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REVIEW

Mechanistic advances and emerging technologies redefining lung aging research

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Abstract

As the population ages, defining how biological processes change over the lifetime has become increasingly important. Acute and chronic lung diseases are more prevalent in older adults, and emerging research is beginning to uncover the mechanistic and cellular pathways that link aging to conditions such as pneumonia and chronic obstructive pulmonary disease (COPD). Additional mechanisms, particularly those involving extracellular vehicles (EVs), the microbiome, and sex differences, are now recognized as potential contributors to age-related changes in lung health, yet remain underexplored. Advances in experimental models and analytical tools have accelerated progress in the field. Three-dimensional lung models such as organoids, precision-cut lung slices, extracellular matrix (ECM) scaffolds, and lung-on-a-chip systems offer more physiologically relevant systems than traditional two-dimensional cultures, improving translatability to in vivo biology. Meanwhile, the expansion of genomics, transcriptomics, proteomics, and metabolomics has enabled comprehensive, multiomics approaches for mapping disease mechanisms, and such datasets are increasingly available. However, deeper integration with patient metadata and spatially resolved methods is still needed to advance precision medicine approaches to exploit aging mechanisms in chronic lung diseases. In this review, we highlight the importance of investigating EVs, the microbiome, and sex differences and their contribution of age-associated mechanisms in the context of pneumonia and COPD, and discuss how innovations in 3-D lung models and omics technologies are reshaping our understanding of the pathological mechanisms that underlie these diseases.

3-D lung models; chronic obstructive pulmonary disease (COPD); lung aging; multiomics; pneumonia

INTRODUCTION

Life expectancy has risen from ~46 yr in 1950 to over 73 yr in 2023 (1), driving a global increase in age-associated diseases. Aging is broadly defined by the progressive loss of physiological function and is generally correlated with chronological age. However, premature aging highlights that it is not exclusively "time-since birth" dependent (2, 3). Acute and chronic lung diseases (CLDs) rank highly among leading causes of death globally, with chronic obstructive pulmonary disease (COPD) being fourth, and lower respiratory tract infections, including pneumonia, ranking fifth (4). Both disproportionately affect older adults, highlighting the lung's vulnerability with age (5, 6). Thirteen hallmarks of aging have been summarized as cellular or molecular changes that affect the lung's structure, immunity, and repair capacity (2, 7).

After lung maturation peaks in early adulthood, physiological function declines steadily. Classic features associated with this decline like reduced elasticity, enlarged alveolar spaces, and decreased mucociliary clearance, are now being mechanistically resolved with multiomics and advanced imaging approaches (8). Aged human lung samples reveal increased expression of fibrillar collagens and extracellular matrix (ECM)-associated proteins (9). Moreover, it has been shown that aged ECM becomes softer at low lung volumes, but notably stiffer when mechanically stretched to mimic breathing strains, indicating strain-dependent vulnerability affecting airway mechanics (10).

At the cellular level, aging reshapes regenerative, immune, and vascular compartments. For example, aged alveolar type-II (AT2) cells show reduced self-renewal capacity and impaired differentiation to AT1 cells, leading to the accumulation of dysfunctional transitional states (11). Moreover, aged AT2's accumulate stress-response and aberrant metabolic signatures consistent with reduced progenitor competence (12). Similarly, within the airways, Jia et al. (13) reported a continuous decrease in the proportion of epithelial progenitors during aging associated with inflammation and aberrant immune responses. These epithelial changes occur alongside immune alterations as aged immune cells

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exhibit reduced pathogen clearance capacity and increased systemic inflammation that has been evidenced in both macrophage and neutrophil populations (14, 15). Single-cell RNA sequencing demonstrates changes in several compartments, including the alveolar epithelium and the endothelium (16), as well as fibroblasts, showing significantly less collagen XIV expression and senescent-associated phenotypes (16–18). Finally, injury models show that aged endothelial populations fail to re-establish quiescent states and instead promote leakage, impaired repair, and fibrotic remodeling (19). This is important considering the parallel loss of the epithelial barrier integrity through lower expression of key junctional genes, including cadherin 1 (*CDH1*), epithelial cell adhesion molecule (*EPCAM*), and transient receptor potential cation channel subfamily V member 4 (*TRPV4*), in aged human lungs (20).

The accumulation of senescent cells is a hallmark of aging, making their identification and characterization essential for studying age-associated lung diseases (7). Senescent cells exhibit stable cell-cycle arrest, increased DNA damage, senescence-associated β-galactosidase activity, and secrete numerous cytokines, chemokines, growth factors, and proteases collectively known as the senescence-associated secretory phenotype (SASP). They also upregulate antiapoptotic pathways, including BCL-2-like protein 1 (BCL-xL), proto-oncogene tyrosine-protein kinase (SRC), and phosphatidylinositol 3-kinase (PI3K) (21). Recent work has focused on developing more specific and robust methods to identify senescent cells, including refined classical markers, new molecular signatures, and advanced reporter systems designed to detect senescence across tissues, species, and physiological contexts (22–24). In parallel, therapeutic strategies targeting senescence have expanded. Among these, senolytics (agents that selectively eliminate senescent cells) and senomorphics (agents that modulate the SASP) have shown promise (25, 26). For instance, the senolytic combination dasatinib and quercetin (D + Q) improves physical function and extends lifespan in mice, and enhances cognitive performance in older humans (27, 28). Senolytics have also been shown to be beneficial in influenza infection by protecting age-related muscle decline and promoting CD8+ T cell dominance and virus-specific IgG production (29, 30). However, studies also report that removing $p16^+$ cells can decrease antibody production in aged mouse lungs, despite improved viral clearance (31). In the context of chronic lung diseases, preclinical work has shown that senolytics induce apoptosis in fibrotic AT2 cells (32), and a phase I found D + Q feasible and generally well-tolerated in patients with idiopathic pulmonary fibrosis (IPF), though further trials are needed (33). Similarly, D + Q reduced inflammation and immune cell infiltration in vitro and in mice exposed to cigarette smoke (34), and navitoclax reduced senescence of AT2 cells derived from patients with COPD (35). Beyond targeting antiapoptotic pathways, immunomodulatory strategies are gaining interest because senescent cells express unique seno-antigens recognizable by the immune system (36). For example, immune checkpoint inhibitors such as anti-PD1 enhance cytotoxic T cell-mediated clearance of senescent cells (37). Moreover, senomorphics such as metformin have been shown to be protective against emphysema in mouse models (38) and slow down disease progression in patients with COPD (39). With some clinical trials ongoing (7, 40) and promising results for other CLDs, senescence-targeting strategies hold promise to advance therapy for age-related diseases such as COPD and pneumonia.

Age-related alterations increase susceptibility to both acute and chronic lung diseases, yet key mechanisms such as extracellular vesicle (EVs) signaling, microbiome interactions, and sex-specific changes in aging remain insufficiently explored. Investigation of these pathways in acute lung injury and fibrosis, through an aging lens, has yielded key insights into inflammatory, immune, and tissue-remodeling processes. The following comprehensive reviews summarize these insights thoroughly (41–43), yet their relevance to COPD and pneumonia remains far less explored. Translating these aging-related findings to these disease contexts offers a promising yet underexplored opportunity to better understand disease susceptibility in older adults. At the same time, advances in experimental models, including organoids, precision-cut lung slices (PCLS), ECM scaffolds, and lung-on-a-chip (LOC) systems, combined with integrative multiomics approaches, have greatly expanded opportunities to study lung aging. This review aims to synthesize current knowledge on these emerging mechanisms, highlight innovative models and multiomics strategies, and discuss how they can deepen our understanding of lung aging and its role in disease susceptibility (Fig. 1).

UNDEREXPLORED MECHANISMS: EXTRACELLULAR VESICLES

EVs are increasingly recognized as key mediators of intercellular communication in aging tissues (44, 45). Senescent cells release elevated quantities of EVs that are enriched in SASP components such as cytokines, chemokines, and microRNAs (46, 47). These EVs facilitate the spread of inflammatory signaling beyond sites of initial cellular injury, suggesting they may amplify inflammaging in the lung (48, 49).

In the aging lungs, EV function is altered and influences disease-relevant outcomes. EVs from young mesenchymal stem cells were able to diminish LPS-induced lung injury, whereas those from the old donor were not (50). Circulating EVs from young mice also slow fibrosis progression and restore a more youthful transcriptional profile in aged mice with bleomycin-induced lung fibrosis (51). Consistent with this, EV biodistribution studies further demonstrate antifibrotic effects in aged lungs, supporting EVs as a potential therapeutic avenue in aging-associated lung disease (52). Together, these findings indicate that aging-focused investigation of EV biology has been fruitful and may be similarly informative when applied to COPD and pneumonia in older individuals.

Indeed, recent studies of EVs in pneumonia indicate they can impair antimicrobial defenses in aging lungs. During severe bacterial pneumonia and sepsis, epithelial, endothelial, and immune cell-derived EVs can modulate different immune processes, including phagocytosis, neutrophil chemotaxis, and cytokine production (53, 54). Endothelial-derived EVs can also promote vascular permeability and procoagulant signaling, which may be responsible for exacerbating the fragility of the alveolar-capillary barrier (55, 56).

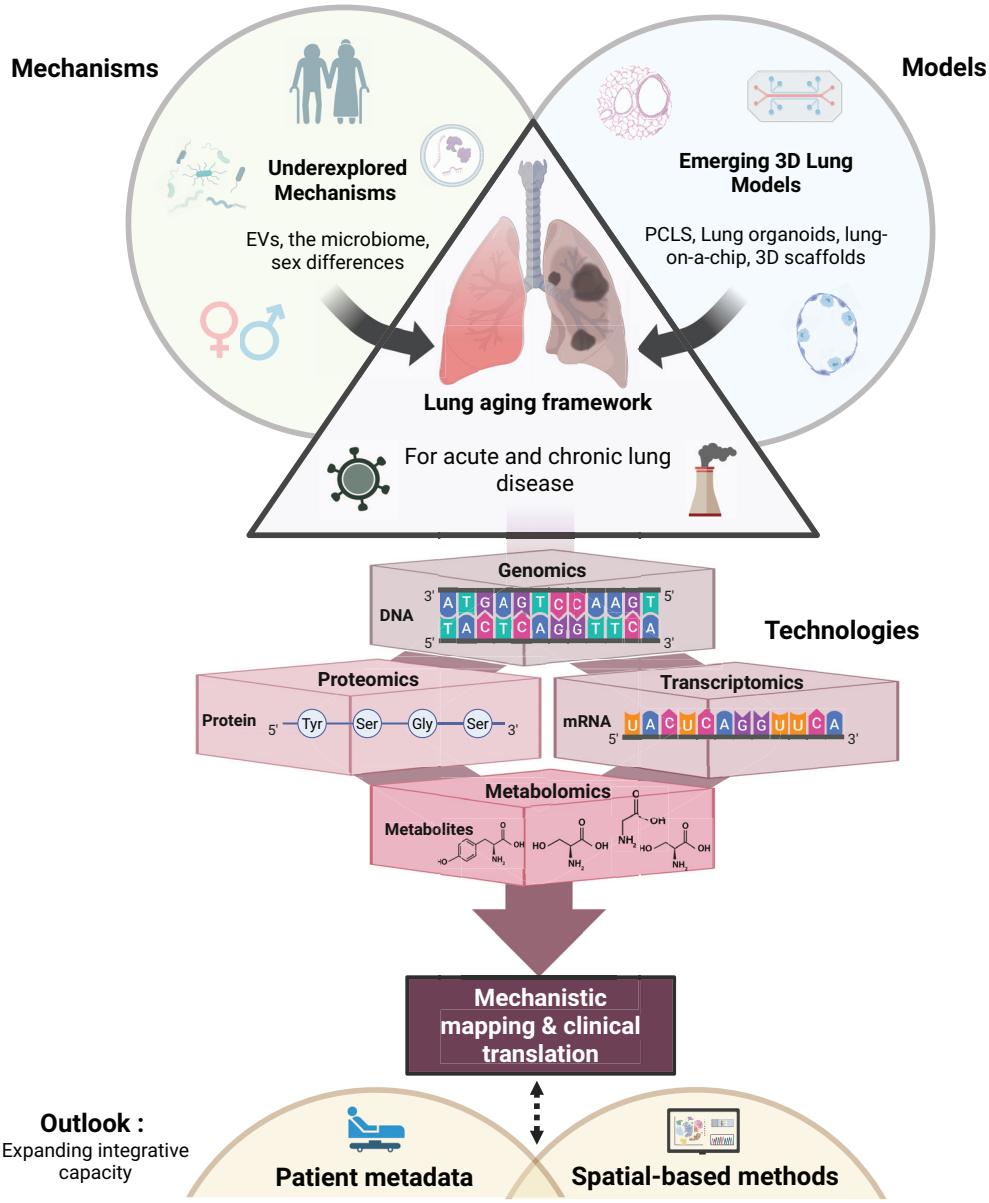

Figure 1. Overview of how emerging mechanisms, methods, and technologies can be integrated for mechanistic mapping in lung aging research. Extracellular vesicles (EVs), the microbiome, and sex differences remain underexplored in the context of acute and chronic disease in the aged lung. Emerging three-dimensional (3-D) lung models can be used for uncovering these underexplored mechanisms. Omics technologies as readouts allow for mechanistic mapping and greater resolution of alterations present. To further improve the current therapeutic strategies, integration of patient metadata and spatial-based technologies is also required. Figure created with a licensed version of BioRender.com.

Infection-induced EVs further impair epithelial barrier repair, amplify inflammatory cascades, or transfer microbial components that alter innate immune signaling (57–59).

In COPD, EVs are emerging as candidate biomarkers of disease activity. It has been reported that lung tissue-derived exosomal miR-125p levels are reduced three- to fivefold in COPD compared with healthy nonsmokers and smokers (60). Thus, EV-associated microRNAs may reflect epithelial injury or persistent inflammatory stress. Further evidence has shown that COPD EVs are able to suppress Sirtuin 1 (SIRT1) in recipient cells via microRNA-34a (61). Given that SIRT1 reduction represents a conserved hallmark of aging across multiple organs, this EV-mediated mechanism is consistent with a broader aging-linked intercellular communication axis that may extend beyond COPD alone (62, 63). In addition to microRNAs, EVs in patients with COPD also show altered proinflammatory and proteolytic protein profiles (56, 64).

Regardless of the advances, few studies incorporate age as a variable when assessing EVs in pneumonia and COPD. Promising emerging work demonstrates that EVs from young organisms can rejuvenate mitochondrial function and restore tissue performance in aged recipients (65). Moreover, a recent study described age-dependent differences in EV cargo (miRNA) and function in response to acute lung injury (66). This further underscores the need to define age-specific EV changes in the lung.

UNDEREXPLORED MECHANISMS: THE MICROBIOME

Dysbiosis, a hallmark of biological aging, is defined as age-associated shifts in microbial diversity, composition, and metabolic output (67, 68). Recent studies have revealed that the respiratory microbiome is dynamic rather than fixed. Longitudinal analyses of infants, children, and young adults show that nasopharyngeal communities in early life are dominated by *Moraxella* and *Dolosigranulum*, whereas in older children and young adults, *Staphylococcus* and *Corynebacterium* become more prevalent (69, 70). Older cohorts of lung microbiome data are lacking; however, microbiome shifts in the gut are more widely reported (71, 72). Moreover, age-related shifts in gut microbiota have been reported in response to influenza infection (73). One study investigating antibiotic resistance genes in the lung microbiome of children compared with adults, a cohort with median age of 63 yr, found significantly higher gene expression of these genes in adults, suggesting that aging may alter not only bacterial diversity but also microbial gene activity (74).

Shifts in bacterial populations have been linked to pneumonia severity in younger populations (75, 76). Infants with recurrent respiratory tract infections showed delayed nasopharyngeal microbiota maturation with reduced numbers of commensal *Corynebacterium* and *Dolosigranulum* and increased shifts toward *Moraxella* dominated communities (77). A comprehensive study of nontuberculosis mycobacterial infection in old and young macaques outlined age-dependent differences in immune responses and microbial communities, including a loss of an uncultured *Tropheryma* species in older animals, highlighting how aging can reshape host-microbe interactions (78). Various studies investigating germ-free and antibiotic-perturbation highlight that the gut microbiota shape alveolar macrophage tone and host response to bacterial pneumonia (79, 80). One specific study investigating the gut-derived bacteria between young and old mice infected with *Streptococcus pneumoniae* highlighted expansion of *Enterobacteriaceae* in the feces of aged mice but not in the young mice and linked age-related intestinal dysbiosis to increased susceptibility to lung infection (81). Similarly, it has been shown that short-chain fatty acid-producing gut bacteria decline in elderly and their restoration reduced lung inflammaging and severity of acute lung injury in old mice (82). Recent studies have only begun to scratch the surface of the microbiome role in pneumonia and provide hope for disease severity predictions and a potential therapeutic avenue through restoration of age-related dysbiosis.

The respiratory microbiome has also been investigated in COPD, where increased *Haemophilus* and decreased *Prevotella* and *Veillonella* correlate with disease severity (83). These alterations are thought to influence lung immunity through the gut-lung axis, where microbial metabolites and immune cell priming play key roles (84). Analyses of the gut microbiome in COPD reveal distinct signatures compared with healthy controls (85). Moreover, fecal microbiota transplantation with the commensal bacterium *Parabacteroides goldsteinii* significantly ameliorated COPD in mice, suggesting a potential therapeutic avenue (86).

Age-related changes of the gut microbiome are becoming more clearly defined, with studies beginning to draw links between these changes and lung-related diseases, particularly infections. In parallel, changes in bacterial gene expression, most notably the higher prevalence of antibiotic resistance genes in aged populations, highlight the broad and clinically relevant influence the microbiome may have, not only in aging biology, but also in disease. Despite these associations, no direct causal links have been established between aging-related microbiome changes and increased susceptibility to pneumonia or COPD, reflecting a broader lack of aging-focused microbiome research, alongside a limited investigation of the respiratory microbiome.

UNDEREXPLORED MECHANISMS: SEX DIFFERENCES

Sex differences in systemic immune aging are increasingly reported, particularly in vaccine responsiveness (87, 88). Recent studies have begun to profile sexual dimorphism in the lungs' resident immune niche, with the lungs of males exhibiting higher levels of alveolar macrophages (89). However, sex differences in lung aging profiles are not as extensively reported. Functionally, studies have shown that men show faster declines in lung function (FEV1) with age, whereas women often show a more gradual decline (90, 91). Beyond lung function, a recent study incorporated both age and sex as factors when investigating responses to LPS-induced lung injury in a mouse model. The study found that age and sex interact to influence outcomes, with old females having the highest mortality risk and old males requiring more noradrenaline to maintain hemodynamic stability (92).

In the case of community-acquired pneumonia, males typically experience more severe outcomes, whereas females mount an earlier innate and adaptive immune response (93, 94). Investigation of pneumonia in male and female mice shows a similar pattern with male mice being more susceptible to infection, having higher mortality, and elevated cytokines secretion, including interleukin-18 (IL-18), tumor necrosis factor-alpha (TNF-α), and IL-1β (95). Given these emerging age- and sex-dependent differences, such factors are likely to influence susceptibility and progression of respiratory immune diseases.

Recent mechanistic investigations have identified sexually dimorphic gene regulatory networks enriched for ECM-related genes, with greater transcription factor targeting observed in females (96). Notably, in a COPD cohort analyzed within the same study, this pattern was reversed, with males exhibiting stronger regulatory targeting of ECM-associated genes. Sex hormones are thought to be able to modulate pathways involved in epithelial barrier integrity, fibroblast collagen synthesis, macrophage activation, and T-cell differentiation (97–99). Given this, the decline of sex hormones in age, particularly menopause-associated estrogen withdrawal, may have a great influence on epithelial repair capacity, innate immune tone, and ECM turnover in women. These studies highlight that there remains a vital gap in understanding the interaction between aging, sex hormones, and ECM regulation in COPD.

Although current single-cell atlases and spatial transcriptomic datasets of the lung integrate age or sex as individual covariates, few systemically integrate both as combined

biological variables. This limits insights into the influence of sex and age on key cell populations such as epithelial progenitors, fibroblast subsets, endothelial cells, alveolar macrophages, and adaptive immune cells. Addressing these gaps will require advanced experimental models that closely mimic the aged in vivo state, integration of patient metadata, and coordinated multiomics, spatial, and longitudinal approaches. These strategies are essential to uncover causal mechanisms, identify biomarkers, and develop interventions to enhance resilience against pneumonia and prevent COPD progression in older adults. In the following section, we discuss emerging models and multiomics technologies that enable these investigations.

ADVANCED 3-D LUNG MODELS AND OMICS

In the past years, several three-dimensional (3-D) models have emerged as important and more physiological options for studying pneumonia and COPD (Table 1). Precision-cut lung slices (PCLS) maintain the native lung architecture and cellular diversity as well as cell-cell and cell-ECM interactions, enabling detailed analysis of tissue responses to pathogens and environmental insults. Lung organoids are three-dimensional structures derived from adult or pluripotent stem cells and recapitulate key aspects of lung development and disease pathology. This system is modular and can be expanded by the addition of specific cell types. Complementing organoid-based systems, lung-on-a-chip (LOC) technologies have recently expanded the toolbox by replicating the dynamic mechanical and cellular microenvironment of the human lung (148, 149). These platforms can simulate airflow, vascular perfusion, and immune cell recruitment, allowing precise modeling of lung disease-associated processes such as airway inflammation, mucus production, and tissue remodeling. In parallel, scaffold-based strategies using decellularized lung matrices or synthetic hydrogels offer additional structural support for cell growth and differentiation, further enhancing physiological relevance (149). Together, these advanced culture systems have significantly advanced our understanding of COPD and pneumonia pathophysiology, bridging gaps left by traditional cell culture and animal models, and providing

Table 1. *Lung models used in pneumonia and COPD research*

Disease	Model	Methods/Omics	References
Pneumonia	PCLS	scRNA-seq; Bulk RNA-seq; cytokine assay; immunofluorescence; proteomics; Raman imaging	(100–106)
	Human airway organoids	Immunofluorescence; Bulk RNA-seq; qPCR	(107–110)
	Human distal lung organoids	scRNA-seq; RNA FISH; live Imaging; Immunofluorescence; qPCR	(111, 112)
	hiPSC-derived alveolar organoids	Immunofluorescence; Bulk RNA-seq; spatial transcriptomics	(113, 114)
	Human nose organoids	Immunofluorescence; multiplex secretome analysis	(115)
	Human embryonic stem cell-derived lung organoids	qPCR; immunofluorescence	(116)
	Bronchial organoids	Bulk RNA-seq; immunofluorescence	(117)
	Organoid-derived bronchioalveolar model	Mass spectrometry; Bulk RNA-seq; scRNA-seq	(118)
	Lung-on-a-chip	qPCR; ELISA; Western blot; immunofluorescence; cytokine array; ELISA; live imaging	(119–123)
	ECM-scaffolds	Bulk-RNA sequencing; proteomics; immunofluorescence	(124–126)
COPD	PCLS	Cytokine assay; immunofluorescence; microarrays; live imaging; proteomics; metabolomics; advanced imaging	(66, 127–132)
	Human embryonic stem cell-derived lung organoids	Live-imaging	(133)
	Mouse distal lung organoids	Western blot; qPCR; bulk RNA-seq; immunofluorescence; ELISA	(134–138)
	Bronchioid model	Western blot; qPCR; single-cell RNA-seq; immunofluorescence; ELISA	(139)
	Bronchospheres	Immunofluorescence; qPCR	(140)
	Human nasopharyngeal and bronchial Organoids	scRNA-seq; bulk RNA-seq; immunofluorescence; cytokine assay	(141)
	Combined models	Immunofluorescence	(142, 143)
	Lung-on-a-chip	qPCR; Western blot; ELISA; cytokine Array; Proteomics; immunofluorescence; transmission electron microscopy	(122, 144–146)
	ECM-scaffolds	qPCR, Western blot, ELISA, immunofluorescence	(147)

Studies were grouped by Model and Methods. COPD, chronic obstructive pulmonary disease; ECM, extracellular matrix; hiPSC, human induced pluripotent stem cell; PCLS, precision-cut lung slices.

increasingly personalized and mechanistically informative platforms for studying disease mechanisms and therapeutic interventions. In this review, we aimed to summarize their applications and contributions to COPD and pneumonia research.

Precision-Cut Lung Slices

Precision-cut lung slices (PCLS) generation involves careful inflation of the lung with low-melting agarose solution, followed by precise sectioning using a vibratome or comprestome to yield slices with a thickness of 300–500 μm, depending on the species (100, 101, 150, 151). Unlike two-dimensional (2-D) cell cultures, PCLS retain the cell-cell interactions within the native 3-D tissue context. Moreover, they preserve alveolar and airway structures, the ECM, and resident cell populations, including AT2 cells and macrophages, which have been implicated in both pneumonia and COPD (100). Therefore, PCLS bridge the experimental gap between simplistic in vitro systems and complex in vivo animal models with limited clinical translation (152). Recent advances in optimizing PCLS viability and storage (153, 154) allowed characterization of dynamic cellular processes over longer periods. This is of great importance to study immune response against respiratory pathogens or lung remodeling relevant for chronic lung diseases (155). PCLS can be derived from multiple species (e.g., mice, pigs, cows) and, most importantly, from healthy or diseased human lung tissue. Although donor history and underlying conditions add complexity and variability, this heterogeneity increases their relevance for translational research and personalized medicine (151, 156–158).

Recent investigations utilizing PCLS have yielded significant insights into the pathogenesis and immunological aspects of pneumonia and respiratory infections. One example is the discovery of the lung's capacity to distinguish between viable and nonviable bacteria, which plays an important role during initial stages of the response to infection (100). Moreover, a recent study explored age-dependent differences in the response to *Pseudomonas aeruginosa,* highlighting that older individuals showed higher inflammation and impaired immune response (159), as previously observed in vivo. Next to bacterial-induced pneumonia, human PCLS have also been extensively used to study viral infections (102–104, 127, 128). Importantly, recent studies suggest age-related variation in viral replication upon SARS-CoV-2 or influenza A infection (105) and exacerbated immune responses after LPS challenge (106) in PCLS. Collectively, these studies underscore the relevance of human PCLS as a physiologically grounded platform that faithfully recapitulates features of respiratory infectious diseases while enabling the dissection of age-dependent differences in host responses in vitro.

PCLS have also been used to model COPD pathology; either by exposing healthy human PCLS to COPD-relevant stimuli such as cigarette smoke or viral infection in vitro (127–129), or by preparing PCLS from COPD animal models such as cigarette smoke (CS) exposure or elastase-induced injury (160). Both approaches allowed the assessment of disease-specific phenotypes, including alveolar destruction, cytotoxicity, metabolic decline, and mitochondrial injury. Moreover, to overcome the absence of circulating immune cells, researchers have added alveolar macrophages (e.g., from bronchoalveolar lavage) to mouse or human PCLS to study macrophage infiltration, phenotype, and epithelial responses after CS exposure (160). PCLS have also been used for preclinical testing of antimicrobial and anti-inflammatory therapies, providing insight into inflammation, immune responses, airway contractility, and tissue remodeling (103, 104, 129). In addition, fibroblast-derived EVs were shown to reverse elastase-induced emphysema in PCLS as observed in vivo (130). Even though there are no studies addressing age-related changes in COPD, results from other disease contexts using PCLS support the idea that these could be used to assess whether aged tissue exhibits impaired repair, exaggerated inflammation, or altered therapeutic response in this context.

Recent advances now allow single-cell sequencing, mass-spectrometry proteomics, and advanced imaging of human PCLS permitting multiomics molecular and structural profiling of lung tissue (102, 103, 131, 157, 161–163). For example, single-cell RNA-sequencing of PCLS exposed to influenza A revealed cell type-specific antiviral and proinflammatory gene expression in epithelial cells, fibroblasts, macrophages, and monocytes, highlighting heterogeneous responses relevant to lung injury (102). Moreover, mass spectrometry-based proteomics has been used to quantify ECM proteins, inflammatory mediators, and signaling molecules in the context of other chronic lung diseases (131), but with high relevance for pneumonia and COPD pathogenesis. By integrating omics technologies with PCLS, researchers can unravel disease mechanisms and treatment responses directly in patient-relevant lung tissue. This powerful combination positions PCLS as a cutting-edge platform for precision-medicine studies in pneumonia and COPD.

Lung Organoids

Lung organoids can be generated either from pluripotent stem cells or adult stem cells (164). Depending on the tissue of origin (nasal, bronchial, and alveolar), lung organoids recapitulate the 3-D organization of their respective anatomical regions, allowing the study of physio-pathological mechanisms in vitro (141, 165). Lung organoids have become indispensable models for investigating viral pneumonia. They were heavily used during the SARS-CoV-2 pandemic, providing insights into viral infection as well as on inflammatory responses (113, 164). Beyond SARS-CoV-2, lung organoids also facilitate the study of other respiratory viruses, such as adenoviruses, influenza A, and respiratory syncytial virus (RSV). In this context, human lung organoids from airways or nasal epithelium were used to study changes in cell and cilia motility, immune cell recruitment, mucus secretion, and inflammatory responses (107, 111, 115, 166) as well as to investigate antiviral effects of potential therapeutics (118). Lung organoids have been used to study bacterial pneumonia with different clinically relevant pathogens [*P. aeruginosa*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Mycobacterium tuberculosis* (167), *Streptococcus pneumoniae*, and *Staphylococcus aureus*] (168). In this way, recent studies have provided mechanistical insights into bacterial adhesion and internalization, the role of surfactant proteins, and temporal and host-specific factors modulating innate responses (108, 116). Moreover, advances in delivery methods into the organoids like microinjection into the

lumen (133) or apical-out culturing (109, 169) methods open now new avenues to study both bacterial and viral low respiratory infections. Organoids and hydrogel-based cocultures further strengthen pneumonia models by enabling controlled study of epithelial-mesenchymal and immune-epithelial interactions involved in pathogen invasion and barrier disruption in viral and bacterial pneumonia (119, 170). They also allow interrogation of ECM-regulated epithelial signaling, including activin receptor-like kinase 5 (ALK5) and integrin αVβ6 pathways relevant to innate immune responses (171).

Lung organoids have also been instrumental in dissecting the complex pathophysiology of COPD. Patient-derived lung organoids accurately replicate changes observed in patients with COPD in vitro, including alterations in cell-cell interactions, cytokine and protein expression, and signaling pathway activation (172). Lung organoids have also helped in understanding the effects of environmental exposures (cigarette smoke or pollution) (134, 135) and inflammatory molecules in stem cell potential and differentiation, highly relevant process in COPD pathogenesis (136). Specifically, highlighting the role of signaling pathways such as wingless-type (WNT), transforming growth factor-β (TGF-β), and receptor for advanced glycation end-products (RAGE), and allowing the testing of strategies to reverse the associated parenchyma deterioration (132, 142, 143, 173–175). Hydrogel-based epithelial-mesenchymal cocultures add complementary insight by recreating ECM stiffness and enabling focused analysis of matrix-dependent epithelial and immune interactions central to airway remodeling and inflammation in COPD (171). In parallel, organoid systems have been useful to study how aging impairs epithelial regeneration and niche support. Cocultures combining epithelial and mesenchymal cells from donors of different ages revealed that aged lung mesenchymal stromal cells display senescence-associated metabolic and redox dysfunction that reduces their ability to support alveolar epithelial stem cell self-organization, whereas young stromal cells can maintain organoid formation regardless of epithelial donor age (137). Aging phenotypes can also be experimentally induced in vitro, for example, by senescence-promoting stimuli. Here, coculture with senescent fibroblasts diminished progenitor potential within lung organoid systems, offering mechanistic insight into how age-related stromal decline contributes to impaired repair in chronic lung diseases (138). Moreover, recent studies have generated immune competent-organoid systems by incorporating macrophages, where the addition of these immune cells, combined with SARS-CoV-2 infection, led to increased epithelial senescence, underscoring the importance of immune-epithelial cross talk in COVID-19 disease pathology (114). Beyond mesenchymal cells and macrophages, the modular nature of organoid platforms also enables the incorporation of additional stromal and immune cell types, such as neutrophils, dendritic cells, endothelial cells, and even adaptive immune cells, thereby reconstructing more faithfully age- and disease-relevant multicellular interactions. Furthermore, the ability of lung organoids to replicate physiological and pathological characteristics even at the individual levels offers a unique opportunity for precision-targeted treatments and the creation of biobanks for individual patient care (141, 176).

Recent single-cell transcriptomic studies showed that organoids derived from patients with COPD retain all major airway cell types while reproducing key disease features, including goblet cell hyperplasia and reduced ciliary beat frequency, reflecting the severity and pathology of the donor tissue. Moreover, it also showed an increased viral replication in COPD organoids, recapitulating clinical observations of poorer outcomes of patients with COPD after SARS-COV-2 infections (141). These results highlight that combining organoid physiology with single-cell transcriptomics and inflammatory readouts can connect cellular phenotypes to infection outcomes and biomarkers of disease severity. Expanding this approach to include broader omics analyses will deepen our insight into disease mechanisms and therapeutic responses. As organoids gain greater cellular complexity, become more standardized, and are adapted for high-throughput assays, organoid-omics platforms will offer powerful, scalable models for accelerated drug discovery and screening in COPD and pneumonia.

Lung-on-a-Chip and ECM-Scaffolds

Advances in in vitro models such as lung-on-a-chip (LOC) systems and scaffold-based 3-D models have substantially improved the physiological relevance by integrating microfluidics, mechanical cues, ECM complexity, and multicellular interactions (120, 122, 147, 148, 177). These platforms support coculture of epithelial, endothelial, fibroblast, and immune cells under airflow, perfusion, and cyclic stretch, enabling controlled exposure to biochemical and mechanical stressors (122, 149, 177, 178). Scaffold-based and bioprinted models further mimic native ECM architecture and support high-resolution multicellular organization, ECM deposition, and fibroblast activation (148, 177).

LOC systems have been especially valuable for studying COPD mechanisms. Chips seeded with patient-derived bronchial epithelial cells at air-liquid interface cell culture (ALI) replicate hallmark COPD phenotypes, including mucociliary dysfunction, oxidative stress responses, and exaggerated IL-8 secretion following cigarette smoke exposure (CSE) delivered under bidirectional flow (122, 145, 149). Moreover, bronchial epithelial cells cultured in a tubular scaffold (bronchioid) maintained mucociliary and contractile functions while recapitulating pathological features of COPD, such as altered cellular composition and cilia beating (139). Epithelial-endothelial cocultures reveal CSE-driven IL-6/TNF-α elevation and tight-junction disruption, indicating barrier impairment (149). Scaffold integration, particularly fibroblast-laden hydrogels, enables modeling of ECM stiffness, epithelial–mesenchymal transition (EMT), apoptosis, and proliferation under controlled smoke dosing (149, 177). Moreover, breathing lung chip models with dynamic breathing mechanisms with an air-liquid interface have been used to study response to inflammatory cytokines (TNF-α) and the therapeutic effect of anti-inflammatory drugs such as nebulized budesonide (146). Moreover, patient-specific COPD chips additionally capture individualized cytokine responses to CSE or IL-13 stimulation, supporting personalized drug-testing strategies (122, 149).

LOC platforms also advance infection and pneumonia research. Early alveolar-capillary interface chips showed bacteria-induced epithelial IL-8 release, endothelial activation, and neutrophil transmigration, all key features of pneumonia pathology (120, 122, 177). More advanced models incorporate breathing-like cyclic stretching or use decellularized ECM or gelatin methacryloyl (GelMA) scaffolds to support 3-D immune cell migration, barrier integrity, and reactive oxygen species (ROS) measurements during infection challenges (149, 177, 179). These systems also model nanoparticle-driven inflammation and ventilator-associated injury (177, 180). Moreover, LOC has been developed to study the airway response to respiratory viruses, reproducing viral cellular pathology and immune cell response and migration (123).

Scaffold-based and bioprinted constructs that mimic native ECM architecture provides controlled environments to study epithelial-mesenchymal communication, fibroblast activation, and matrix deposition (124, 125, 177). Stretchable-alveoli LOC systems add mechanical realism, reproducing ECM-dependent barrier stability and epithelial-endothelial communication relevant to COPD airway damage and pneumonia-associated barrier breakdown (121, 144). Furthermore, these models enabled detailed characterization of the inflammatory responses to infection and the dynamics of cell-pathogen communication, not only in mono-infections but also in multikingdom (bacterial-fungal-human) interactions (126), which more closely reflect the complex environment of the human airways.

The integration of omics technologies with lung-on-a-chip and scaffold-based 3-D systems is increasingly demonstrating how these platforms capture disease-relevant signatures in COPD and pneumonia (149, 177). By pairing physiologically realistic microenvironments with transcriptomic, proteomic, and cytokine profiling approaches, these models provide a deeper view of how cellular interactions, inflammation, and tissue remodeling unfold under disease-like conditions (122, 147). Similar strategies in infection-focused chips reveal epithelial and endothelial responses to viral and bacterial challenges, whereas stretchable alveoli systems resolve barrier instability under mechanical load (120–122, 144). Together, these omics-integrated platforms mirror the analytical depth achieved in organoid transcriptomic studies and highlight advanced in vitro lung models as powerful tools for uncovering mechanisms and accelerating therapeutic development in COPD and pneumonia.

FUTURE DIRECTIONS AND CONCLUSIONS

As outlined earlier, advanced human ex vivo models have substantially expanded our understanding of chronic and acute lung diseases. However, most studies have not systematically integrated sex or age as analytical parameters. Incorporating these dimensions will be essential in the future, as they fundamentally influence disease susceptibility, cellular phenotypes, and treatment responses. The integration of advanced single-cell and spatial omics technologies with detailed patient metadata will be critical for deciphering genetic, epigenetic, and exposure-related determinants of disease predisposition. These approaches mark a major step toward precision medicine strategies that leverage the full potential of human ex vivo models (Fig. 1). For example, resolving cell-type-specific aging signatures will enable us to differentiate between regenerative and pathological cellular programs, such as those associated with cellular senescence, and ultimately allow specific reprogramming of these pathological pathways. Looking ahead, computational frameworks that combine multimodal omics, systemic perturbation experiments, and patient-derived metadata may enable personalized prediction of treatment response. In this way, these emerging technologies create a bidirectional bridge between bench and bedside, allowing clinical observations to guide experimental design and experimental insights to directly inform clinical decision-making (Fig. 1).

Despite their strengths, current organotypic models face important challenges. For PCLS, limitations include the absence of recruited inflammatory cells, lack of ventilation and innervation, suboptimal exposure paradigms, and difficulties in defining reversible structural or functional impairments within the constraints of culture duration. Moreover, complex conditions such as acute respiratory distress syndrome (ARDS) cannot be fully recapitulated ex vivo, as PCLS do not capture systemic factors, including hypoxia, hyperoxia, or dynamic disease progression, that critically shape ARDS pathology. Lung organoid models, while powerful, represent only selected aspects of disease biology. They effectively model progenitor activity, mucus hyperplasia, and retain patient-specific genetic and phenotypic traits, enabling personalized disease modeling and drug testing. Nonetheless, they lack full disease complexity, certain functional readouts, and a complete representation of terminal and respiratory bronchiolar regions. Finally, ECM scaffolds face challenges such as variability introduced during decellularization, mechanical mismatch with native lung tissue, immunogenicity risks, limited scalability and reproducibility, and difficulties aligning scaffold degradation with new tissue formation. On the other hand, lung-on-a-chip models offer controlled microphysiological environments, but they still have important limitations. They cannot fully mimic the 3-D structure or cellular complexity of the lung, especially in the alveolar region. They are also affected by matrix-related molecule absorption, often lack key cell types and mechanical cues, and remain low-throughput and difficult to standardize. Both technologies also struggle to incorporate systemic immune responses and to maintain long-term, stable, and primary multicellular cultures, underscoring the need for continued technological innovation.

Adapting and expanding these models to address these limitations, through the addition of autologous or engineered cell types, advanced coculture approaches, or the incorporation of mechanical and environmental cues, will further enhance their physiological relevance. These developments hold considerable promise for broadening the applicability of human ex vivo systems and deepening mechanistic insights into age-related lung disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.F.W., M.L., and M.C.M.-N. prepared figures; L.F.W., M.L., and M.C.M.-N. drafted manuscript; L.F.W., M.L., and M.C.M.-N. edited and revised manuscript; L.F.W., M.L., and M.C.M.-N. approved final version of manuscript.

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