugs for their activity against SARS-CoV-2	Significant prophylactic and therapeutic activity of amodiaquine against SARS-CoV-2 was shown
3D model of human alveolar type 2 cells (3D hAT2) (Youk et al. 2020)	Primary human lung tissue	Characterizing the pathogenesis of SARS-CoV-2 infection	A robust endogenous innate immune response was observed, following rapid viral replication and overexpression of interferon-associated genes plus proinflammatory genes
Differentiated air–liquid interface cultures and 3D organoids (Mulay et al. 2020)	Proximal and distal lung epithelium	COVID-19 modeling and drug discovery	Efficacy of remdesivir was validated to suppress viral replication and infection. Epithelial cell-autonomous proinflammatory response was also noted
Human bronchial organoids (Suzuki et al. 2020)	Cryopreserved human bronchial epithelial cells	SARS-CoV-2 research and drug discovery	Angiotensin converting enzyme 2 (ACE2) and transmembrane serine proteinase 2 (TMPRSS2) were significantly expressed. Camostat, an inhibitor of TMPRSS2 caused a reduction in viral copies by 2% compared to the control group
Lung organoid models (Han et al. 2020)	Human pluripotent stem cells (hPSCs)	Transcriptomic analysis	Cytokine and chemokine induction noted, with type I/III interferon signaling
Distal human lung progenitor organoids (Salahudeen et al. 2020)	Adult human alveolar epithelial type II (ATII) cells	Molecular investigations and identification of possible novel therapeutic targets	Unsuspected basal cell functional heterogeneity observed; club cells were identified to be novel targets for drug treatment
Mammalian airway organoids (Elbadawi and Efferth 2020)	Progenitor basal cells, secretory cells and alveolar epithelial cells	Studying infectivity and cytopathy of SARS-CoV-2	Virus propagation was effectively studied but histology and function of the respiratory tract was poorly defined

to the circumstances of cell culture and frequently yields inconsistently differentiated cells. Another major drawback is the lack of effective differentiation (Choi et al. 2013). Complex changes in the cellular micro-environment can be achieved by a combination of printing and imprinting. It has been reported that 4D spatiotemporal cues would be able to imitate the topographical and mechanobiological milieu of NSCs (neural stem cells). This principle may be applied to cell culture techniques, to advance the simulation of pulmonary infections. Such developments in 4D cultures are anticipated to have a significant impact on other bioengineered tissues as well (Miao et al. 2020). Matrigel is popularly used in the cell cultures over the past few decades. However, batch-to-batch inconsistencies and potential antigenicity hinder the throughput and reproducibility of these models. These limitations have called for the development of xenogeneic-free scaffolds (Aisenbrey and Murphy 2020; Ma et al. 2008). Synthetic hydrogel-based platforms have been evolved to resemble the in vivo conditions and generate physiologically similar systems for efficient organoid development. These hydrogels are better than 3D cultures at mimicking the in vivo cell development and regeneration. Cell culture substrates that promote colony growth and differentiation in space and time, i.e., 4 four-dimensional materials, are expected to enhance the scope of organoid culture progression (Weber et al. 2017). Despite of the advancements in these 3D models, they do not satisfactorily represent the dynamic stress stimuli in gastric carcinomas (GC). As a measure to overcome this shortcoming, Zhao and colleagues developed a 4D cell culture model using an alginate-based hydrogel. 4D models may provide an essential platform not just for GC translational research but also for drug screening for other conditions, like pulmonary infections (Zhao et al. 2021). Even though multi-well plates are easily available for high-throughput 3D culture, the arduous histology methods make evaluating the internal architecture of 3D cell cultures laborious. A 4D-printed transformable tube array has recently been developed using a shape-memory polymer, which corresponds the histology of 3D cultures. This model supplemented the inventions in 3D cell culture applications (C. Yang et al. 2020). Despite organ-on-chip models being excellent candidates for drug testing and development, it has certain limitations. Kim et al. applied 3D bioprinting to develop a three-layered alveolar lung-on-chip microfluidics model that was shown to be physiologically relevant and could potentially be used to study pulmonary infections due to its versatility and customizable models. Using 3D inkjet bioprinter, cells were cultured on a microfluidic chip at the air–liquid interface. It overcomes the limitations of the conventional organ-on-chip model and enables reconstitution of the cells in a very thin fashion (W. Kim et al. 2023). Another study bio-fabricated a model that mimics the air-blood barrier, forming layers of endothelial cells, basement membrane, and epithelial cells through bioprinting. The study shows promising results and can be proven an excellent platform for development of pulmonary models providing high-throughput screening of drugs, which can be incorporated for pulmonary infections in the future (Horváth et al. 2015). Several other models incorporating the principle of four-dimensions are currently under investigation for translational and biomolecular research, driving toward more reliable and sustainable sources to study pathologies and develop novel therapies.

Outlook and conclusion

Despite the complex developmental processes of human pulmonary 3D models, there lies an intriguing prospect for these models. The most essential aspect to be taken care of is the validation of clinical and translational relevance. Robust and efficient designing of pulmonary 3D models would provide a valuable scientific toolset as an alternative to animal models, overcoming several ethical and scientific issues of animal-based studies. Standardization and methodological improvements in 3D models are envisaged to offer advancing possibilities in biomedical science. They range from simple spheroids which offer co-culturing with different cell types to complex scaffold-based systems, allowing the recapitulation of pathogenic heterogeneity of pulmonary diseases. In addition to in vitro models, ex vivo perfused and ventilated human lung models are also gaining popularity, owing to their advantage of studying processes like lung oedema formation, oxygenation, bacterial infections, and bacterial reactivity. Deriving cell-specific mechanistic answers is yet to be explored in detail. Exhaustive and comprehensive implementation of these systems and relentless progress would help uplift the competence of organoid-based models in pulmonology. Industrial expansion of 3D culture models would be facilitated with high-quality cryopreservation and culture techniques. Lastly, improved feasibility of these unique models would require a concentrated effort on part of the researchers, making ultimate use of inter-laboratory experiences and scientific publications.

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