The importance of mechanical forces in chronic respiratory diseases

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Optimal lung performance requires orchestrated and multiscale mechanical forces, disruption of which can contribute to disease-driving mechanisms. Considering mechanics may advance treatment possibilities for lung diseases https://bit.ly/48BhMmB

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Abstract

The lung may be the organ whose mechanical environment needs to be most finely tuned to achieve optimal function. These needs have to be fulfilled at multiple scales, from proper force transmission between the chest wall and the parenchyma to reduction of surface tension by surfactants inside the alveoli. In addition, a plethora of mechanical loads and forces takes place within the lung, from the passive stretch withstood by epithelial cells lining the alveoli, to active forces generated by smooth muscle cells to control airway calibre or cilia beating by ciliary cells in the bronchi to clear debris. Furthermore, the acellular structures in the lung are finely tuned in composition and mechanical properties, from the viscoelastic properties of the mucus to trap pathogens, to the collagen- and elastin-rich extracellular matrix that enables the lung to display elastic recoil at resting volumes but stiffen as it approaches total lung capacity. In this review, we describe the mechanical interplay between the cell types found in the lung, as well as cellular responses to their mechanical niche. We further describe how these responses are altered in diseases such as asthma, COPD, pulmonary fibrosis and lung cancer. In addition, key proteins in mechanotransduction events are detailed, stressing their potential role as therapeutical targets for lung diseases. Finally, we also include a sex perspective to lung pathologies and highlight engineered model systems that may be used to advance our understanding of mechanical forces in experimental investigations or towards lung regeneration.

Introduction

The lungs are made up of a complex structure of airways and alveoli that are optimised for efficient gas exchange. To generate this complex structure, defined, specialised cell types are located in precise microenvironmental niches that maintain the cell homeostatic state in health or provide signals for appropriate behaviours to enable necessary repair processes after injury. The characteristics of these niches have been explored in depth when considering the cellular constituents and the pro- or anti-inflammatory factors (cytokines, chemokines, growth factors) that regulate cellular behaviour [1–4]. However, less attention has been paid to the cues for regulating cellular response in health and disease that are provided by the physical characteristics and the biomechanical environment [5, 6].

The nature of the lung makes it a biomechanical environment, at multiple spatial scales. This review aims to highlight the importance of considering the mechanical characteristics and their impact on

responses, at all levels, when searching for underlying mechanisms and possible therapeutic targets for chronic lung diseases.

The lung mechanical environment is complex and occurs at multiple spatial scales

The main role of the lung is gas exchange between the air and blood. While the exchange carried out *via* O_2 and CO_2 diffusion within the alveoli is passive, a number of events involving active force transmission need to take place in multiple regions and spatial scales in the lung so that fresh air reaches the alveoli (and CO_2-rich air leaves them). As a result, the human lung is a complex organ at multiple scales that is continuously subjected to external forces throughout life. Any pathological changes that alter the structure or the mechanical behaviour of the airways or lung parenchyma may lead to its dysfunction (figure 1).

The requirements for gas exchange are primarily a structure with large surface areas and small distances between air in the alveoli and blood in the capillaries (figure 2). Such an optimised structure is fulfilled by a fractal organisation with 480 million alveoli, each reaching 200 µm in diameter when the lung is maximally inflated, all packed within the volume of the lung. The resulting structure has <8 µm between septal walls separating neighbouring alveoli, a space partially occupied by a complex extracellular matrix (ECM) [7].

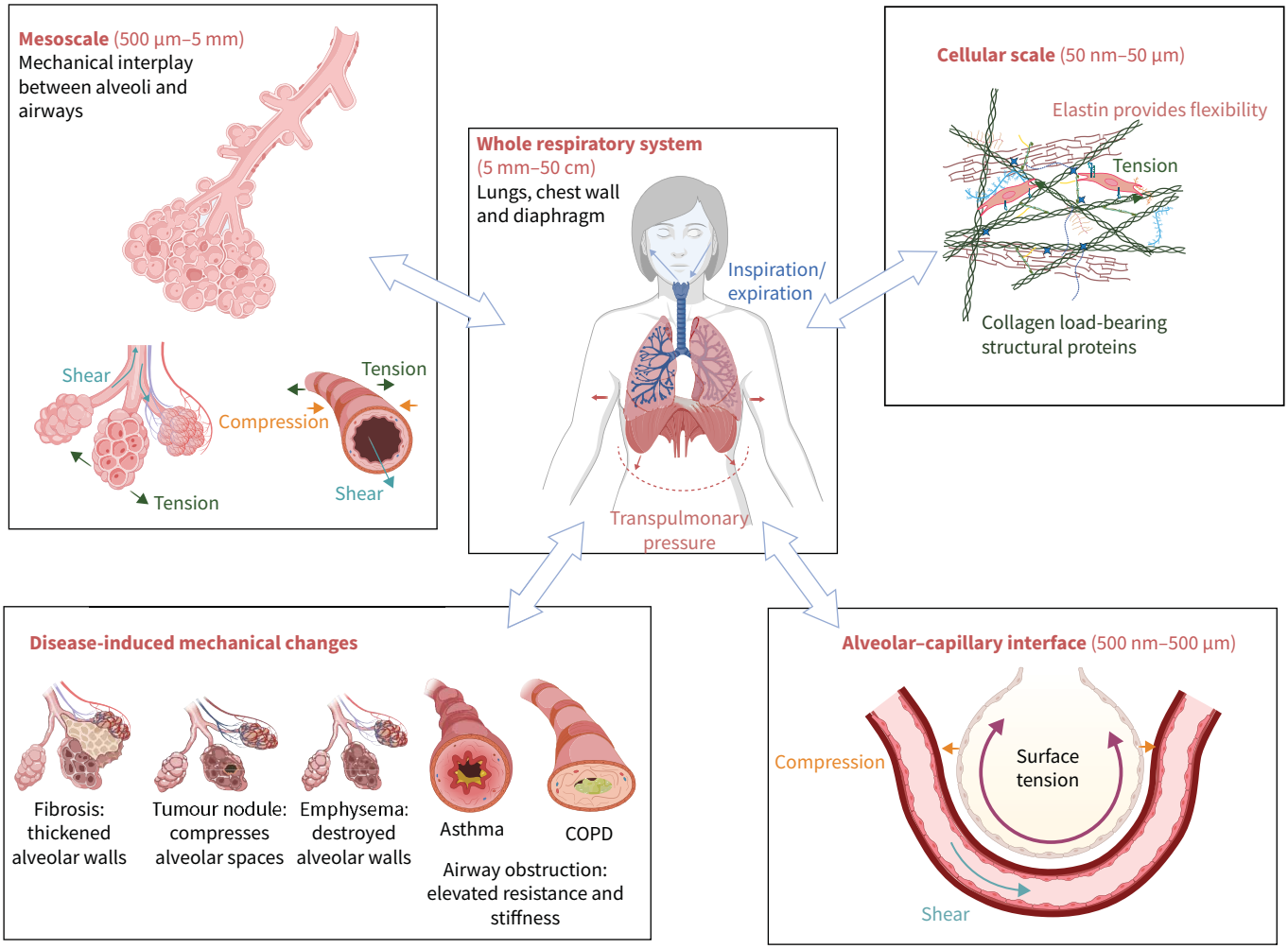

FIGURE 1 The lung is a complex organ that is continuously subjected to external multiscale forces, such as transpulmonary pressure at the whole organ level, stretch of the alveoli at the mesoscale, surface tension at the epithelial side of the air–blood barrier, and shear and compression at the endothelial side of the air–blood barrier. In addition, its elastin and collagen rich extracellular matrix (ECM) confers the lung with unique nonlinear mechanical responses at increasing lung volumes. At the cellular scale, cells adhere to the ECM in their immediate microenvironment and thus sense mechanical changes from tension within the ECM fibres and also the inherent mechanical properties of the ECM itself. Prevalent lung diseases such as fibrosis, cancer, asthma or COPD alter the lung's ECM composition, multicellular organisation and overall architecture, thus leading to altered mechanical properties and aberrant force transmission. Figure created with BioRender.com (Burgess, 2025).

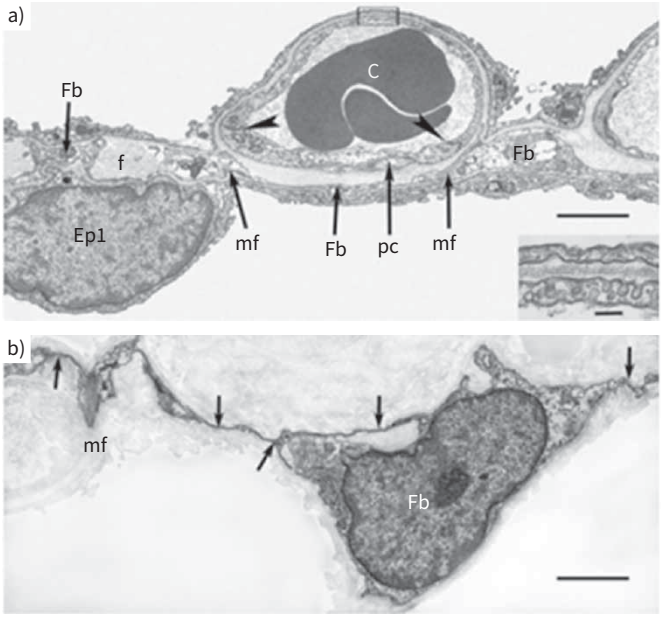

FIGURE 2 Electron micrograph of an alveolar septum of human lung showing a) a capillary (C) with two intercellular junctions of the endothelium (arrowheads) and a pericyte process (pc), a type 1 alveolar epithelial cell (Ep1) and fine extensions of a fibroblast (Fb) that is tightly related to fibres (f) and forms myofibrils (mf) that span across the septum. The box at the top of the panel marks the minimal barrier whose structure is shown in the inset: the epithelium and endothelium are joined by their fused basement membranes. b) Touched up image of a fibroblast to show its very slim cytoplasmic extensions (arrows). Scale bars: 2 µm, 0.1 µm (inset). Reproduced from [8] with permission.

During normal breathing, collagen types I and III are the main load-bearing structural proteins in the alveolar ECM, while elastin fibres provide the majority of recoil pressure [9]. Remarkably, the entropic nature of elastin extension and its dense crosslinking in the lung ECM lead to a structure that behaves like a linear spring, with minimal dissipation of energy in the ranges of cyclic stretch and low to medium lung volumes associated with normal breathing [10]. Collagen fibres, though, are wavy at low and intermediate lung volumes and become straighter and thus stiffer as the lung inflates to approach total lung capacity (TLC) [9]. This nonlinear mechanical behaviour of the collagen ECM results in a multiplicity of spatial structures that display ever larger resistances when forced to distend further, such as when the load acting upon them or their volume are increased. As will be discussed below, this phenomenon gives rise to heterogeneities in force transmission, tissue deformations and furthermost ventilation levels, from the alveoli scale up to the whole organ scale.

Heterogeneities in lung structural arrangements

Not all alveoli are created equal, and large heterogeneity in their mechanical behaviour has been recently demonstrated, made possible through the development of a crystal ribcage that allows multiscale imaging and mechanical probing of a fully functional healthy whole lung [11]. Further distinct local heterogeneities in ECM composition, alveoli structure and subsequent mechanical properties are associated with pathologies. In fibrosis, which has been extensively studied, the injured alveoli display thickened septal walls and reduced diameters at higher alveolar pressures, all consistent with local stiffening of the structure [12]. Such local heterogeneities in force transmission may lead to functional impairments beyond the affected region. As an example, the presence of a nodular tumour within an alveolus renders it unable to inflate and thus nonfunctional for gas exchange [11]. Remarkably, this functional impairment extends to one or two layers of the adjacent alveoli. Similarly, the local neighbourhood (up to 200 µm) of larger nodules displays alveoli that are either fully stretched or compacted [13].

Structural heterogeneity and thus diminished physical performance of the lung can also be found at the airway level, and this is best exemplified in pathologies such as asthma and COPD (for detail see below). A reduction in airway calibre observed in both asthma and COPD leads to elevated resistance and stiffness of the whole system.

Force transmission within the lungs: at multiple scales

Connections between airways and alveoli

At the mesoscale, the lung is a functionally interconnected organ, as highlighted by the mechanical interplay between alveoli and airway changes during breathing. A number of studies have shown that increasing tidal volume reduces airway resistance and can attenuate or reverse bronchoconstriction events [14]. This phenomenon is not observed in patients with asthma, who lack stress-induced smooth muscle relaxation [15]. Similarly, situations that limit operational lung volumes, such as chest strapping, obesity or resting in a supine position, lead to worsening of bronchoconstriction events [16–18]. Furthermore, small changes in calibre between peripheral airways of the same generation could result in airflow being diverted from more to less constricted airways, thus further increasing the size of the latter and the associated structural and functional heterogeneity of the lung [13].

Forces at the whole respiratory system scale

One scale up, when we consider the whole respiratory system, the already-discussed airways and alveolar compartment are joined by the chest wall and the respiratory muscles. In physiological conditions and in terms of balance of force transmission, the lungs and the chest wall move together owing to the virtual adhesion conferred by the pleura. Furthermore, the chest wall is an elastic compartment directly connected to the respiratory muscles. Accordingly, the pleura transmits the mechanical forces associated with transpulmonary pressure to the subpleural alveolar septal walls, which are further transmitted to the adjacent alveoli, and their next adjacent neighbours, and so forth. At the end of a normal tidal breath, the balanced opposing forces of the lung (inward) and chest wall (outward) generate a negative intrapleural pressure in the space located between the chest wall and the lung, maintaining it in a stretched (or prestressed) state [19]. Therefore, the dynamic force transmission associated with breathing is in fact superimposed onto this static prestress. Of note, such prestress is crucial in preventing small airways from collapsing in the normal lung. The lungs can be further considered to be a freely hanging structure inside the thorax. Therefore, accounting for the presence of gravity, the upper lobes of the lung are more distended than their basal counterparts, which are mainly supported by the diaphragm [20]. As a result, if breathwork is carried out in an upright position, the upper parts of the lung, being already stretched, will appear less compliant and so less prone to air intake. In contrast, the basal regions of the lung, being less stretched initially, will deform more and fill in with more air. The result is that fresh air preferentially reaches the lower regions of the lung, yielding another source of ventilation heterogeneity, in this case at the whole organ scale. In connection to this, the onset of idiopathic pulmonary fibrosis (IPF) tends to take place in the subpleural space of the lower lung lobes [21, 22], corresponding to lung areas where alveoli are more greatly stretched during breathwork. This spatial correlation may thus suggest alveoli stretch as a trigger or driver of fibroblast activation and aberrant matrix deposition [23].

The importance of surface tension within the lungs

Finally, if we consider the remarkable structure of the lung, a number of additional physical phenomena contribute further to force transmission events. Being such a massive surface containing an air–liquid interface, the lung would be plagued with large surface tensions, especially at low lung volumes. However, pulmonary surfactant lowers surface tension as lung volume decreases, preventing alveoli from collapsing at end-expiration volumes [24]. That being said, the remaining surface tension still contributes significantly to the elastic recoil of the lung. In addition, the cells resident within the alveolar walls also exert contractile forces onto their underlying ECM. Other contractile cells that are found in the lung parenchyma include fibroblasts, myofibroblasts and smooth muscle cells. Their contractility gives rise to a mechanical and tensional interplay that will be discussed in detail in a specific section below.

Mechanics within the blood vessels

Last but not least, the blood vessels in the lung are also a remarkable mechanical structure, acting as low-resistance, highly compliant blood conduits [25]. All blood vessels bear a number of mechanical loads due to their functional nature. First, their cells undergo cyclic uniaxial stretch caused by blood pulse pressure. In the particular case of endothelial cells, fluid shear stress is also present owing to the circulating viscous blood. In the specific case of alveolar vessels, their calibre is highly influenced by alveolar pressure, with vessels undergoing compression at higher alveolar pressures [25]. In addition, the mechanical connection of alveolar vessels to the lung parenchyma subjects them to similar and substantial tissue forces. Of note, pulmonary vascular tone is also altered by hypoxia in a manner opposite to that in systemic circulation. Reductions in alveolar and arterial oxygen content cause profound constriction in the pulmonary vasculature, a response rapidly reversible when normoxia is restored [26]. Nevertheless, if the hypoxic stimulus is maintained over a prolonged period, as can occur with pulmonary hypertension or other chronic lung diseases, contraction is accompanied by extensive remodelling of the vasculature. Heterogeneities in structure, mechanical forces, ventilation and gas exchange have already been discussed

from the alveolar perspective. It is worth mentioning here that the blood vessels in the lung are able to provide compensatory mechanisms so that blood flow can match alveolar ventilation as much as possible. For example, when breathing in the upright position, the net result of forces leads to an overall increase in blood flow in the basal lobes as compared to the apical ones, thus matching the preferred destination of fresh air entering the lung [27].

How the lung mechanical environment changes in disease states

Asthma

Asthma has historically been defined as an airway disease, with airway hyperresponsiveness (AHR) as a pathophysiological trait that contributes to variable airflow obstruction, causing dyspnoea and wheezing [28]. AHR is assessed through bronchial provocation tests using methacholine or histamine. The resulting dose–response curve can be characterised by three related indices of airway responsiveness: airway sensitivity (the provoking dose or concentration that elicits a pre-specified fall in forced expiratory volume in 1 s (FEV_1), typically 20%), airway reactivity (the slope of the dose–response curve reflecting how quickly FEV_1 falls per incremental dose) and the maximal response (the plateau or highest bronchoconstriction achievable during the provocation) [29–32].

In the last decades, asthma has started to be considered an immune dysfunction caused by an abnormal reaction to stimuli (allergens, virus, airway irritants) that leads to the secretion of cytokines and the induction of an inflammatory microenvironment in the airways [33, 34]. Ultimately, this leads to major hallmarks of the disease, such as AHR and airway narrowing by bronchoconstriction. However, the pathobiology of asthma is not that simple, given that specific structural changes such as airway remodelling and mucus hypersecretion are also observed, adding additional complexity to the relationship between inflammation and airway narrowing [32].

Airway remodelling involves subepithelial fibrosis, airway smooth muscle (ASM) hypertrophy/hyperplasia, angiogenesis and altered ECM composition [35–38]. This leads to obstruction of the airway lumen, especially due to collagen deposition or fibrosis and mucus hyperplasia. It also results in changes in the compliance of the airway wall, making it less distensible and reducing the ability to expand upon breathing, which could lead to a fatal closure of the airways [32, 39, 40], although there are also conflicting reports on this phenomenon [41, 42].

Furthermore, alterations in the airway ECM, such as increased collagen I and III deposition in severe asthma [43], eventually result in increased mechanical compression of the airway epithelium [44]. This augments ASM proliferation and contractility, further contributing to the disease [32]. Indeed, the force of the ASM, arranged concentrically around the airway wall, mechanically constricts the airways, resulting in airway narrowing. In this context of abnormal bronchoconstriction, airway epithelial cells activate mechanotransduction pathways and release mediators that further increase airway remodelling [45]. The role of integrins as mechanosensors that mediate asthma disease progression has also recently been described [46].

Moreover, an “unjamming transition” in epithelial cells, a phenomenon that facilitates the migration of these cells in response to a compressive stress, further described in the section on “Importance of lung mechanics in homeostasis and injury responses”, has also been proposed as a driving factor of airway remodelling in asthma [47]. The basement membrane, the ECM layer that is located directly below the epithelial cells and provides support and regulatory signals, is thickened in airways from patients with asthma compared to controls [48, 49]. The composition and dysregulation of the basement membrane influences epithelial differentiation and regulates repair processes following injury [50]. However, its role in activating mechanotransduction pathways in asthma has not yet been investigated.

Interestingly, severe asthma has been linked to microscopic centrilobular emphysema surrounding terminal bronchioles (but not in the alveolar sacs), with loss of elastic recoil that is only noticeable with high-resolution imaging methods [49, 51]. This leads to the loss of airway-parenchymal interdependence, which means that the parenchyma cannot counteract the constrictive forces of the ASM in the airways, as happens in a healthy state.

There are some studies pointing to specific mechanisms that link mechanical forces to structural and functional changes in the ASM in asthma, such as the role of Piezo channels and Ca^{2+} regulatory proteins like stromal interaction molecule 1 in abnormal cyclic stretch conditions [39, 52, 53]. However, the precise mechanotransduction pathways by which ASM senses and adapts to chronic stretch or compressive forces

in asthma need further elucidation. This suggests possibilities for the development of drugs that target the mechanobiological pathways that mediate asthma pathophysiology.

Chronic obstructive pulmonary disease

COPD is mainly caused by cigarette smoke, pollution and indoor cooking without ventilation (especially in developing countries) [54]. In all these cases, exposure to noxious particles provokes a chronic inflammatory response in the lung, inducing airway and parenchyma remodelling, involving changes in ECM composition and in the balance between ECM synthesis and degradation [55–57]. These changes also involve a dysregulation in the expression of different lysyl oxidases (LOX), with higher LOX-like protein 1 (LOXL1) and lower LOX protein levels in the small airways of patients with COPD [58].

COPD has two distinct characteristics. In the initial stages, airway remodelling is observed in the small airways, involving airway wall thickening due to fibrosis and higher contraction of the ASM. This leads to the obstruction of the airways, which impairs breathing. Similar to asthma, airway remodelling alters mechanical forces, causing a compression of the epithelial cells, and modifies the stretch sensitivity of the ASM [6, 57]. As the disease progresses, it is also characterised by elastin degradation and subsequent loss of elastic recoil, and the enlargement of the alveolar spaces in the parenchyma, known as emphysema. In emphysema, in which the alveolar walls are disrupted, the lung volume increases. In these emphysematous regions the distribution of forces is very heterogenous, with a proportion of the remaining septal walls experiencing high tensile stresses [59]. Disorganised ECM fibres and reduced proteoglycan content contribute to the remodelling [60–62]. The mechanical forces in the emphysematous lung regions have been suggested to disrupt programmed ECM maintenance and, in so doing, drive the processes that lead to emphysema progression/pathogenesis [59]. Emphysematous regions of human precision-cut lung slices (PCLS) have stiffness ranges of 0.1–2 kPa (measured using a novel approach applying equibiaxial stretch and tracking fluorescent bead displacement at a membrane–tissue sample interface) [63], which means they are around half the stiffness measured in PCLS from control donors. The combination of both pathophysiological features results in an increase in the stiffness of the airway wall that impairs the ability of ASM to relax with bronchodilators, but a decreased overall stiffness in the lung [57].

In addition to the changes in tissue stiffness, both vascular and airway remodelling, together with mucus hypersecretion, lead to increased blood, air and mucus flow shear stress, which may damage endothelial cells in blood vessels and airway epithelial cells [64–68]. Moreover, the loss of elastic recoil in lung parenchyma increases the likelihood of non-physiological stretch in the lungs, including abnormal stretching frequency [57]. Furthermore, lower elasticity leads to lung hyperinflation due to an imbalance between inspiratory and expiratory mechanics that results in the entrapment of air in the enlarged alveoli. This aspect has been correlated with a higher risk of dyspnoea, exacerbations, cardiovascular disease and lung cancer, among others [69]. However, the effect of mechanical stretch from hyperinflation on cell senescence, an important observation in various cell populations in COPD lungs [70, 71], and emphysema progression is an open question. Overall, a pathological mechanical stretch in COPD worsens inflammation and changes cellular behaviour by affecting cell shape and adhesion [72, 73]. Further research is needed to fully understand mechanotransduction in COPD and propose novel therapeutical targets.

Pulmonary fibrosis

Pulmonary fibrosis is a type of interstitial lung disease that includes several chronic lung disorders, such as IPF, one of the most studied forms of lung fibrosis without a known cause [21, 74]. The common characteristic of all fibrotic lung diseases is the increased deposition of collagens and the consequent scarring of the parenchymal lung tissue [74–77]. The degree of crosslinking of ECM proteins (*e.g.* collagens) is also higher in fibrosis, where the expression of different members of the LOX family, such as LOXL1 and LOXL2, is increased [78, 79]. From a mechanical perspective, all these changes raise the stiffness of the lung, from around 1 kPa to >10 kPa (reported in studies using atomic force microscopy [80, 81]). The changes in stiffness of the parenchymal regions of fibrotic lungs are very heterogeneous, but these changes significantly affect the mechanics of breathing, resulting in the lung being prone to experiencing non-physiological stresses during normal breathing and mechanical ventilation. This results in a restrictive lung disease in which alveoli cannot expand upon breathing, with extremely impaired lung function and gas exchange. There is increasing evidence pointing towards an association between non-physiological mechanical forces experienced by the parenchymal tissues in fibrotic lungs and abnormal mechanotransduction, which may lead to progression of pulmonary fibrosis. The reader is referred to [23] for further discussion of these concepts.

In IPF, increased lung stiffness influences cellular mechanotransduction and progression of the disease through a positive feedback loop [75, 82–84]. For instance, a rigid interstitial matrix induces the activation

of fibroblasts to myofibroblasts [85, 86], where Yes-associated protein (YAP) signalling in the Hippo pathway plays a key role (see "Fibroblast responses to mechanics" section) [87]. Moreover, increased pulling of the ECM by cells, mainly fibroblasts, promotes the release of latent transforming growth factor-β (TGF-β), which triggers the synthesis of collagens, further contributing to the feedback loop [88, 89]. Alveolar epithelial cells may also become affected by the increased rigidity, undergoing epithelial–mesenchymal transition (EMT), a phenomenon also observed in cancer that contributes to tissue fibrosis [90, 91]. Whether this process is active in lung fibrosis is controversial [92]. In this regard, the higher mechanical tension has been shown to activate TGF-β signalling in alveolar type II epithelial cells (AT2) [93, 94].

In addition to remodelling of the parenchymal lung tissue, IPF often involves impaired mucociliary clearance, leading to an overaccumulation of mucus especially in the small airways. This is associated with retention of injurious particles and resulting persistent injury and repair cycles [95]. Two known contributors to mucociliary dysfunction in IPF include overproduction of the mucin MUC5B and acidification of the mucus *via* altered proton transport in the small airways [96]. While more research is needed to understand how IPF progression is affected by mucociliary dysfunction, it has been recognised as a potential driver and not merely a bystander of the disease [97].

The emergence of an increasing number of *in vitro* models that mimic the increased tissue stiffness and enable investigation of cellular mechanotransduction pathways observed in pulmonary fibrosis has allowed some elucidation of mechanisms underlying the disease [85, 98]. However, there is an increasing need for more complex models that can recapitulate features of fibrosis, including the altered three-dimensional (3D) mechanical, compositional and topographical microenvironment, to contribute to the discovery of drugs that target mechanobiological pathways to stop disease progression [99, 100]. Furthermore, while some mutations in surfactant proteins and telomerase genes influence fibrosis risk [101], the interplay of these underlying factors with tissue mechanics has been poorly characterised.

Lung cancer

Lung cancer is the main cause of death among cancer-related deaths worldwide and it is mainly caused by mutations induced by cigarette smoke in lung cells in airways and/or parenchyma [102]. While the cause is genetic, changes in the mechanical properties in the ECM in the tumour microenvironment (TME) strongly contribute to the progression of solid tumours [103, 104]. The excessive growth and proliferation of cancer cells can lead to compression of the tissue, inducing the activation of fibroblasts to cancer-associated fibroblasts (CAFs) within the TME [5, 105–107]. CAFs synthesise larger amounts of collagens and increase their degree of crosslinking compared to normal fibroblasts, resulting in an overall increase in TME stiffness, which promotes cancer growth [108, 109]. This is mediated by the Hippo/YAP pathway in mouse models of lung cancer [110]. In addition, a recent study has shown that activating transcription factor 5 is activated by ECM stiffening and promotes the proliferation of lung cancer cells [111].

EMT has also been described as a hallmark of lung cancer, for instance related to the enhancement of Snail1, a transcription factor involved in this process [112]. However, it has been reported that TME stiffness drives cancer cell migration through adhesion signalling pathways rather than through EMT [113]. Moreover, stretching has also been proposed to activate cation channels in lung adenocarcinoma cells, which may increase their metastatic capacity [114]. Integrin α11 deficiency also reduces tumour growth in A549 adenocarcinoma cell cultures, highlighting the importance of mechanotransduction pathways in cells other than CAFs [115]. YAP has been implicated in the resistance of tumour cells to chemotherapeutic drugs and in immune evasion, which stresses the importance of TME stiffening [116]. However, the complex interactions and molecular crosstalk underlying the mechanobiology in lung tumorigenesis are still unclear [117].

A note on mucus

Adding to the profound mechanical remodelling of respiratory tissues, mucus transport from the airways is often impaired in chronic respiratory disease, including COPD, IPF, asthma, cystic fibrosis and many more. While the clinical symptoms and aggravating factors, such as mucus plugging, are often shared between different pathologies, the underlying mechanical mechanisms are typically disease-specific, and we refer the interested reader to comprehensive reviews on the topic [64, 118].

To provide an example, while both COPD and IPF promote mucus plugging, the underlying mechanical mechanisms differ. In COPD, this is thought to result from a combination of increased mucus secretion, mucus dehydration and altered mucus composition [118, 119], in particular increased levels of MUC5AC relative to MUC5B [120, 118], which reduces mucus flowability [121]. By contrast, mucus plugging in

IPF is associated with not only increased production of mucus, but also impaired function of cilia [122], and an overall shift towards increased MUC5B production relative to MUC5AC [95], which can lead to uncoordinated mucociliary transport [121].

Both sets of factors favour the formation of hyperconcentrated mucus plugs and the associated occlusion of the small airways, which is associated with faster lung function decline and early death in COPD patients [123–125]. Emergent evidence suggests that mucus plugs are not only mechanical obstructions but also active drivers of pathogenesis [126, 127]. While disease-specific mechanisms remain understudied, mucus plugs are thought to generally aggravate disease progression by fundamentally changing the microenvironment. For example, the underlying airway epithelial cells may experience limited access to oxygen, hence driving epithelial hypoxia and associated epithelial necrosis [128]. Moreover, macrophages trapped in the thickened mucus release interleukin-1β, which further stimulates mucin secretion [129], and mucus plugs can also exacerbate microbial infection and chronic inflammation [130]. This chronically inflamed, hypoxic microenvironment created by the mucus plug may even promote the development of lung cancer in patients with COPD [131]. Taken together, the mechanical blockage by mucus plugs is likely to be an active driver of pathogenesis in many respiratory diseases.

Overarching role of mechanics in lung disease

While the detailed discussion above provides a glimpse into the complex roles that mechanics play in major respiratory diseases, most, if not all, lung pathologies are shaped by mechanical changes that cause debilitating symptoms and often even drive disease progression. To highlight this fact and encourage further exploration by the reader, we compiled a summary of a diverse range of lung diseases in which mechanobiology is known to contribute to disease pathology (table 1). A key question for the field for the future will be defining exactly "what is the role of mechanics in the onset, development and progression of chronic lung diseases?"

Sex perspective in chronic lung diseases

Sex differences in the prevalence and risks of chronic lung diseases are mainly influenced by the different anatomical features of male and female lungs and hormonal diversity between the sexes [154, 155]. For instance, in asthma, there are clear differences in the pathogenesis (oestrogen and progesterone increase airway responsiveness and inflammation as compared to testosterone), severity (higher morbidity and mortality in women) and treatment (women are less likely to receive preventive therapies) [156]. However, it is not known whether and how sex can affect the disease from a mechanobiological point of view.

In the case of COPD, while it has historically been seen as a male disease due to smoking trends in the 20th century [157–159], its prevalence is now higher in females [160, 161]. Considering that women smoke less, this statistic suggests that women are more susceptible to smoke-related COPD [162, 163]. This could be due to anatomical differences, given that women have smaller airways and there is a higher concentration of deposited smoke particles per unit of area, in comparison to men [157, 164–166]. Such an effect has been confirmed in mice exposed to cigarette smoke, where there was an increase in small airways disease in female animals. However, male mice showed a higher degree of emphysema, and it has been suggested that oestrogen protects against this pathophysiological feature of COPD [167–169]. Interestingly, males have larger lungs, which may contribute to the increased prevalence of emphysema from a mechanobiological perspective, although this theory requires further investigation. Overall, women experience a faster loss of FEV_1 [170], have a higher risk of severe dyspnoea and exacerbations [171–173], and increased hospitalisation and mortality rates [174]. Moreover, women have a higher risk of early onset COPD and are more likely to develop non-cigarette COPD [175, 176]. Nonetheless, there is no evidence that supports the use of sex-specific pharmacological and non-pharmacological treatments for COPD [160].

Regarding sex differences in pulmonary fibrosis, male sex confers a 1.5–2-fold higher risk compared to female sex, partly due to exposures such as smoking and asbestos. This includes both the risk of death and occurrence of lung transplantation [74, 177–180]. In addition, preclinical models have shown that male mice develop more severe fibrosis compared to female mice upon bleomycin challenge [181]. However, how the altered ECM stiffness in pulmonary fibrosis interacts with sex differences is unknown. A recent study explored the potential of a dynamic 3D model using cells and sex hormones from female subjects to explore how responses to microenvironmental stiffness may explain a sex dimorphism in susceptibility and survival in pulmonary hypertension [182]. Sex differences in drug-induced modulation of matrix stiffness (*e.g.* antifibrotics) have not been systematically studied.

Lung cancer risk also differs by sex, with women showing a higher susceptibility to tobacco carcinogens and more frequent p53/KRAS mutations, with nearly triple odds at equivalent smoking exposure [183, 184].

TABLE 1 Overview of respiratory diseases with known contributions of mechanics to the pathology

Respiratory disease	Primary region	Example roles of mechanics in pathology	Key reference
Asthma	Airways	Bronchoconstriction triggers remodelling (goblet cell hyperplasia, subepithelial fibrosis) and airway smooth muscle cell proliferation, further aggravating compressive stresses	[39, 132, 133]
COPD/emphysema	Parenchyma+ small airways	Protease-driven matrix failure lowers recoil; stress redistribution causes progressive alveolar wall failure and airspace enlargement	[57, 134, 135]
Idiopathic pulmonary fibrosis	Interstitial/ parenchyma	Stiffer ECM activates fibroblasts and perpetuates fibrosis *via* mechanotransduction	[136]
Lung cancer (NSCLC)	Tumour stroma	Increased collagen stiffness of NSCLC tumour stroma increases tumorigenicity and metastatic potential	[115]
Acute respiratory distress syndrome	Alveoli/interstitial	Reduced surfactant levels increase alveolar surface tension, which is assumed to cause alveolar instability and collapse	[137]
Pulmonary arterial hypertension	Pulmonary vasculature	Increased fluid shear stress induces the stiffening of pulmonary vascular ECM, which drives endothelial dysfunction and smooth muscle proliferation/remodelling	[138]
Chronic thromboembolic pulmonary hypertension	Pulmonary vasculature	Obstruction increases shear and pressure and promotes microvasculopathy distal to thrombi	[139]
Cystic fibrosis	Airways	Hyperconcentrated mucus dehydrates and compresses the periciliary “brush”, stalls ciliary beat and increases mucus plugging risk	[140]
Non-cystic fibrosis bronchiectasis	Airways	Excessive sputum and airway dilation reduce clearance, enabling an infection–inflammation–remodelling cycle	[141]
Primary ciliary dyskinesia	Airways	Defective cilia mechanics lead to stasis, infection and downstream airway remodelling/bronchiectasis	[142]
Pulmonary alveolar proteinosis	Alveoli	Surfactant accumulation in the alveoli physically obstructs airflow, impairing gas exchange	[143]
Lymphangioleiomyomatosis	Parenchyma+ airways	Abnormal smooth muscle-like cells weaken ECM and constrict airways, leading to airflow obstruction, trapping and cyst formation	[144]
Bronchopulmonary dysplasia (neonatal)	Developing lung	Cyclic stretch disrupts septation and ECM assembly, causing simplified alveoli and altered mechanics	[145, 146]
Hypersensitivity pneumonitis (fibrotic form)	Interstitial	Chronic antigen exposure plus stiff ECM biases towards fibroblast activation and fibrosis	[147]
Interstitial lung diseases	Interstitial	Stiffness cues push fibroblast lineage towards myofibroblasts, sustaining fibrosis independent of inflammation	[148]
Silicosis/pneumoconiosis	Interstitial	Increased stiffness in silicotic lungs activates fibroblasts, driving immune cell activation associated with disease progression	[149]
Pneumonia/COVID-19 (viral)	Alveoli	Infection of surfactant producing alveolar type II cells leads to surfactant deficiency and hence increases the risk of alveolar collapse	[150]
Alpha-1 antitrypsin deficiency emphysema	Parenchyma	Elastin degradation reduces recoil and increases mechanical strain during the respiratory cycle, accelerating alveolar wall failure and emphysema	[151]
Surfactant protein deficiencies (SFTPB, SFTPC, ABCA3)	Alveoli	High surface tension collapses alveoli and magnifies shear during reopening, causing atelectasis and injury	[152]
Congenital trachea/bronchomalacia	Large airways	High collapsibility causes flow-limitation, air-trapping and ineffective cough, predisposing to poor mucus clearance and recurrent infections	[153]

For brevity, only selected mechanisms are listed. ABCA3: ATP binding cassette subfamily A member 3; ECM: extracellular matrix; NSCLC: nonsmall cell lung cancer; SFTPB/C: surfactant protein B/C.

Moreover, environmental exposures like indoor pollutants disproportionately affect women [185], and hormonal influences, particularly oestrogen, modulate carcinogenesis, potentially promoting lung cancer growth [186]. Treatment outcomes are mixed: women experience higher chemotherapy toxicity but equal or superior responses; immunotherapy benefits vary, with men responding better to checkpoint inhibitors alone and women to combination treatments [183]. Despite these disparities, women, particularly older and minority groups, are under-enrolled in research, limiting tailored guidelines [183].

In summary, there is a clear knowledge gap in the effect of sex differences in mechanotransduction pathways, and how these affect the observed dissimilarities in, *e.g.* prevalence and mortality, for different chronic lung diseases.

Effects of ageing on pulmonary mechanics

Ageing is a major driver of cellular senescence in the lung, a hallmark characterised by cell-cycle arrest and a unique autocrine and paracrine secretory phenotype that leads to dysregulation of ECM homeostasis [187]. Ultimately, this results in structural changes, overall decreased lung function and a greater predisposition to pulmonary diseases [187, 188]. The concept of fibroageing, a tendency to develop tissue fibrosis in connection with ageing, has been suggested as a potential mechanism related to chronic lung diseases [189]. With regard to the ECM, aged lungs show increased fibril crosslinking, especially of collagen, which increases stiffness (*i.e.* lowers mechanical compliance) and reduces elasticity/elastic recoil [190]. Moreover, collagen synthesis and degradation decrease with age, pointing to its accumulation, and the mean thickness of collagen fibres in the septal walls is reported to increase from 0.97 µm at 20 years to 1.17 µm at 80 years old [190, 191]. Similarly, elastic fibres become wider (0.97 to 1.27 µm) and their lysine content increases with age, allowing a larger degree of crosslinking [192]. At the same time, elastin becomes more sensitive to proteolytic digestion, which supports the view of ageing as a minor version of emphysema [193].

More recent studies have studied ECM changes in the ageing lung in more depth. For instance, Koloko *et al*. [194] performed a comparative analysis of transcriptomic and proteomic data, showing a higher expression of seven ECM proteins in aged human peripheral lung tissue, including collagen (COL)1A1, COL6A2, COL14A1 and lumican (LUM). These data were complemented with extensive immunohistochemistry of the selected lung ECM proteins in specific tissue compartments. Their findings show that the higher protein levels are region specific, with COL6A2 increased in whole tissue, parenchyma, airway wall and blood vessels; COL14A1 and LUM in bronchial epithelium; and COL1A1 in lung parenchyma [194]. In another study, Ulldemolins *et al*. [195] found that laminin was decreased in aged mice compared to young controls, while fibronectin and COL1 were increased. A similar result regarding higher fibronectin and COL1 levels was found in pulmonary artery smooth muscle cells obtained from aged donors compared to younger ones [196].

Disruption of ECM homeostasis in ageing lungs leads to alterations in biomechanics, including tissue stiffness. To study this effect, Sicard *et al*. [196] used atomic force microscopy to measure the mechanical properties of airway and parenchymal regions of normal lungs from donors ranging from 11 to 60 years old. They observed a higher stiffness in vessels (3.6% per year increase) and parenchyma (1.8% per year) in subjects 41–60 years old compared to those 11–30 years old, which is in line with the accumulation of collagen and elastin and a higher degree of crosslinking. Interestingly, Ulldemolins *et al*. [195] found that the mechanical properties of mice lung slices measured by atomic force microscopy differ depending on how they were inflated upon collection, either to reach residual or functional volume. They observed that aged mice lungs were stiffer in samples prepared in functional volume inflation conditions compared to young mice, but there was an opposite trend for residual volume samples, highlighting the need for standardised measurements when possible but certainly clear methodological descriptions to enable comparison across the data in the field. In general, although different measurements may give varying stiffness values, most studies suggest stiffer tissue in aged compared to young lungs [190].

Finally, alterations in the biomechanical properties with ageing lead to lung function impairments. Early works have reported several changes, including a fall in static recoil pressure due to changes in elasticity, which results in an increased functional residual capacity and a decrease in FEV_1/force vital capacity ratio [193, 197]. Overall, older adults are more vulnerable to ventilatory failure during high demand states (heart failure, pneumonia, physical exercise, *etc*.) and to poorer outcomes [198].

Importance of lung mechanics in homeostasis and injury responses: interplay of cell types in the lung and response to mechanics

Mechanical forces are essential to lung biology throughout life, even prenatally. During development, the branching morphogenesis of the lungs is regulated by the relative pressure of the fluid within the lumen of the lung. The so-called transmural pressure induces retinoic acid signalling that modulates the relative rates of epithelial growth and smooth muscle differentiation, which in turn controls branching rate [199]. Intriguingly, lung region-dependent pressure differences may also contribute to the proximal-distal patterning of the epithelium *via* differential retinoic acid signalling [199]. Activity of contractile cell types also contributes to lung development. Contraction of smooth muscle wrapped around the stem of budding lung tubes is essential for the terminal bifurcation of the epithelium during normal branching morphogenesis [200]. Furthermore, contractions of interconnected, “fishnet”-forming myofibroblasts drive the formation of secondary septa in the development of alveoli, subdividing alveolar saccules, which increases the gas-exchange surface area [201].

After birth, normal breathing imposes cyclic stretch on the airways and alveoli, which is crucial for lung homeostasis. The lung's ECM provides an elastic scaffold that transmits these mechanical forces and, with them, information on the microenvironment, to resident cells. For example, lung fibroblasts normally sense these mechanical cues and replace or reorganise ECM proteins in response, preserving the structural and mechanical properties of alveoli and airways, which in turn regulate fibroblast activity in an intrinsic feedback loop [202]. Cyclic stretch is also a physiological trigger for surfactant release by AT2 epithelial cells, which reduces surface tension and prevents collapse of alveoli [203].

In addition to stretching, lung tissue is also subject to fluid shear stresses from blood and air flow. Airway epithelial cells show accelerated differentiation and planar polarisation in response to combined cyclic stretch and air flow [204]. Pulmonary capillary endothelial cells, which together with the epithelium form the alveolar–capillary barrier, experience blood shear stress, which regulates the endothelial phenotype, exerting anti-inflammatory and anti-thrombotic effects and maintaining barrier integrity [205]. A less well understood mechanism is the so-called unjamming transition by airway epithelial cells in response to mechanical compression, such as during bronchoconstriction, wherein cells switch from the typical tightly "jammed" arrangement to collective migration and swirls reminiscent of fluid flow [206]. In contrast to cells undergoing EMT, unjammed cells maintain cell–cell junctions, apico-basal polarity and barrier function, possibly activating regenerative programmes [207].

In addition to cell-specific responses, mechanical signalling between different lung cells and tissues is a key aspect of homeostasis. For example, bronchial epithelial cells stimulated by mechanical stress release mediators that can activate both unstimulated fibroblasts, eliciting matrix remodelling [208], and unstimulated ASM, triggering proliferation and contraction [209]. Different lung tissues are also mechanically coupled. As the lung expands on inspiration, the alveolar walls pull the airways apart by "radial traction" *via* connective tissue attachments [210]. Deep inspirations can stretch ASM to extend by 25–30%, which reduces muscle tone and dilates the airways [211]. Taken together, physiological mechanical forces (stretch, matrix elasticity and shear flow) regulate epithelial, mesenchymal and endothelial cell responses, while keeping the airways and alveoli open for gas exchange.

Mechanical forces also play a major role in the lung's responses to injury. Loss of epithelial integrity or ECM continuity alters the local tissue mechanics, which surrounding cells can detect as a cue for the need to repair. For example, epithelial cells can sense a wound site *via* the disruption of the epithelial sheet force balance [212, 213], and free edges trigger increased epithelial motility [214]. These mechanisms likely underlie the ability of AT2 epithelial cells to proliferate after injury and differentiate into AT1 cells to re-epithelialise denuded areas, a process critical for alveolar repair [215]. Similarly, airway epithelial cells respond to injury and free edges with extensive cell spreading and migration to cover the denuded area [216]. Fibroblasts are also highly mechanosensitive "first responders" in wound repair. They normally exist in a quiescent state maintaining the ECM, but tissue injury or stress (*e.g.* tension at the wound edge) stimulates fibroblasts to proliferate, migrate and differentiate into myofibroblasts [217]. Myofibroblasts produce new ECM and generate contractile forces, effectively pulling injured tissue edges together [218]. Extensive crosstalk between epithelium and fibroblasts plays an important role in wound healing and maintaining lung homeostasis, especially with respect to ECM deposition and associated matrix mechanics [219]. However, repeatedly damaged lung epithelium may trigger fibroblast activation and drive the initiation of fibrosis, which causes further epithelial damage in a vicious cycle [219]. Taken together, lung cells use mechanical forces both to sense injury and to initiate the healing response; derailments of these processes may also underlie major lung diseases. The question "how are cellular responses in health and disease altered by the biomechanical environment?" will be a key focus for future research in the field of lung disease.

Cellular responses to their mechanical niche

Cells interact with the ECM, the scaffold that supports them within lung tissue, through various cell surface receptors (figure 3a). Of these receptors, the most well-recognised class of receptors is the integrins. These heterodimeric receptors consist of an α and a β subunit, of which to date 18 α subunits and eight β subunits have been identified, with β1 (CD29) identified in almost every cell in the body. The 24 different combinations of α and β subunits enable specificity for binding integrins to different ECM components *via* defined short amino acid combinations (*e.g.* RGD (fibronectin-binding) and GROGER (collagen-binding)) [220–223]. Upon sensing a mechanical force or ligand, the inactive integrin receptor undergoes a conformational change to an active state that can then interact with the ligand [6, 224–226]. Extracellular or intracellular forces result in integrins clustering with similar molecules or growth factor receptors, which leads to the recruitment of cytoplasmic proteins to the short intracellular tail of the integrin complex. This clustering of mechanoresponsive proteins, including vinculin, talins, lamin,

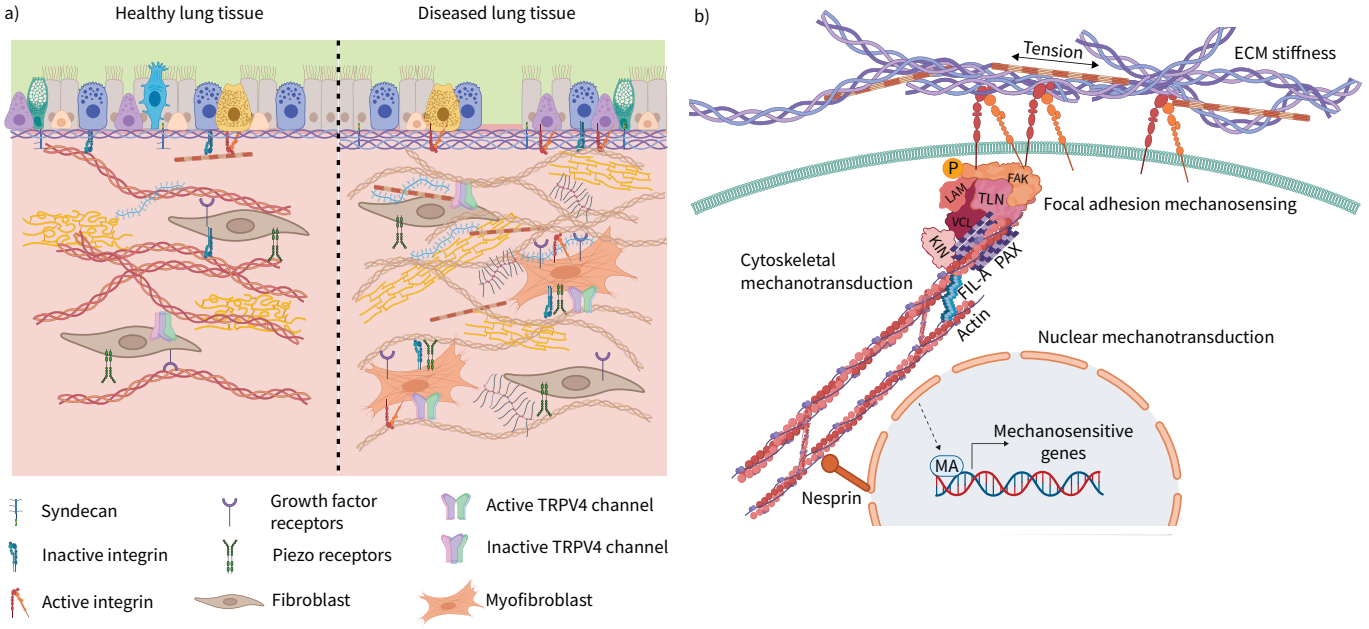

FIGURE 3 Cell–extracellular matrix (ECM) interactions for sensing biomechanical signals. a) Schematic representation of the organisation of the ECM in healthy and diseased lung tissues, showing the epithelium and the underlying connective tissue layer. Epithelial cells are connected to the basement membrane through a variety of cell surface receptors, including integrins. The basement membrane anchors to the interstitial matrix through a variety of collagen fibrils, including collagens VI and VII (not shown). The main collagen type in the interstitial matrix are the heterotypic fibrils of collagens I, III and V. Short leucine rich repeat proteins and fibronectin participate in collagen assembly and fibrillogenesis. Many other components contribute in interstitial matrix organisation, including elastin, proteoglycans and hyaluronic acid. Cell surface receptors of stromal cells such as integrins, syndecans, mechanosensitive channels and growth factor receptors interact with ECM components and growth factors. The composition of the interstitial matrix is altered in diseased lung tissues, influencing the bioavailability of epitopes for stromal cell surface receptors to bind, the number and density of which are also altered in fibroblasts and myofibroblasts in this environment. FAK: focal adhesion kinase; FIL-A: filamin-A; KIN: kindlin; LAM: lamin; MA: mechano-activation; P: phosphorylation; PAX: paxillin; TLN: talin; TRPV4: transient receptor potential vanilloid-4; VCL: vinculin. Figure adapted from [228] and [229]. Figure created in BioRender (Burgess, 2025).

kindlins, adapter proteins like paxillin, actin linker proteins such as filamin A, and signalling molecules including focal adhesion kinase (FAK) forms a focal adhesion complex. Once the focal adhesion matures, it acts as a conduit that is important for transmission of the forces in both directions between the cell surface and the nucleus, *via* cytoskeletal protein networks and signalling pathways, referred to as mechanotransduction (figure 3b) [224, 227, 229].

The majority of our understanding of cell sensing of mechanical signals comes from two-dimensional (2D) culture systems, involving cells grown on surfaces of tuneable stiffness. After binding of the integrin complex and maturation of the focal adhesion complex when cells are on a stiff surface, actomyosin contraction, FAK and the Rho signalling pathway are activated. Mechanical activation of talin, vinculin and lamin influences their activation and interactions, thereby converting mechanical to biological signals. Tensioning of stress fibres within the cell leads to deformation and stretching of the nucleus, which opens pores and allows translocation of dephosphorylated YAP into the nucleus (part of the Hippo signalling pathway), which regulates transcriptional responses.

Another family of cell surface proteins that are now recognised as playing an important mechanosensory role in the lung are mechanosensitive ion channels. Through stretch-regulated activity, these proteins enable transduction of cellular secondary messengers such as calcium (Ca^{2+}). This is a structurally diverse superfamily in mammalian cells, including transient receptor potential vanilloid (TRPV) channel proteins (particularly TRPV4) and Piezo channels [230–234]. The sensing of extracellular cues, including mechanical forces, by TRPV4 or Piezo family receptors results in structural rearrangement and opening of the channels, which triggers Ca^{2+}-influx, leading to activation of various intracellular signalling cascades in a spatiotemporal manner. The functional impact of activation of TRPV4 appears to be cell-type-specific, with important regulatory roles being reported in epithelial, endothelial and mesenchymal cells in the lungs [6, 231, 232, 235]. For an overview of the impact of TRPV4 mechanosensing on ECM remodelling, the

reader is referred to [235]. Piezo channels have also been recently recognised as key drivers of mechanical signal transduction in respiratory cellular signalling [230]. Recent reports have identified crosstalk between Piezo and FAK signalling cascades in response to biomechanical stimuli [232].

Constituents of mechanosensitive pathways are altered in chronic lung diseases [6]. The expression and activity of integrins involved in cell–ECM interactions are altered in different lung diseases, where they switch to a more activated and clustered state as the matrix stiffens, amplifying mechanotransduction pathways through the Hippo pathway and activating latent TGF-β [236, 237]. In IPF, there is an upregulation of $\alpha_v\beta_6$ integrins in alveolar epithelium, which, together with $\alpha_v\beta_1/\beta_3/\beta_5/\beta_8$ activation, both in the mesenchyme and in the epithelium, leads to the activation of TGF-β and an increase in ECM secretion and stiffening [236, 238]. In asthma, $\alpha_5\beta_1$ integrins are the main fibronectin-binding receptors in ASM, and blockade of the $\alpha_5\beta_1$-fibronectin interaction ameliorates severe AHR [239]. Given the role of integrins in pathological mechanosensing, they have been proposed as therapeutic targets to treat fibrosis [240]. FAK has also been suggested as a potential therapeutic target downstream of integrin signalling owing to its strong activation in fibrosis leading to a positive feedback loop of activation-ECM stiffening, which is also reported in other diseases like COPD and asthma [241]. Mechanosensitive ion channels are also dysregulated in lung diseases [242]. Specifically, PIEZO1 activation upregulates TGF-β expression and subsequent EMT in epithelial cells, suggesting a role in lung fibrosis [243]. As regards TRPV4, it can promote myofibroblast differentiation, with a link to fibrosis [244].

2D versus *3D mechanotransduction*

Cells in a 3D environment experience different forces than those in a 2D environment (figure 4). In two dimensions, cells interact with the ECM in only one direction, giving them polarity, while in three dimensions they can interact in all directions. Recent studies have suggested that the formation of focal adhesions may also be different in 2D *versus* 3D models, dependent upon the nature of the hydrogel used, with less distinct adhesive complexes observed in 3D models. The confinement of the cells is greater in a 3D environment, potentially altering the cell signalling responses. Cell-generated forces (contractile forces, protrusive forces and cell volume change-related forces) are all relevant in the 3D environment but not to the same extent in the 2D environment. Some of these forces can be sensed by neighbouring cells at a distance from the cell of origin [246]. The range for sensing of such signals is dependent on multiple factors that include the state of the ECM, the geometry, orientation of the cells and their coupling to the matrix, but can be up to at least five cells diameter distance in the ECM [247]. For an excellent overview of the current understanding of mechanotransduction in 2D and 3D environments, the reader is referred to [245].

When considering the lung cellular environment, it is important to consider the natural environment for the cell being studied. Epithelial and endothelial cells are naturally polar, interacting with their basement membrane only *via* one surface, while having interactions with other cells through their junctional proteins. By contrast, mesenchymal cells that reside within the airway, alveolar and vessel ways and interstitial regions of the tissue are surrounded by ECM, which they interact with on all sides. Cells that naturally reside in the tissue are dependent upon connections with the ECM for their survival. When there is disruption of their cell–matrix adhesion, anoikis (a form of programmed cell death) ensues. Cells that are resident in a stiffer matrix environment, such as in fibrotic tissue (*e.g.* in IPF or around a tumour) appear to develop resistance to anoikis [248, 249]. Such cell adaptation, *i.e.* a response to adherence to an altered ECM, has been suggested to enable resistance to adverse effects usually induced through exposure to chemotherapeutic and other agents or radiation [250–252]. Therefore, the forces experienced by cells will differ under different conditions and should be considered when exploring mechanotransduction.

Cellular responses to mechanical properties

With breathing, the lung volume increases, elicited through the unfolding of pleats in epithelial layers of the alveoli. This increase in surface area exerts stress and strain on the components of the ECM. These stresses are transmitted to stress-bearing components within cells *via* the cell–matrix and cell–cell contacts. At higher lung volumes, it has been suggested that epithelial and endothelial cells, *via* their attachment to their basement membranes, start bearing stresses in their cytoskeleton and plasma membranes [253]. When cells contract, they can also alter the local tension exerted on ECM fibres, although this change in force is not considered to be large [254–256].

Cellular mechanical forces in the lung can be influenced by major forces exerted at the organ or tissue level, including tension, compression, bending and shear [100]. These forces can result from fluid flow, constriction and/or tissue stretch or occur within a cell through active rearrangement of the cytoskeleton. Cells also experience mechanical forces elicited by the stiffness of the material (tissue ECM scaffold) that

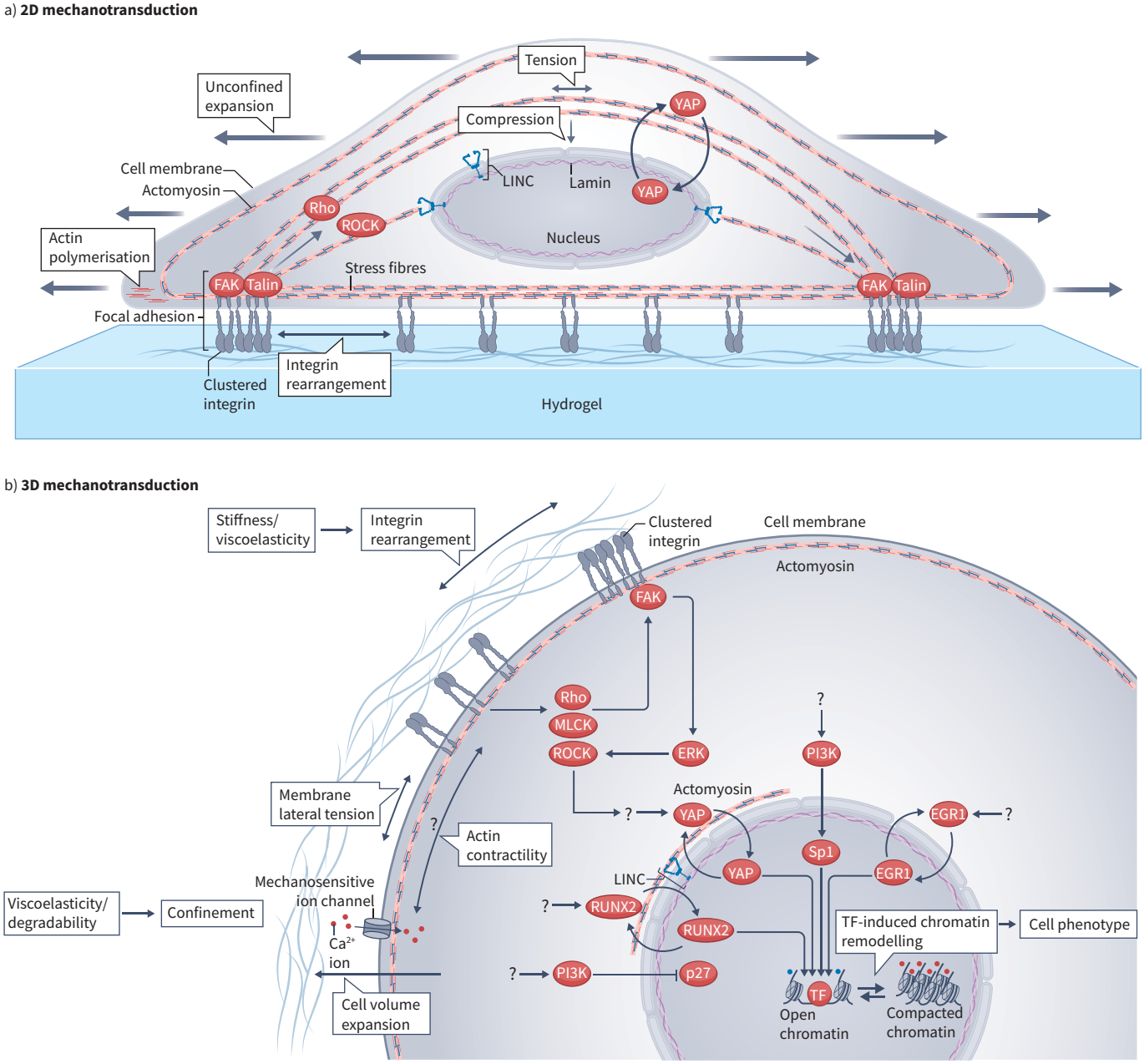

FIGURE 4 Mechanotransduction in two-dimensional (2D) and three-dimensional (3D) environments. a) Cells sense substrate stiffness by exerting contractile forces on 2D substrates with stress fibres through focal adhesions, which activates various proteins, such as focal adhesion kinase (FAK), talin, Rho and Rho-associated coiled-coil containing protein kinase (ROCK), at the adhesion site. Activation of these proteins leads to adhesion maturation and stress fibre formation and contractility, which in turn transmits forces to the nucleus *via* the linker of nucleoskeleton and cytoskeleton (LINC) complex, resulting in changes in nuclear envelope tension and nuclear pore opening. This allows the nuclear entry of proteins such as the transcription regulator Yes-associated protein (YAP), leading to a downstream impact on cell phenotype. Moreover, in a 2D model, a cell can spread laterally without encountering any mechanical confinement. b) Cells embedded in the extracellular matrix (ECM) sense stiffness and viscoelastic properties of the matrix through integrin binding, activation and clustering, while sensing confinement, viscoelasticity and plasticity through cell volume changes and ion channel activation, which leads to Ca^{2+} ion influx. Additionally, ECM stiffness/viscoelasticity and confinement regulate activation of various proteins, such as FAK, ROCK, myosin light chain kinase (MLCK); pathways, such as those involving phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) and Rho; and transcription regulators, such as YAP, p27, Sp1, Runt-related transcription factor 2 (RUNX2) and early growth response 1 (EGR1). However, clear mechanistic links between the ECM properties and activation of these proteins, pathways and transcription regulators remain unclear. Unknown connections in the pathways are indicated by question marks. Both mechanisms of mechanotransduction converge on the nucleus and regulate the activation of transcription factors (TFs), which are facilitated by chromatin remodelling and control cell behaviour. Reproduced from [245] with permission.

they are in contact with and the viscoelasticity of these materials [257–260]. Stiffness (Young's modulus) is defined as the resistance of a material to deformation, while viscoelasticity (stress relaxation) reflects how quickly a material rearranges to dissipate a force that has been applied upon it.

Cellular responses to stiffness

ECM stiffening, as occurs in areas of fibrotic deposition during tissue remodelling in all of the above-described lung diseases, can establish a feedback loop in which the cells respond to the increased tissue stiffness by enhancing their ECM production and deposition, further stiffening the tissue environment. Many different cell types respond to changes in stiffness (within the range of pathological stiffening in the lung) by increasing ECM gene expression, production, deposition and remodelling [80, 84, 261, 262]. When compared to stiffness of physiologically normal tissues, pathological stiffness can induce many characteristics of activated mesenchymal cells, including proliferation, differentiation, migration, contractility and resistance to apoptosis [263–267]. More recently it has emerged that the stiffness of the ECM also has a regulatory role in cellular inflammatory and metabolic states [268–271]. The stiffness of the ECM in the TME also enhances cancer cell metastatic capacity, progression and therapy resistance [90, 109, 272–275].

Altering stiffness, either dynamically or by seeding mesenchymal cells in a matrix of a different stiffness to that of their origin, can change the cellular response to the environment [276, 277]. Increasing stiffness readily leads to cell activation; in some cases, matrix softening can partially reverse the activation state of the cells [82, 278–280]. However, cells also display mechanomemory [84, 281, 282], with fibroblasts from donors with IPF showing contrasting results and not always losing their activation state when exposed to environments of greater physiological stiffness [84, 283].

Cellular responses to viscoelasticity

In addition to stiffness (elasticity), the viscoelasticity of a material in which a cell is embedded independently influences their biological responses [284–286]. An environment with a higher rate of (*i.e* faster) stress relaxation enables greater cell spreading and proliferation of mesenchymal stem cells. The viscoelasticity also influences the differentiation potential of these cells [287]. Fibroblasts demonstrate enhanced spreading on substrates that have stress relaxation in comparison to those with the same elastic modulus (stiffness) but no stress relaxation (no viscous component) [288]. Fibroblasts and tumour cell line cells also elongate, migrate and proliferate to a greater extent when in an environment with a higher stress relaxation amplitude [289, 290].

The lung is a viscoelastic organ, with a unique stress relaxation profile compared to other organs [291]. The composition and organisation of the ECM are important for establishing the viscoelastic properties of the tissue. Alteration of these properties in chronic lung diseases impacts the viscoelastic stress relaxation capacity of the tissue [81, 291]. The impact of the altered viscoelasticity of the ECM environment in chronic lung diseases, although likely to have significant consequences in their underlying mechanisms, has not been examined to date.

Cellular responses to topography

The micro- and nanoscale features of the ECM, which are primarily generated from the fibrous proteins but can also be influenced by the matricellular proteins dictating the assembly profile of these larger fibrous proteins, can also provide topographical stimuli that influence cellular behaviours [292]. Such topographical features, which can range from tens of nanometres to some hundreds of micrometres, are recognised to be an important parameter in native ECM [293–295]. At the nanoscale, parameters including surface roughness or smoothness and thickness of fibres influences the surface topography sensed by cells as they adhere to that surface. At the microscale, characteristics of the ECM fibre network, including bundle size and thickness, protrusions and valleys in the overall assembly of the network, and patterns such as pillars, grooves or grates have been shown in the tissue engineering field to influence cellular responses to the surfaces they encounter [292, 296, 297].

Many studies have reported that cell adhesion and spreading are influenced by surface topography (*e.g.* [298–301]). These studies have examined a variety of adhesive cell-type responses (fibroblasts, endothelial cells, mesenchymal stromal cells, osteoblasts) to different surfaces and the results are not always consistent, suggesting that different cell types respond differently to topographies. The morphology adopted by cells on a surface is also influenced by the topography. A well-studied aspect is the parallel alignment of many cell types to nanogrooves when there is a large axel dimension (along the longitudinal axis of the groove) and a nanoscale lateral dimension (perpendicular to the primary axis: the side-to-side measurement of the groove). Interestingly, when osteoblast-like cells were exposed to a chemical patterned topography

(fibronectin), they aligned strongly with this pattern. However, when the fibronectin pattern was superimposed perpendicular to mechanical grooves, the cells preferentially aligned with the mechanical topography (figure 5), indicating that the biomechanical cues were stronger than the biochemical ones [302].

Topography is also important for regulating cell migration, with wavelength and amplitude of the topography combining to regulate speed and direction of migration [303–305]. There are also some data suggesting topography can influence cell proliferation, but results are conflicted so the picture is not as clear for this cellular response [292].

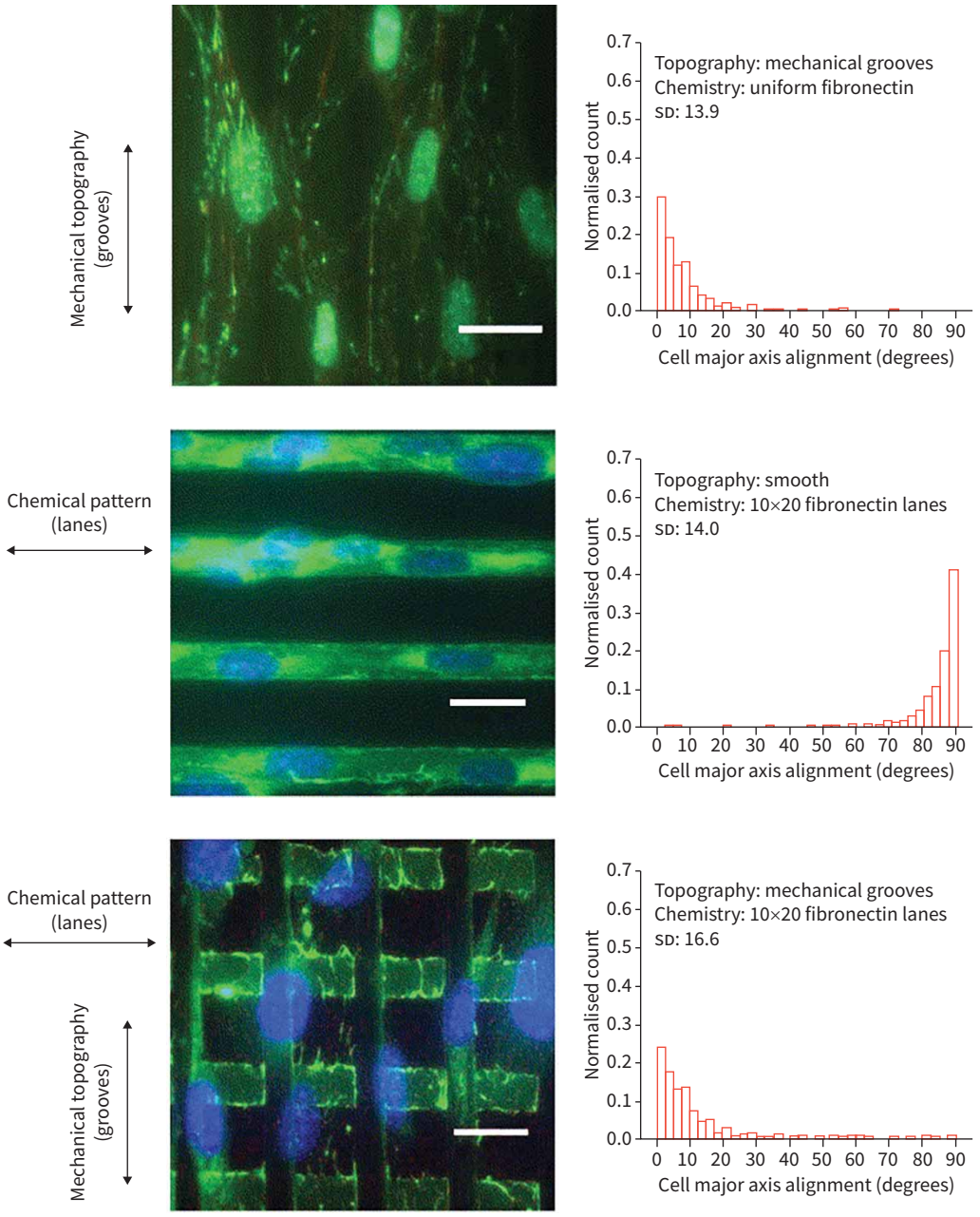

FIGURE 5 Immunofluorescence images of cells on patterned substrates with corresponding histograms of cell alignment angle. Grooves are vertical and lanes are horizontal. Cells showed strong alignment to mechanical grooves (top) and stronger alignment to protein lanes (middle) when presented separately. When presented with combined mechanical grooves and orthogonal protein lanes on one substrate, the cells align to the mechanical grooves (bottom). Scale bars: 20 µm. Reproduced from [302] with permission.

In diseased lung tissues the topographical network of ECM is altered, compared to healthy lung tissues [76]. Using advanced imaging techniques, including second harmonic generation, high-dimensional imaging mass cytometry, collagen fibre visualisation and electron microscopy techniques, studies are visualising the altered structural landscape in the tissues in asthma [306], COPD [307] and fibrosis [79, 295, 308]. How these altered topographical microenvironments in diseased lung tissues impact cellular responses that then contribute to underlying disease processes is a question that remains to be explored.

Lung surface fluid flow for epithelial cell differentiation and maintenance

Lung surface fluids and their interactions with epithelial cells play essential roles in the homeostasis of alveoli and airways. In alveoli, the presence of surfactant and a thin fluid lining is tied to epithelial cell stability *via* biochemical and mechanical signalling mechanisms. Surfactant protein A, for example, interacts with alveolar epithelial receptors and immune cells, helping modulate inflammation and preserve normal cell turnover [309]. The mechanical role of surfactants is to prevent the formation of fluid bridging and collapse of the alveoli by reducing surface tension [310]. If alveolar fluid mechanics are disturbed (*e.g.* increased surface tension from surfactant loss), the resulting collapse and reopening of alveoli can injure cells and alter the normal type I/II cell balance [311].

In the mammalian airways, ciliated epithelial cells are essential for homeostasis by continuously clearing inhaled pollution and pathogens through their beat activity, as well as their emergent role in setting and maintaining the organisation and integrity of the entire epithelium [312]. These functions are tightly regulated *via* mechanical interactions with the overlaying surface fluids. In the healthy trachea and bronchi, the airway surface is lined by a periciliary liquid (PCL) layer about 5–15 µm in depth, topped by a thicker mucus gel layer that can reach up to 70 µm [313]. The PCL is a watery, low-viscosity layer bathing the cilia, while the overlying mucus (~97% water with ~3% mucins and proteins) forms a viscoelastic gel [314]. The mucus traps particles and pathogens, which are continuously propelled toward the pharynx by ciliary beating. Cilia beat at a rate of 12–15 Hz, with their tips gripping the underside of the mucus layer. The mucus gel's viscoelasticity is high enough to transmit ciliary forces but low enough to flow [314]. The underlying PCL acts as a lubricant and spacer: it prevents the mucus from sticking to cell surfaces and enables the cilia to beat without obstruction [315]. In sum, the trachea and bronchi sustain a layered fluid lining in which ciliary beating, PCL and mucus interact to mechanically clear debris and protect epithelium. Beyond this clearance mechanism, the mechanical interaction between cilia and surface fluids also mediates important self-regulatory feedback and homeostatic processes that actively shape epithelial organisation and function. For example, the mechanical forces due to mucus resistance induce airway epithelia to actively regulate the PCL volume *via* ion transport (epithelial NA channel-mediated Na^+ absorption and cystic fibrosis transmembrane conductance regulator (CFTR)-dependent Cl^- secretion) to maintain the height of the airway surface liquid [315]. This mechanical feedback loop prevents dehydration of the mucus (which would make it too viscous) or excessive dilution (which would cause mucus to flow in the direction of gravity) [316].

Further, coordinated mucociliary clearance depends on planar cell polarity (PCP) that aligns ciliary beat across the epithelium in the same direction. Recent *in vitro* work suggests that the hydrodynamic shear forces created by ciliary beating and transmitted to neighbouring cells by the transport of the mucus is essential for the initial maturation as well as maintenance of airway epithelial-wide PCP. Reducing or increasing mucus viscosity, and hence the hydrodynamic forces, results in a slow remodelling and loss of tissue-wide PCP [317]. Consistently, the directionality of particle transport in human *in vitro* airway epithelia is found to be a direct function of secretory cell abundance, providing evidence of the important role of mucus in aligning ciliary beating during differentiation [318]. This mechanism can also be induced artificially. Applying external, directed hydrodynamic shear forces *via* medium perfusion aligned the PCP and ciliary beat of human *in vitro* airway epithelia along the flow axis [319]. Similarly, applying directed airflow on top of the mucus layer accelerated the maturation of PCP signalling in differentiating *in vitro* airway epithelia and polarised the mucociliary transport along the flow axis [204]. Intriguingly, while full submersion in medium suppresses airway ciliated cell differentiation *via* hypoxic signalling, adding a small amount of apical medium increases ciliogenesis [320]. The mechanisms have not been resolved but, in light of the above findings, may be related to changes in surface fluid forces.

Taken together, these results suggest that the shear forces exerted by surface fluid flow promote the maturation and alignment of airway cilia, which is consistent with findings in other ciliated epithelia. For example, experiments in mouse ependyma and frog skin epithelia demonstrated that applying an external fluid flow can reorient ciliary beating direction [321, 322]. Despite these insights, many questions remain, such as whether PCP and associated ciliary beat orientation in airway epithelia remains plastic and subject to remodelling by external forces, as suggested by some studies [317], or whether there is a limited time

window during differentiation in which ciliary beat orientation can be refined, as seen in ciliated ependymal epithelia [322]. This question is further complicated by the long timeframe of ciliary beat differentiation. On the single cilia level, a stepwise variation in waveform development was observed during ciliogenesis, taking up to 60 days to complete [323]. Broadly, waveform parameters evolved as cilia length increased and different dynein motor proteins started to localise to specific sections of the cilium. Assuming a slow but constant turnover of airway epithelial cells over 30–50 days [324], which would be sped up in injury, a fraction of ciliated cells would always be within this window of ciliary beat maturation. It would be interesting to know whether ciliary beat orientation is susceptible to external forces during this maturation period, suggesting that maintenance of tissue-level ciliary beat alignment may be an active process throughout life.

Fibroblast responses to mechanics

In 1858, Virchow described for the first time the "spindle-shape cells of the connective tissue", which were thereafter named fibroblasts [325, 326]. They are found in different organs, such as skin, heart and lung, showing shared commonalities but also tissue-specific phenotypes [326]. In the lung, fibroblasts are found both in the subepithelial layer of the conducting airways and in the interstitial spaces in the lung parenchyma, between the alveoli [327]. Several lung fibroblast subpopulations have been identified, with greater granularity in this population being developed recently with the advent of single-cell transcriptomics [328, 329]. These subtypes are found in specific cell niches in homeostasis, or they can arise as a response to an insult (*e.g.* an injury or an infection) [326]. Importantly, specific fibroblast subpopulations are not terminally differentiated, with fibroblasts being highly plastic, changing their phenotype depending on biochemical and mechanical signalling cues [326, 330].

One of the most studied pathways involved in mechanotransduction in fibroblasts is the Hippo pathway, where YAP and the transcriptional coactivator with PDZ-binding motif (TAZ) act as transcriptional effectors (figure 6) [331]. When the Hippo pathway is activated, YAP and TAZ are phosphorylated *via* a kinase signalling cascade that involves mammalian sterile 20-like kinase 1/2 (MST1/2) and large tumour suppressor kinase 1/2 (LATS1/2). The phosphorylated YAP/TAZ complex is then degraded through the proteosome pathway, preventing its translocation to the nucleus. When fibroblasts sense a high stiffness mechanical stimulus, MST and LATS are inactive and subsequently the YAP/TAZ complex is not phosphorylated, resulting in it translocating to the nucleus. This induces the expression of downstream mechanosensitive genes, such as ECM genes and genes regulating ECM remodelling, cell proliferation and apoptosis inhibition. The Hippo pathway cooperates with many other intracellular and extracellular pathways for regulating fibroblast (and other cell type) mechanoresponses, including TGF-β, Wnt and integrin-mediated signalling (figure 6) [332, 333]. The formation of focal adhesions through integrin clustering initiates FAK activation, facilitating its interaction with the actin cytoskeleton and promoting changes in cytoskeletal tension, which is thought to modulate YAP/TAZ subcellular localisation [334]. In 2D environments, stiff substrates promote the formation of focal adhesions in fibroblasts that results in the activation of FAK. This triggers an intracellular cascade that regulates the expression of genes related to migration and ECM synthesis, among others, leading to the activation of fibroblasts towards myofibroblasts [233]. This leads to the transition of fibroblasts to myofibroblasts that show a mechanical memory and have an important role in lung fibrosis [282]. Owing to the significance of ECM stiffening in the progression of fibrosis, especially in IPF, this is the main context for the study of fibroblast mechanosensing. One of the initial works on this topic showed that physiological stiffness matrices can inactivate IPF fibroblasts [82], while other studies have reported the activation of normal fibroblasts when cultured in ECM from patients with IPF [266, 335]. Similarly, how fibroblasts organise the ECM fibres around them is influenced by the characteristics of the ECM microenvironment in which they are embedded. In one study, control fibroblasts did not change the alignment of collagen fibres in either a fibrotic or a non-fibrotic environment, whereas fibrotic fibroblasts increased the alignment of the fibres in both environments, but to a greater extent in the fibrotic one. Both the control and fibrotic fibroblasts increased the stiffness of the fibrotic environment, but no change occurred in the mechanical properties of the non-fibrotic environment [84]. Moreover, an upregulation of genes related to senescence and fibrosis (smooth muscle α2 actin (*ACTA2*), *COL1A1* and fibulin 1 (*FBLN1*)) has been described when fibroblasts are cultured on stiff matrices (15 kPa) [267]. Interestingly, in line with the increased ECM stiffness in IPF, diseased fibroblasts showed a greater cellular stiffness [336]. Given that fibroblast activation protein is associated with ECM and integrin signalling [337, 338], it is likely that its expression levels may be altered with changes in ECM stiffness. Nevertheless, specific data about this, as well as studies on its potential as a drugable target for IPF, are still lacking. All these studies highlight the key role of matrix stiffness in directing the fate of lung fibroblasts, especially in fibrosis. Similarly, these observations indicate that microenvironment changes, both in composition and mechanical properties, impart a powerful signal that dictates cellular responses.

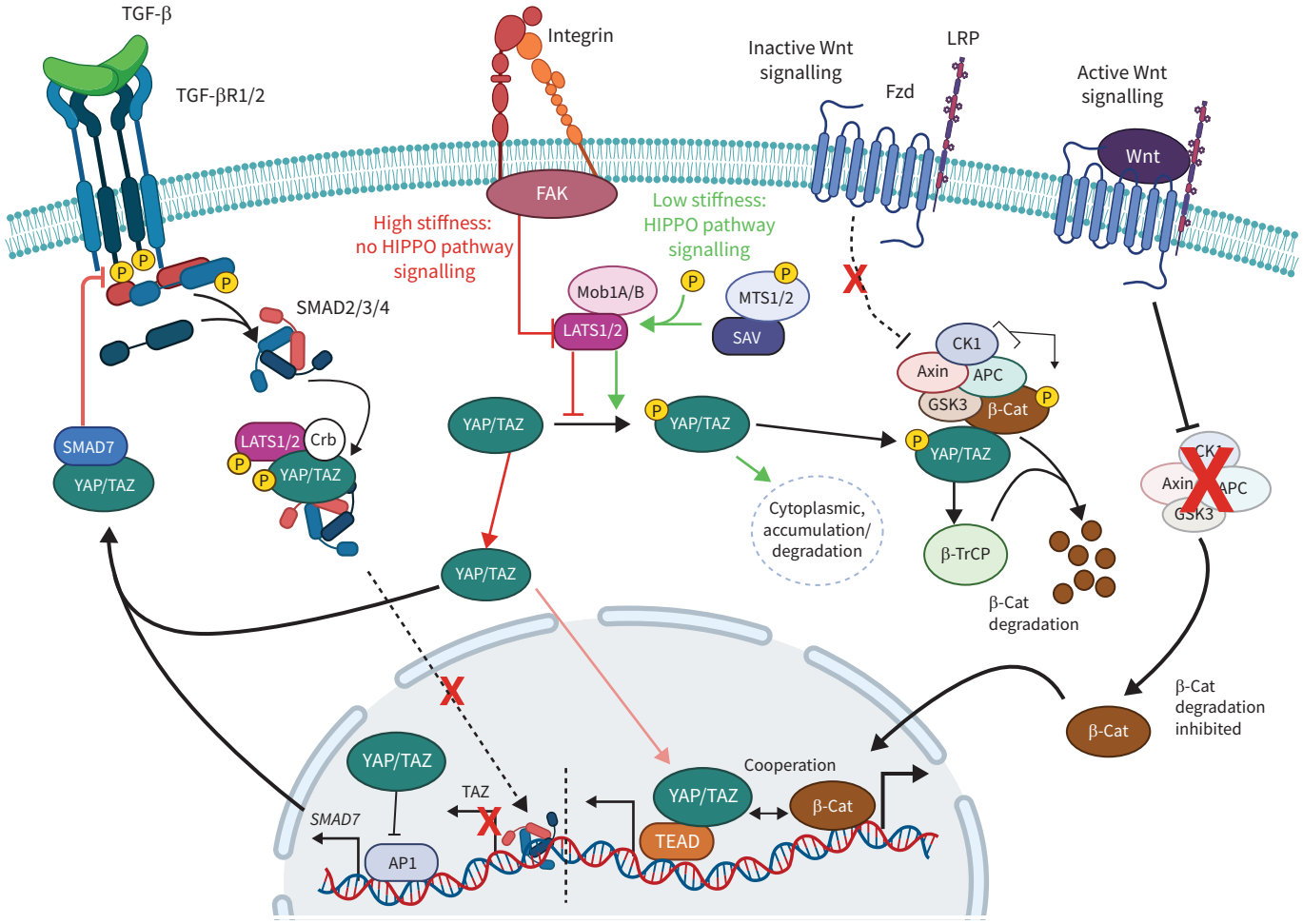

FIGURE 6 Crosstalk between Hippo/Yes-associated protein (YAP) and transforming growth factor-β (TGF-β) and wingless-related integration site (Wnt) signalling in response to mechanical stimuli. At low stiffnesses, the Hippo pathway is activated, YAP and transcriptional coactivator with PDZ-binding motif (TAZ) are phosphorylated (P) *via* a kinase signalling cascade that involves mammalian sterile 20-like kinase 1/2 (MST1/2) and large tumour suppressor kinase 1/2 (LATS1/2) and adapter proteins *Drosophila* Sal-vador (SAV) and *Drosophila* Mob 1A/B (Mob1A/B). The phosphorylated YAP/TAZ complex then accumulates in the cytoplasm and is degraded through the proteosome pathway, preventing its translocation to the nucleus. When cells sense a high stiffness, MST and LATS are inactive and subsequently the YAP/TAZ complex is not phosphorylated, resulting in nuclear translocation. This induces the expression of downstream mechanosensitive genes. Hippo–YAP crosstalk with TGF-β signalling: TGF-β receptor (TGF-βR) activation leads to SMA and mothers against decapentaplegic homolog 2/3/4 (SMAD2/3/4) complex formation, which induces *TAZ*. Crumbs (Crb)-dependent YAP/TAZ phosphorylation induces binding to SMAD2/3/4, which drives sequestration in the cytoplasm, blocking TGF-β-dependent target gene expression. Nuclear YAP inhibits activator protein 1 (AP-1)-induced *SMAD7* induction. YAP–SMAD7 interactions inhibit TGF-βR-induced signalling. Hippo–Wnt crosstalk occurs at multiple levels. When Wnt signalling is inactive, phosphorylated YAP/TAZ induces β-catenin (β-Cat) degradation through its interaction with β-Cat in the β-Cat degradation complex and recruitment of β-transducin repeats-containing proteins (β-TrCP). When Wnt signalling is active, the β-Cat degradation complex is inhibited, allowing β-Cat to translocate to the nucleus. YAP/TAZ and β-Cat cooperate in the nucleus to induce Wnt target genes. APC: APC regulator of Wnt signalling pathway; CK1: casein kinase 1; FAK: focal adhesion kinase; Fzd: frizzled; GSK3: glycogen synthase kinase 3; LRP: lipoprotein receptor-related protein; TEAD: TEA domain transcription factor family members. Reproduced and modified from [332] with permission. Created in BioRender (Burgess, 2025).

Model systems for advancing understanding of mechanical forces in experimental investigations

As recently highlighted, there is an urgent need to develop relevant *in vitro* models of chronic lung diseases that include the use of matrices with a controlled mechanical environment [339, 340]. To address this challenge, several models have been described for the culture of fibroblasts, usually harnessing the potential of combining natural ECM with biomaterials and/or advanced chemistry methods. For example, it is possible to stiffen lung ECM-derived hydrogels using a ruthenium crosslinking approach, driving the activation of fibroblasts to myofibroblasts [341]. Other works have described the possibility of incorporating a human decellularised ECM into a poly(ethylene glycol) (PEG) hydrogel, allowing

modulation of the stiffness and showing fibroblast activation on stiffened matrices [342]. Pure biomaterial matrices have also been used to study the effects of stiffness on lung fibroblasts. For instance, it has been shown that fibroblasts cultured on stiff polyacrylamide hydrogels (25 kPa) spread and migrate more than those cultured on softer ones (2 kPa), and that this is mediated by a higher expression of α-smooth muscle actin [343]. In addition, PEG hydrogels have been used to culture murine fibroblasts in 3D environments, resulting in a higher number of activated cells in the stiff hydrogels [344]. Overall, these models mimic the fibrotic microenvironment to direct fibroblasts towards an activated phenotype, showing a strong potential for use as *in vitro* models for the evaluation of novel antifibrotic drugs.

Other key mechanotransduction pathways in lung fibroblasts are mediated by calcium channels, namely Piezo 1/2 and TRPV4, which sense not only matrix stiffness, but also the mechanical stretch induced in the lungs during breathing [233, 234]. Different models have been developed to study the effect of stretch, including several based on the Flexcell system [345]. Using this strategy, an important role of Piezo 1 channels in the modulation of fibroblast-to-myofibroblast activation has recently been described [346]. Furthermore, a custom cyclic stretch bioreactor that makes use of synthetic hydrogels has been used to examine the effects of strain, stiffness and substrate composition on lung fibroblasts [347]. In addition, a calcium-binding protein, S100A4, has shown a mechanoeffector role in fibroblast activation to myofibroblast, highlighting its potential role in fibrogenesis and the importance of calcium channels such as TRPV4 in fibroblast mechanosensing [348].

Beyond these systems, recent years have seen the emergence of a host of platforms capable of delivering mechanical cues to a variety of cell and tissue formats (table 2). While their operation usually requires more complex workflows and microfabrication resources than insert-based designs such Flexcell, organ chips containing flexible membranes can also be dynamically stretched, which has been shown to exacerbate barrier dysfunction in a model of pulmonary oedema [349]. Additionally, organ chips lend themselves to the application of fluid shear forces, *e.g.* to achieve the physiological elongation and barrier formation of endothelial cells [350] but also to test the role of air flow in airway epithelial differentiation [351]. A variety of bulk and microfluidic devices exist to apply compressive forces to living cells, using weights or hydraulics [353], along with shear stress-generating parallel-plate and cone-and-plate flow chambers [354]. More recently developed models attempt to better represent the 3D mechanical microenvironment, such as stretching of PCLS [355] and vascularised and/or perfused constructs with tuneable stiffness, *e.g.* hydrogel tubes [356], bioprinted 3D tissues [357] and so-called organoid-on-chips [360]. The most advanced systems combine dynamic, multimodal mechanical stimulation with physiological 3D environments, including alveolar chips made from stretchable, biomimetic ECM scaffolds [352], dynamically stiffening cell-loaded hydrogels that switch modulus on demand, capturing processes such as progressive fibrosis or tumour stiffening [359], and actuated organoids whose lumens are cyclically inflated to drive morphogenesis and cell-type patterning [358].

A consensus response is needed to the question “what are the minimum requirements for a model system that will elucidate the role of the mechanical environment as a driver of lung disease?” This will further the advance of incorporating biomechanical elements in a wider range of research approaches.

Mechanics and therapeutic interventions for pulmonary diseases: future perspective

It is clear that the mechanical nature of the lung affects the responses of the organ, tissue and cells that are present within this complex, multiscale structure. It is then logical to ask the question as to whether the mechanical status of the system should also be considered when seeking therapeutic possibilities for managing or treating chronic lung diseases. This leads us to ask “how can we best therapeutically target the altered mechanical environment and mechanosignalling in chronic lung disease?”

When considering mechanisms underlying induction of chemoresistance, the mechanical state of the TME has been recognised as a significant contributor to this process [361, 362]. As cancer cells proliferate, they alter the mechanical properties of the ECM surrounding them. This stiffer ECM induces greater proliferation of the cancer cells, further increasing the mass of the tumour. The host tissue then exerts reciprocal compression on the tumour mass, and at the interface between the tumour and the host tissue, tensile stress is exerted [363]. Cells within this environment sense the compressive force, which alters their proliferation (cell-cycle progression), migration and invasiveness [364–366]. At the mesoscale the compression leads to squeezing of blood and lymphatic vessels, which can impair oxygenation and nutrient transfer but also drug access to the target cells within the tumour [367]. The dense ECM can also act as a physical barrier that blocks immune cell access to the tumour, leading to immune-excluded tumours [368, 369]. New blood vessels form in response to the lack of oxygen in that region of the tissue;

TABLE 2 Emerging model systems for examining mechanical influences in the context of the lung

Type of model	Mechanical feature modulated	Key novelty	Reference
Flexible-membrane stretch systems (2D)			
Flexcell	Stretch	Use of the Flexcell system to address the effect of the mechanical lung environment on fibroblast phenotype	[345, 346]
Microfluidic lung-on-a-chip devices			
Lung-on-chip	Shear stress and stretch	Dynamic cyclic stretch of a flexible membrane to combine stretch and the shear stress inherent of microfluidic devices	[349, 350]
Airway-on-chip	Airflow-induced shear stress	Combination of an oscillatory bidirectional airflow that induces a breathing-like shear stress on airway epithelial cells and a thin matrix-derived (collagen I and Cultrex BME) membrane that simulates the airway ECM	[351]
Alveolar lung-on-chip	Stretch	Array of alveoli formed by epithelial and endothelial cells seeded on each side of a stretchable biological membrane made of collagen and elastin	[352]
Mechanical compression devices			
Flexible microdevices/ microfluidic systems	Compressive stress	Reviews different examples of microfluidics-based microdevices used to study the effects of mechanical compression on living cells	[353]
Parallel-plate and cone-and-plate flow chambers			
Modified parallel-plate flow chamber	Shear stress	Flow chamber design creates local zones of low/disturbed shear to study endothelial dysfunction under these conditions, with a focus on atherosclerosis	[354]
PCLS stretch bioreactors			
Biaxial stretch device	Stretch	Biaxial cyclic stretch device specifically developed to study the effects of physiological and pathological breathing stretch on PCLS and decellularised lung scaffolds or 3D platforms in general (*e.g.* hydrogels)	[355]
Tuneable-stiffness 3D hydrogel and organoid systems			
ECM-derived hydrogel	Stiffness	Controlled light-induced crosslinking of the ECM hydrogel to simulate lung fibrosis progression	[341]
Hybrid ECM-PEG hydrogel	Stiffness	Chemical modification of natural ECM to achieve hybrid hydrogels of tuneable stiffness to mimic tissue fibrosis	[280, 342]
PEG hydrogel	Stiffness	Biomaterial-based approach to mimic lung fibrosis progression *in vitro*	[343, 344]
3D (multicell type) vascularised and/or perfused constructs			
3D-bioprinted perfusable scaffolds for organ-on-chips	Shear stress and stiffness	Use of 3D bioprinting to render perfusable scaffolds made of collagen-based bioinks at different concentrations (modulating stiffness) and combination with a microfluidic system for 3D organ-on-chip development	[356, 357]
Emerging multimodal devices			
Cyclically inflatable organoids	Inflation–collapse (pressure-driven stretch/ compression)	Demonstrates that rhythmic lumen dynamics mechanically pattern epithelium during morphogenesis in intestinal organoids	[358]
Uniaxial stretch device (ADMET BioTense Bioreactor)	Stretch and stiffness	Cyclic stretch of hydrogels of varying stiffness and compositions to evaluate the contribution of each mechanical feature to pulmonary fibrogenesis	[347]
Hydrogels with tuneable stiffness	Temporal stiffness changes	Dynamically stiffening cell-loaded hydrogels that switch modulus on demand	[359]

This table describes examples of model systems for studying mechanical stimuli but does not completely capture all current reports within this field. 2D: two-dimensional; 3D: three-dimensional; BME: basement membrane extract; ECM: extracellular matrix; PCLS: precision-cut lung slices; PEG: poly(ethylene glycol).

however, these vessels do not mature appropriately, leading to leakiness of the vessels. The transfer of fluids from the vasculature into the extracellular space of the tumour causes an increase in the interstitial fluid pressure (another mechanical force) and also washes drugs out of the TME [370–372].

Many of the changes in mechanical properties described in the TME are also seen in fibrotic regions in chronic lung diseases. Whether similar responses to the altered tissue mechanical environment, compared to those seen in the TME, are also active in cells in chronic lung diseases has recently been considered

TABLE 3 Emerging and preclinical compounds for targeting elements within mechanotransduction pathways

Compound	Proposed action	Target molecule	Target pathology	Current status	References
Therapeutic					
BG00011	Therapeutic	Integrin $\alpha_v\beta_6$	Fibrosis	Clinical trial terminated: NCT03573505	[381]
GSK3008348	Therapeutic	Integrin $\alpha_v\beta_6$	Fibrosis	Clinical trial terminated: NCT03069989	
Cilengitide	Therapeutic	Integrin $\alpha_v\beta_3/\alpha_v\beta_5$	NSCLC	Clinical trial phase 1 completed: NCT01118676	
GC1008	Therapeutic	TGF-β	Fibrosis	Clinical trial phase 1 completed: NCT00125385	[382]
GSK-2126458	Therapeutic	PI3K/mTOR	Fibrosis	Clinical trial phase 1 completed: NCT01725139	[383, 384]
HEC68498	Therapeutic	PI3K/mTOR	Fibrosis	Clinical trial phase 1 completed: NCT03502902	
IDL-2965	Therapeutic	Integrin $\alpha_5\beta_1/\alpha_v\beta_3/\alpha_v\beta_6$	Fibrosis	Clinical trial phase 1 terminated: NCT03949530	
PXS-5382A	Therapeutic	LOXL2	Fibrosis	Clinical trial phase 1 completed: ACTRN12617001564347	[385]
Bexotegrast (PLN-74809)	Therapeutic	$\alpha_v\beta_6/\alpha_v\beta_1$	Fibrosis	Clinical trial phase 2 completed: NCT04396756	[386, 387]
PBI-4050	Therapeutic	CTGF inhibitor, FFAR1 (GPR-40) agonist, GPR84 antagonist	Fibrosis	Clinical trial phase 2 completed: NCT02538536	[388]
Simtuzumab	Therapeutic	LOXL2	Fibrosis	Clinical trial phase 2 terminated: NCT01769196	[389]
Tipelukast (MN-001)	Therapeutic	Leukotriene receptor antagonist, PDE3/4 inhibition, 5-LO inhibition, phospholipase C inhibition, thromboxane A2 inhibition	Fibrosis	Clinical trial phase 2 completed: NCT02503657	
Anlotinib	Therapeutic	Receptor tyrosine kinase inhibitor (VEGFR2, VEGFR3, PDGFR-β and c-KIT)	NSCLC, fibrosis	Clinical trial phase 2 completed: NCT01924195 Clinical trial phase 2/3 currently recruiting: NCT05828953	
Imatinib (Glivec/Gleevec)	Therapeutic	Tyrosine kinase	Fibrosis, systemic sclerosis, PH	Clinical trial phase 2/3 completed: NCT00131274	[390]
Nerandomilast (BI 1015550)	Therapeutic	PDE4B inhibitor	Fibrosis	Clinical trial phase 3 completed: NCT05321069	[391, 392]
Pamrevlumab (FG-3019)	Therapeutic	CTGF	Fibrosis	Clinical trial phase 3 terminated: NCT04419558	[393]
Pentraxin-2 (rhPTX-2; PRM-151)	Therapeutic	Recombinant pentraxin 2	Fibrosis	Clinical trial phase 3 terminated: NCT04594707, NCT02550873	[394–396]
Treprostinil	Therapeutic	PPARβ agonist	PH, fibrosis	Clinical trial phase 4 completed: NCT03497689 Clinical trial phase 3 currently enrolling: NCT04905693, NCT05943535	[397, 398]

Continued

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TABLE 3 Continued

Compound	Proposed action	Target molecule	Target pathology	Current status	References
Preclinical					
Cyclo (RGDfC) peptide-decorated zeolitic imidazolate framework-8 nanoparticles	Therapeutic	Reduces mechanical tension in AEC IIs, releases zinc ions and 7,8-dihydroxyflavone	Fibrosis	Preclinical	[377]
Dual ROCK1/2 inhibitor (compound 1)	Therapeutic	ROCK	Fibrosis	Preclinical	[399]
Fasudil	Therapeutic	ROCK	Fibrosis, PH	Preclinical	[400–402]
Verteporfin	Therapeutic	YAP/TEAD	Fibrosis	Preclinical	[403]
Diagnostic					
$\alpha_v\beta_6$ cystine knot PET tracers	Diagnostic	$\alpha_v\beta_6$	Lung cancer, fibrosis		[404]
[^{18}F]FAPI-74 PET tracer	Diagnostic, theranostic	Fibroblast activation protein	Lung cancer, fibrosis, long COVID		[405–408]
[^{111}In]In-DOTAGA-AB0023	Diagnostic	LOXL2	Fibrosis		[409]

5-LO: 5-lipoxygenase; AEC: alveolar epithelial cells; CTGF: connective tissue growth factor; FFAR: free fatty acid receptor; GPR: G-protein-coupled receptor; LOXL2: lysyl oxidase-like 2; mTOR: mechanistic target of rapamycin; NSCLC: nonsmall cell lung cancer; PDE: phosphodiesterase; PDGFRβ: platelet-derived growth factor receptor-β; PET: positron emission tomography; PH: pulmonary hypertension; PI3K: phosphoinositide 3-kinase; PPARβ: peroxisome proliferator-activated receptor β; ROCK: Rho-associated protein kinase; TEAD: TEA domain family member; TGF-β: transforming growth factor-β; VEGFR: vascular endothelial growth factor receptor; YAP: Yes-associated protein.

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[6, 100, 272]. To date, the response of cells to antifibrotic therapies in different mechanical environments has not been explored. It has been suggested that the poor transfer of preclinical drug targets to success in clinical trials may relate to the lack of replication of the mechanical environment in the models being used for preclinical development of targets/candidate compounds [6]. In this respect, important differences in mechanical properties, force transmission patterns and overall lung architecture exist between humans and the majority of animal models used in preclinical studies. To briefly name a few, human lungs are stiffer than those of mice and have a greater number of alveoli generations [373]. In addition, the larger airway lumens and lack of submucosal glands make mice less prone to inflammatory processes [374]. In the case of rats, they lack respiratory bronchioles and have a thinner visceral pleural, thus making them less likely than humans to develop emphysema and fibrosis, but more prone to oedema [375]. Finally, in the case of pigs, the dissimilar composition of their ECM, with a larger relative balance of elastin *versus* collagen, give their lungs a greater tendency to collapse at low volumes, greater compliance at TLC and an overall more linear response of their pressure–volume curves [376].

The potential for targeting the mechanical environment for treating lung diseases is an emerging concept [377]. While a range of drugs are emerging in the cancer field that target mechanosensitive receptors, ion channels, mechanosensitive pathways or components of the ECM [361], some with US Food and Drug Administration/European Medicines Agency approval, it remains to be seen whether any of these approaches are viable for crossover to chronic lung diseases. One pathway that is receiving particular attention for its potential to disrupt mechanotransduction in lung fibrosis relates to integrin signalling [378–380]. Table 3 provides a snapshot of emerging and preclinical compounds for targeting elements within mechanotransduction pathways. Whether these studies translate to novel approaches for therapy in lung fibrosis will be interesting to monitor.

Final conclusion

The lung is a multiscale mechanical organ, with many different types of mechanical forces and stresses needed for the maintenance of a healthy, functional, homeostatic state across whole organ, tissue and cellular levels. The advent of disease in the lung changes this mechanical environment, consequently impacting the disease process and potentially the scope of cells to respond to therapeutic treatment. Previously, limited attention has been paid to the importance of the mechanical state of the lung, but this is now changing. There is increasing recognition of the need to incorporate a mechanics viewpoint into model systems to understand chronic lung diseases, identify their underlying mechanisms and develop novel treatments that will more successfully be translated from preclinical to clinical testing.

The questions for future research highlighted in this review represent important focuses for research programmes that will advance our knowledge relating to the importance of mechanical forces in chronic respiratory diseases.

Questions for future research

- What is the role of mechanics in the onset, development and progression of chronic lung diseases?
- How are cellular responses in health and disease altered by the biomechanical environment?
- What are the minimum requirements for a model system that will elucidate the role of the mechanical environment as a driver of lung disease?
- How can we best therapeutically target the altered mechanical environment and mechanosignalling in chronic lung disease?

Provenance: Commissioned article, peer reviewed.

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References

1 Sutherland TE, Dyer DP, Allen JE. The extracellular matrix and the immune system: a mutually dependent relationship. *Science* 2023; 379: eabp8964.
2 Joglekar MM, Nizamoglu M, Fan Y, *et al.* Highway to heal: influence of altered extracellular matrix on infiltrating immune cells during acute and chronic lung diseases. *Front Pharmacol* 2022; 13: 995051.
3 Joulia R, Lloyd CM. Location, location, location: spatial immune-stroma crosstalk drives pathogenesis in asthma. *Immunol Rev* 2025; 330: e70013.
4 Rahal Z, El Darzi R, Moghaddam SJ, *et al.* Tumour and microenvironment crosstalk in NSCLC progression and response to therapy. *Nat Rev Clin Oncol* 2025; 22: 463–482.
5 Xia T, Pan Z, Wan H, *et al.* Mechanisms of mechanical stimulation in the development of respiratory system diseases. *Am J Physiol Lung Cell Mol Physiol* 2024; 327: L724–L739.
6 Burgess JK, Gosens R. Mechanotransduction and the extracellular matrix: key drivers of lung pathologies and drug responsiveness. *Biochem Pharmacol* 2024: 228: 116255.
7 Ochs M, Nyengaard JR, Jung A, *et al.* The number of alveoli in the human lung. *Am J Respir Crit Care Med* 2004; 169: 120–124.
8 Weibel ER. Lung morphometry: the link between structure and function. *Cell Tissue Res* 2017; 367: 413–426.
9 Toshima M, Ohtani Y, Ohtani O. Three-dimensional architecture of elastin and collagen fiber networks in the human and rat lung. *Arch Histol Cytol* 2004; 67: 31–40.
10 Suki B. Structure and Function of the Extracellular Matrix. London, Academic Press, 2022.
11 Banerji R, Grifno GN, Shi L, *et al.* Crystal ribcage: a platform for probing real-time lung function at cellular resolution. *Nat Methods* 2023; 20: 1790–1801.
12 Murphy DM, Hall DR, Petersen MR, *et al.* The effect of diffuse pulmonary fibrosis on lung mechanics. *Bull Eur Physiopathol Respir* 1981; 17: 27–41.
13 Anafi RC, Wilson TA. Airway stability and heterogeneity in the constricted lung. *J Appl Physiol (1985)* 2001; 91: 1185–1192.
14 Gobbi A, Antonelli A, Dellaca R, *et al.* Effects of increasing tidal volume and end-expiratory lung volume on induced bronchoconstriction in healthy humans. *Respir Res* 2024; 25: 298.
15 Noble PB, Jones RL, Cairncross A, *et al.* Airway narrowing and bronchodilation to deep inspiration in bronchial segments from subjects with and without reported asthma. *J Appl Physiol (1985)* 2013; 114: 1460–1471.
16 Torchio R, Gulotta C, Ciacco C, *et al.* Effects of chest wall strapping on mechanical response to methacholine in humans. *J Appl Physiol (1985)* 2006; 101: 430–438.
17 Meinero M, Coletta G, Dutto L, *et al.* Mechanical response to methacholine and deep inspiration in supine men. *J Appl Physiol (1985)* 2007; 102: 269–275.
18 Pellegrino R, Gobbi A, Antonelli A, *et al.* Ventilation heterogeneity in obesity. *J Appl Physiol (1985)* 2014; 116: 1175–1181.
19 Mead J, Takishima T, Leith D. Stress distribution in lungs: a model of pulmonary elasticity. *J Appl Physiol* 1970; 28: 596–608.
20 Shi L, Herrmann J, Bou Jawde S, *et al.* Modeling the influence of gravity and the mechanical properties of elastin and collagen fibers on alveolar and lung pressure–volume curves. *Sci Rep* 2022; 12: 12280.
21 Raghu G, Remy-Jardin M, Richeldi L, *et al.* Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* 2022; 205: e18–e47.
22 Podolanczuk AJ, Thomson CC, Remy-Jardin M, *et al.* Idiopathic pulmonary fibrosis: state of the art for 2023. *Eur Respir J* 2023; 61: 2200957.
23 Marchioni A, Tonelli R, Cerri S, *et al.* Pulmonary stretch and lung mechanotransduction: implications for progression in the fibrotic lung. *Int J Mol Sci* 2021; 22: 6443.
24 Smith JC, Stamenovic D. Surface forces in lungs. I. Alveolar surface tension-lung volume relationships. *J Appl Physiol (1985)* 1986; 60: 1341–1350.
25 Suresh K, Shimoda LA. Lung circulation. *Compr Physiol* 2016; 6: 897–943.
26 Swenson ER. Hypoxic pulmonary vasoconstriction. *High Alt Med Biol* 2013; 14: 101–110.

27 Hughes M, West JB. Gravity is/is not the major factor determining the distribution of blood flow in the human lung. *J Appl Physiol (1985)* 2008; 104: 1531–1533.
28 Porsbjerg C, Melen E, Lehtimaki L, *et al.* Asthma. *Lancet* 2023; 401: 858–873.
29 Brannan JD, Lougheed MD. Airway hyperresponsiveness in asthma: mechanisms, clinical significance, and treatment. *Front Physiol* 2012; 3: 460.
30 Chapman DG, Irvin CG. Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. *Clin Exp Allergy* 2015; 45: 706–719.
31 Coates AL, Wanger J, Cockcroft DW, *et al.* ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017; 49: 1601526.
32 Kaminsky DA, Chapman DG. Asthma and lung mechanics. *Compr Physiol* 2020; 10: 975–1007.
33 Umetsu DT, DeKruyff RH. Immune dysregulation in asthma. *Curr Opin Immunol* 2006; 18: 727–732.
34 Lambrecht BN, Ahmed E, Hammad H. The immunology of asthma. *Nat Immunol* 2025; 26: 1233–1245.
35 Bai TR, Knight DA. Structural changes in the airways in asthma: observations and consequences. *Clin Sci (Lond)* 2005; 108: 463–477.
36 Boulet L-P. Airway remodeling in asthma: update on mechanisms and therapeutic approaches. *Curr Opin Pulm Med* 2018; 24: 56–62.
37 Papi A, Brightling C, Pedersen SE, *et al.* Asthma. *Lancet* 2018; 391: 783–800.
38 Varricchi G, Brightling CE, Grainge C, *et al.* Airway remodelling in asthma and the epithelium: on the edge of a new era. *Eur Respir J* 2024; 63; 2301619.
39 Grainge CL, Lau LC, Ward JA, *et al.* Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 2011; 364: 2006–2015.
40 Gelb AF, Licuanan J, Shinar CM, *et al.* Unsuspected loss of lung elastic recoil in chronic persistent asthma. *Chest* 2002; 121: 715–721.
41 Williamson JP, McLaughlin RA, Noffsinger WJ, *et al.* Elastic properties of the central airways in obstructive lung diseases measured using anatomical optical coherence tomography. *Am J Respir Crit Care Med* 2011; 183: 612–619.
42 Al-Alwan A, Bates JH, Chapman DG, *et al.* The nonallergic asthma of obesity. A matter of distal lung compliance. *Am J Respir Crit Care Med* 2014; 189: 1494–1502.
43 Benayoun L, Druilhe A, Dombret M-C, *et al.* Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; 167: 1360–1368.
44 Veerati PC, Mitchel JA, Reid AT, *et al.* Airway mechanical compression: its role in asthma pathogenesis and progression. *Eur Respir Rev* 2020; 29: 190123.
45 Park JA, Fredberg JJ, Drazen JM. Putting the squeeze on airway epithelia. *Physiology (Bethesda)* 2015; 30: 293–303.
46 Joseph C, Tatler AL. Pathobiology of airway remodeling in asthma: the emerging role of integrins. *J Asthma Allergy* 2022; 15: 595–610.
47 Park JA, Kim JH, Bi D, *et al.* Unjamming and cell shape in the asthmatic airway epithelium. *Nat Mater* 2015; 14: 1040–1048.
48 Payne DN, Rogers AV, Adelroth E, *et al.* Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003; 167: 78–82.
49 Mauad T, Silva LF, Santos MA, *et al.* Abnormal alveolar attachments with decreased elastic fiber content in distal lung in fatal asthma. *Am J Respir Crit Care Med* 2004; 170: 857–862.
50 Dekkers BGJ, Saad SI, van Spelde LJ, *et al.* Basement membranes in obstructive pulmonary diseases. *Matrix Biol Plus* 2021; 12: 100092.
51 Gelb AF, Yamamoto A, Verbeken EK, *et al.* Further studies of unsuspected emphysema in nonsmoking patients with asthma with persistent expiratory airflow obstruction. *Chest* 2018; 153: 618–629.
52 Yao Y, Zheng M, Borkar NA, *et al.* Role of STIM1 in stretch-induced signaling in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2024; 327: L150–L1l9.
53 Yao Y, Borkar NA, Zheng M, *et al.* Interactions between calcium regulatory pathways and mechanosensitive channels in airways. *Expert Rev Respir Med* 2023; 17: 903–917.
54 Christenson SA, Smith BM, Bafadhel M, *et al.* Chronic obstructive pulmonary disease. *Lancet* 2022; 399: 2227–2242.
55 Burgess JK, Mauad T, Tjin G, *et al.* The extracellular matrix – the under-recognized element in lung disease? *J Pathol* 2016; 240: 397–409.
56 Lomas DA. Does protease–antiprotease imbalance explain chronic obstructive pulmonary disease? *Ann Am Thorac Soc* 2016; 13: S130–S137.
57 Bidan CM, Veldsink AC, Meurs H, *et al.* Airway and extracellular matrix mechanics in COPD. *Front Physiol* 2015; 6: 346.
58 Migulina N, Tjin G, Faiz A, *et al.* Differential roles for lysyl oxidase (like), family members in chronic obstructive pulmonary disease; from gene and protein expression to function. *FASEB J* 2022; 36: e22374.

59 Suki B, Sato S, Parameswaran H, *et al.* Emphysema and mechanical stress-induced lung remodeling. *Physiology (Bethesda)* 2013; 28: 404–413.
60 Kononov S, Brewer K, Sakai H, *et al.* Roles of mechanical forces and collagen failure in the development of elastase-induced emphysema. *Am J Respir Crit Care Med* 2001; 164: 1920–1926.
61 Merrilees MJ, Ching PS, Beaumont B, *et al.* Changes in elastin, elastin binding protein and versican in alveoli in chronic obstructive pulmonary disease. *Respir Res* 2008; 9: 41.
62 van Straaten JF, Coers W, Noordhoek JA, *et al.* Proteoglycan changes in the extracellular matrix of lung tissue from patients with pulmonary emphysema. *Mod Pathol* 1999; 12: 697–705.
63 Kim JH, Schaible N, Hall JK, *et al.* Multiscale stiffness of human emphysematous precision cut lung slices. *Sci Adv* 2023; 9: eadf2535.
64 Hill DB, Button B, Rubinstein M, *et al.* Physiology and pathophysiology of human airway mucus. *Physiol Rev* 2022; 102: 1757–1836.
65 Lo Bello F, Leni A, Hansbro PM, *et al.* Role of the mucins in pathogenesis of COPD: implications for therapy. *Expert Rev Respir Med* 2020; 14: 465–483.
66 Knudsen L, Ochs M. The micromechanics of lung alveoli: structure and function of surfactant and tissue components. *Histochem Cell Biol* 2018; 150: 661–676.
67 Barak OF, Mladinov S, Hoiland RL, *et al.* Disturbed blood flow worsens endothelial dysfunction in moderate-severe chronic obstructive pulmonary disease. *Sci Rep* 2017; 7: 16929.
68 Liu H, Fan P, Jin F, *et al.* Targeting biophysical microenvironment for improved treatment of chronic obstructive pulmonary disease. *Trends Mol Med* 2023; 29: 926–938.
69 Koopman M, Posthuma R, Vanfleteren L, *et al.* Lung hyperinflation as treatable trait in chronic obstructive pulmonary disease: a narrative review. *Int J Chron Obstruct Pulmon Dis* 2024; 19: 1561–1578.
70 Brandsma CA, de Vries M, Costa R, *et al.* Lung ageing and COPD: is there a role for ageing in abnormal tissue repair? *Eur Respir Rev* 2017; 26: 170073.
71 Melo-Narvaez MC, Stegmayr J, Wagner DE, *et al.* Lung regeneration: implications of the diseased niche and ageing. *Eur Respir Rev* 2020; 29: 200222.
72 Tang Z, Hu Y, Wang Z, *et al.* Mechanical forces program the orientation of cell division during airway tube morphogenesis. *Dev Cell* 2018; 44: 313–25.
73 Pairet N, Mang S, Fois G, *et al.* TRPV4 inhibition attenuates stretch-induced inflammatory cellular responses and lung barrier dysfunction during mechanical ventilation. *PLoS One* 2018; 13: e0196055.
74 Martinez FJ, Collard HR, Pardo A, *et al.* Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 2017; 3: 17074.
75 Nizamoglu M, Burgess JK. The multi-faceted extracellular matrix: unlocking its secrets for understanding the perpetuation of lung fibrosis. *Curr Tissue Microenviron Rep* 2021; 2: 53–71.
76 Burgess JK, Harmsen MC. Chronic lung diseases: entangled in extracellular matrix. *Eur Respir Rev* 2022; 31: 210202.
77 Haak AJ, Tan Q, Tschumperlin DJ. Matrix biomechanics and dynamics in pulmonary fibrosis. *Matrix Biol* 2018; 73: 64–76.
78 Tjin G, White ES, Faiz A, *et al.* Correction: Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. *Dis Model Mech* 2017; 10: 1545.
79 Tjin G, White ES, Faiz A, *et al.* Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. *Dis Model Mech* 2017; 10: 1301–1312.
80 Booth AJ, Hadley R, Cornett AM, *et al.* Acellular normal and fibrotic human lung matrices as a culture system for *in vitro* investigation. *Am J Respir Crit Care Med* 2012; 186: 866–876.
81 de Hilster RHJ, Sharma PK, Jonker MR, *et al.* Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue. *Am J Physiol Lung Cell Mol Physiol* 2020; 318: L698–L704.
82 Marinkovic A, Liu F, Tschumperlin DJ. Matrices of physiologic stiffness potently inactivate IPF fibroblasts. *Am J Respir Cell Mol Biol* 2013; 48: 422–430.
83 Tschumperlin DJ. Matrix, mesenchyme, and mechanotransduction. *Ann Am Thorac Soc* 2015; 12: Suppl. 1, S24–S29.
84 Nizamoglu M, Alleblas F, Koster T, *et al.* Three dimensional fibrotic extracellular matrix directs microenvironment fiber remodeling by fibroblasts. *Acta Biomater* 2024; 177: 118–131.
85 Deng Z, Fear MW, Suk Choi Y, *et al.* The extracellular matrix and mechanotransduction in pulmonary fibrosis. *Int J Biochem Cell Biol* 2020; 126: 105802.
86 Mascharak S, Guo JL, Griffin M, *et al.* Modelling and targeting mechanical forces in organ fibrosis. *Nat Rev Bioeng* 2024; 2: 305–323.
87 Sun M, Sun Y, Feng Z, *et al.* New insights into the Hippo/YAP pathway in idiopathic pulmonary fibrosis. *Pharmacol Res* 2021; 169: 105635.
88 Froese AR, Shimbori C, Bellaye P-S, *et al.* Stretch-induced activation of transforming growth factor-β1 in pulmonary fibrosis. *Am J Respir Crit Care Med* 2016; 194: 84–96.

89 Liu G, Cooley MA, Jarnicki AG, *et al.* Fibulin-1c regulates transforming growth factor-β activation in pulmonary tissue fibrosis. *JCI Insight* 2019; 5: e124529.
90 Zhang M, Zhang B. Extracellular matrix stiffness: mechanisms in tumor progression and therapeutic potential in cancer. *Exp Hematol Oncol* 2025; 14: 54.
91 Sacco JL, Gomez EW. Epithelial–mesenchymal plasticity and epigenetic heterogeneity in cancer. *Cancers (Basel)* 2024; 16: 3289.
92 Gabasa M, Duch P, Jorba I, *et al.* Epithelial contribution to the profibrotic stiff microenvironment and myofibroblast population in lung fibrosis. *Mol Biol Cell* 2017; 28: 3741–3755.
93 Sainz de Aja J, Kim CF. May the (mechanical) force be with AT2. *Cell* 2020; 180: 20–22.
94 Wu H, Yu Y, Huang H, *et al.* Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. *Cell* 2020; 180: 107–121.
95 Schwartz DA. Idiopathic pulmonary fibrosis is a genetic disease involving mucus and the peripheral airways. *Ann Am Thorac Soc* 2018; 15: Suppl. 3, S192–S197.
96 Abdelgied M, Uhl K, Chen OG, *et al.* Targeting ATP12A, a nongastric proton pump α subunit, for idiopathic pulmonary fibrosis treatment. *Am J Respir Cell Mol* 2023; 68: 638–650.
97 Zabner J, Birket SE. ATP12A: connecting mucus and fibrosis in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2023; 68: 603–604.
98 Nho RS, Ballinger MN, Rojas MM, *et al.* Biomechanical force and cellular stiffness in lung fibrosis. *Am J Pathol* 2022; 192: 750–761.
99 Schmitt S, Hendricks P, Weir J, *et al.* Stretching mechanotransduction from the lung to the lab: approaches and physiological relevance in drug discovery. *Assay Drug Dev Technol* 2012; 10: 137–147.
100 Krishnan R, Park JA, Seow CY, *et al.* Cellular biomechanics in drug screening and evaluation: mechanopharmacology. *Trends Pharmacol Sci* 2016; 37: 87–100.
101 Kropski JA, Blackwell TS, Loyd JE. The genetic basis of idiopathic pulmonary fibrosis. *Eur Respir J* 2015; 45: 1717–1727.
102 Leiter A, Veluswamy RR, Wisnivesky JP. The global burden of lung cancer: current status and future trends. *Nat Rev Clin Oncol* 2023; 20: 624–639.
103 Linke JA, Munn LL, Jain RK. Compressive stresses in cancer: characterization and implications for tumour progression and treatment. *Nat Rev Cancer* 2024; 24: 768–791.
104 Silver FH. The role of connections between cellular and tissue mechanical elements and the importance of applied energy in mechanotransduction in cancerous tissue. *Biomolecules* 2025; 15: 457.
105 Glabman RA, Choyke PL, Sato N. Cancer-associated fibroblasts: tumorigenicity and targeting for cancer therapy. *Cancers* 2022; 14: 3906.
106 Kim BG, Gao M-Q, Kang S, *et al.* Mechanical compression induces VEGFA overexpression in breast cancer *via* DNMT3A-dependent miR-9 downregulation. *Cell Death Dis* 2017; 8: e2646.
107 Najafi M, Farhood B, Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. *J Cell Biochem* 2019; 120: 2782–2790.
108 Zeltz C, Pasko E, Cox TR, *et al.* LOXL1 is regulated by integrin α11 and promotes non-small cell lung cancer tumorigenicity. *Cancers* 2019; 11: 705.
109 Xin Y, Li K, Huang M, *et al.* Biophysics in tumor growth and progression: from single mechano-sensitive molecules to mechanomedicine. *Oncogene* 2023; 42: 3457–3490.
110 Maehama T, Nishio M, Otani J, *et al.* The role of Hippo-YAP signaling in squamous cell carcinomas. *Cancer Sci* 2021; 112: 51–60.
111 Ishihara S, Enomoto A, Sakai A, *et al.* Stiff extracellular matrix activates the transcription factor ATF5 to promote the proliferation of cancer cells. *iScience* 2025; 28: 112057.
112 Sala L, Franco-Valls H, Stanisavljevic J, *et al.* Abrogation of myofibroblast activities in metastasis and fibrosis by methyltransferase inhibition. *Int J Cancer* 2019; 145: 3064–3077.
113 Shukla VC, Higuita-Castro N, Nana-Sinkam P, *et al.* Substrate stiffness modulates lung cancer cell migration but not epithelial to mesenchymal transition. *J Biomed Mater Res A* 2016; 104: 1182–1193.
114 Li Y, Yu W-K, Chen L, *et al.* Electrotaxis of tumor-initiating cells of H1975 lung adenocarcinoma cells is associated with both activation of stretch-activated cation channels (SACCs) and internal calcium release. *Bioelectrochemistry* 2018; 124: 80–92.
115 Navab R, Strumpf D, To C, *et al.* Integrin α11β1 regulates cancer stromal stiffness and promotes tumorigenicity and metastasis in non-small cell lung cancer. *Oncogene* 2016; 35: 1899–1908.
116 Liang H, Xu Y, Zhao J, *et al.* Hippo pathway in non-small cell lung cancer: mechanisms, potential targets, and biomarkers. *Cancer Gene Ther* 2024; 31: 652–666.
117 Gargalionis AN, Papavassiliou KA, Basdra EK, *et al.* Update on the relevance of mechanobiological mechanisms in lung cancer. *Transl Oncol* 2025; 55: 102375.
118 Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med* 2010; 363: 2233–2247.
119 Lin VY, Kaza N, Birket SE, *et al.* Excess mucus viscosity and airway dehydration impact COPD airway clearance. *Eur Respir J* 2020; 55: 1900419.

120 Radicioni G, Ceppe A, Ford AA, *et al.* Airway mucin MUC5AC and MUC5B concentrations and the initiation and progression of chronic obstructive pulmonary disease: an analysis of the SPIROMICS cohort. *Lancet Respir Med* 2021; 9: 1241–1254.

121 Song D, Iverson E, Kaler L, *et al.* MUC5B mobilizes and MUC5AC spatially aligns mucociliary transport on human airway epithelium. *Sci Adv* 2022; 8: eabq5049.

122 Peng Y, Wang ZN, Xu AR, *et al.* Mucus hypersecretion and ciliary impairment in conducting airway contribute to alveolar mucus plugging in idiopathic pulmonary fibrosis. *Front Cell Dev Biol* 2021; 9: 810842.

123 Mettler SK, Nardelli P, Campo MI, *et al.* Longitudinal changes in airway mucus plugs and FEV_1 in COPD. *N Engl J Med* 2025; 392: 1973–1975.

124 Hogg JC, Chu FS, Tan WC, *et al.* Survival after lung volume reduction in chronic obstructive pulmonary disease: insights from small airway pathology. *Am J Respir Crit Care Med* 2007; 176: 454–459.

125 Mohamady YK, Geudens V, De Fays C, *et al.* Computational fluid dynamics of small airway disease in chronic obstructive pulmonary disease. *EBioMedicine* 2025; 114: 105670.

126 Hsieh A, Vasilescu DM, Barker-Mulleder J, *et al.* Mucus plugs correlate with small airway remodelling in asthma: a case-control study. *Am J Respir Crit Care Med* 2025; in press [https://doi.org/10.1164/rccm.202501-0310OC].

127 Aegerter H, Lambrecht BN. The pathology of asthma: what is obstructing our view? *Annu Rev Pathol Mech Dis* 2023; 18: 387–409.

128 Fritzsching B, Zhou-Suckow Z, Trojanek JB, *et al.* Hypoxic epithelial necrosis triggers neutrophilic inflammation via IL-1 receptor signaling in cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2015; 191: 902–913.

129 Chen G, Sun L, Kato T, *et al.* IL-1β dominates the promucin secretory cytokine profile in cystic fibrosis. *J Clin Invest* 2019; 129: 4433–4450.

130 Worlitzsch D, Tarran R, Ulrich M, *et al.* Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002; 109: 317–325.

131 Kim SJ, Park H, Lee HJ, *et al.* Mucus plug and lung cancer incidence in patients with COPD. *Sci Rep* 2025; 15: 30193.

132 Hough KP, Curtiss ML, Blain TJ, *et al.* Airway remodeling in asthma. *Front Med (Lausanne)* 2020; 7: 191.

133 Gosens R, Grainge C. Bronchoconstriction and airway biology. *Chest* 2015; 147: 798–803.

134 Suki B, Lutchen KR, Ingenito EP. On the progressive nature of emphysema: roles of proteases, inflammation, and mechanical forces. *Am J Respir Crit Care Med* 2003; 168: 516–521.

135 Maarsingh H, Bidan CM, Brook BS, *et al.* Small airway hyperresponsiveness in COPD: relationship between structure and function in lung slices. *Am J Physiol Lung Cell Mol Physiol* 2019; 316: L537–L546.

136 Freeberg MAT, Perelas A, Rebman JK, *et al.* Mechanical feed-forward loops contribute to idiopathic pulmonary fibrosis. *Am J Pathol* 2021; 191: 18–25.

137 Günther A, Ruppert C, Schmidt R, *et al.* Surfactant alteration and replacement in acute respiratory distress syndrome. *Respir Res* 2001; 2: 353–364.

138 Thenappan T, Chan SY, Weir EK. Role of extracellular matrix in the pathogenesis of pulmonary arterial hypertension. *Am J Physiol Heart Circ Physiol* 2018; 315: H1322–H1331.

139 Simonneau G, Torbicki A, Dorfmüller P, *et al.* The pathophysiology of chronic thromboembolic pulmonary hypertension. *Eur Respir Rev* 2017; 26: 160112.

140 Button B, Cai LH, Ehre C, *et al.* A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. *Science* 2012; 337: 937–941.

141 Murray MP, Hill AT. Non-cystic fibrosis bronchiectasis. *Clin Med (Lond)* 2009; 9: 164–169.

142 Tilley AE, Walters MS, Shaykhiev R, *et al.* Cilia dysfunction in lung disease. *Annu Rev Physiol* 2015; 77: 379–406.

143 Trapnell BC, Nakata K, Bonella F, *et al.* Pulmonary alveolar proteinosis. *Nat Rev Dis Primers* 2019; 5: 16.

144 Harari S, Torre O, Moss J. Lymphangioleiomyomatosis: what do we know and what are we looking for? *Eur Respir Rev* 2011; 20: 34–44.

145 Hilgendorff A, Reiss I, Ehrhardt H, *et al.* Chronic lung disease in the preterm infant. Lessons learned from animal models. *Am J Respir Cell Mol Biol* 2014; 50: 233–245.

146 Ambalavanan N, Deutsch G, Pryhuber G, *et al.* The evolving pathophysiology of bronchopulmonary dysplasia. *Physiol Rev* 2026; 106: 197–237.

147 Barnes H, Troy L, Lee CT, *et al.* Hypersensitivity pneumonitis: current concepts in pathogenesis, diagnosis, and treatment. *Allergy* 2022; 77: 442–453.

148 Selman M, Pardo A. When things go wrong: exploring possible mechanisms driving the progressive fibrosis phenotype in interstitial lung diseases. *Eur Respir J* 2021; 58: 2004507.

149 He Y, Yang F, Yang L, *et al.* Mechanics-activated fibroblasts promote pulmonary group 2 innate lymphoid cell plasticity propelling silicosis progression. *Nat Commun* 2024; 15: 9770.

150 Calkovska A, Kolomaznik M, Calkovsky V. Alveolar type II cells and pulmonary surfactant in COVID-19 era. *Physiol Res* 2021; 70: S195–S208.

151 Gooptu B, Ekeowa UI, Lomas DA. Mechanisms of emphysema in α1-antitrypsin deficiency: molecular and cellular insights. *Eur Respir J* 2009; 34: 475–488.
152 Wert SE, Whitsett JA, Nogee LM. Genetic disorders of surfactant dysfunction. *Pediatr Dev Pathol* 2009; 12: 253–274.
153 Wallis C, Alexopoulou E, Antón-Pacheco JL, *et al.* ERS statement on tracheomalacia and bronchomalacia in children. *Eur Respir J* 2019; 54: 1900382.
154 Bellemare F, Jeanneret A, Couture J. Sex differences in thoracic dimensions and configuration. *Am J Respir Crit Care Med* 2003; 168: 305–312.
155 Silveyra P, Fuentes N, Rodriguez Bauza DE. Sex and gender differences in lung disease. *In*: Wang Y-X (ed). Lung Inflammation in Health and Disease, Volume II. Cham, Springer International Publishing, 2021; pp. 227–258.
156 Jenkins CR, Boulet L-P, Lavoie KL, *et al.* Personalized treatment of asthma: the importance of sex and gender differences. *J Allergy Clin Immunol Pract* 2022; 10: 963–71.
157 Somayaji R, Chalmers JD. Just breathe: a review of sex and gender in chronic lung disease. *Eur Respir Rev* 2022; 31: 210111.
158 Barnes PJ. Sex differences in chronic obstructive pulmonary disease mechanisms. *Am J Respir Crit Care Med* 2016; 193: 813–814.
159 Han MK, Postma D, Mannino DM, *et al.* Gender and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007; 176: 1179–1184.
160 Milne KM, Mitchell RA, Ferguson ON, *et al.* Sex-differences in COPD: from biological mechanisms to therapeutic considerations. *Front Med (Lausanne)* 2024; 11: 1289259.
161 Aryal S, Diaz-Guzman E, Mannino DM. Influence of sex on chronic obstructive pulmonary disease risk and treatment outcomes. *Int J Chron Obstruct Pulmon Dis* 2014; 9: 1145–1154.
162 Sørheim I-C, Johannessen A, Gulsvik A, *et al.* Gender differences in COPD: are women more susceptible to smoking effects than men? *Thorax* 2010; 65: 480–485.
163 Amaral AFS, Strachan DP, Burney PGJ, *et al.* Female smokers are at greater risk of airflow obstruction than male smokers. UK Biobank. *Am J Respir Crit Care Med* 2017; 195: 1226–1235.
164 LoMauro A, Aliverti A. Sex differences in respiratory function. *Breathe* 2018: 14: 131–140.
165 Bennett WD, Zeman KL, Kim C. Variability of fine particle deposition in healthy adults: effect of age and gender. *Am J Respir Crit Care Med* 1996; 153: 1641–1647.
166 Sheel AW, Guenette JA, Yuan R, *et al.* Evidence for dysanapsis using computed tomographic imaging of the airways in older ex-smokers. *J Appl Physiol (1985)* 2009; 107: 1622–1628.
167 Tam A, Churg A, Wright JL, *et al.* Sex differences in airway remodeling in a mouse model of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2016; 193: 825–834.
168 Sverzellati N, Calabrò E, Randi G, *et al.* Sex differences in emphysema phenotype in smokers without airflow obstruction. *Eur Respir J* 2009; 33: 1320–1328.
169 Martinez FJ, Curtis JL, Sciurba F, *et al.* Sex differences in severe pulmonary emphysema. *Am J Respir Crit Care Med* 2007; 176: 243–252.
170 Gan WQ, Man SFP, Postma DS, *et al.* Female smokers beyond the perimenopausal period are at increased risk of chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Respir Res* 2006; 7: 52.
171 McGarvey L, Lee AJ, Roberts J, *et al.* Characterisation of the frequent exacerbator phenotype in COPD patients in a large UK primary care population. *Respir Med* 2015; 109: 228–237.
172 Fan VS, Ramsey SD, Giardino ND, *et al.* Sex, depression, and risk of hospitalization and mortality in chronic obstructive pulmonary disease. *Arch Intern Med* 2007; 167: 2345–2353.
173 de Torres JP, Casanova C, Hernández C, *et al.* Gender and COPD in patients attending a pulmonary clinic. *Chest* 2005; 128: 2012–2016.
174 Prescott E, Bjerg A, Andersen P, *et al.* Gender difference in smoking effects on lung function and risk of hospitalization for COPD: results from a Danish longitudinal population study. *Eur Respir J* 1997; 10: 822–827.
175 Foreman MG, Zhang L, Murphy J, *et al.* Early-onset chronic obstructive pulmonary disease is associated with female sex, maternal factors, and African American race in the COPDGene study. *Am J Respir Crit Care Med* 2011; 184: 414–420.
176 Yang IA, Jenkins CR, Salvi SS. Chronic obstructive pulmonary disease in never-smokers: risk factors, pathogenesis, and implications for prevention and treatment. *Lancet Respir Med* 2022; 10: 497–511.
177 Kawano-Dourado L, Glassberg MK, Assayag D, *et al.* Sex and gender in interstitial lung diseases. *Eur Respir Rev* 2021; 30: 210105.
178 Raghu G, Weycker D, Edelsberg J, *et al.* Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2006; 174: 810–816.
179 Abramson MJ, Murambadoro T, Alif SM, *et al.* Occupational and environmental risk factors for idiopathic pulmonary fibrosis in Australia: case–control study. *Thorax* 2020; 75: 864–869.

180 Zaman T, Moua T, Vittinghoff E, *et al.* Differences in clinical characteristics and outcomes between men and women with idiopathic pulmonary fibrosis: a multicenter retrospective cohort study. *Chest* 2020; 158: 245–251.
181 Redente EF, Jacobsen KM, Solomon JJ, *et al.* Age and sex dimorphisms contribute to the severity of bleomycin-induced lung injury and fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2011; 301: L510–L518.
182 Mueller MC, Blomberg R, Tanneberger AE, *et al.* Female fibroblast activation is estrogen-mediated in sex-specific 3D-bioprinted pulmonary artery adventitia models. *ACS Biomater Sci Eng* 2025; 11: 2935–2945.
183 Mederos N, Friedlaender A, Peters S, *et al.* Gender-specific aspects of epidemiology, molecular genetics and outcome: lung cancer. *ESMO Open* 2020; 5: e000796.
184 Stapelfeld C, Dammann C, Maser E. Sex-specificity in lung cancer risk. *Int J Cancer* 2020; 146: 2376–2382.
185 Florez N, Kiel L, Riano I, *et al.* Lung cancer in women: the past, present, and future. *Clin Lung Cancer* 2024; 25: 1–8.
186 Siegfried JM. Sex and gender differences in lung cancer and chronic obstructive lung disease. *Endocrinology* 2021; 163: bqab254.
187 Schneider JL, Rowe JH, Garcia-de-Alba C, *et al.* The aging lung: physiology, disease, and immunity. *Cell* 2021; 184: 1990–2019.
188 Wang Y, Huang X, Luo G, *et al.* The aging lung: microenvironment, mechanisms, and diseases. *Front Immunol* 2024; 15: 1383503.
189 Selman M, Pardo A. Fibroageing: an ageing pathological feature driven by dysregulated extracellular matrix-cell mechanobiology. *Ageing Res Rev* 2021; 70: 101393.
190 Suki B, Bates JHT, Bartolák-Suki E. Remodeling of the aged and emphysematous lungs: roles of microenvironmental cues. *Compr Physiol* 2022; 12: 3559–3574.
191 Sobin SS, Fung YC, Tremer HM. Collagen and elastin fibers in human pulmonary alveolar walls. *J Appl Physiol* 1988; 64: 1659–1675.
192 John R, Thomas J. Chemical compositions of elastins isolated from aortas and pulmonary tissues of humans of different ages. *Biochem J* 1972; 127: 261–269.
193 Pride NB. Ageing and changes in lung mechanics. *Eur Respir J* 2005; 26: 563–565.
194 Koloko Ngassie ML, De Vries M, Borghuis T, *et al.* Age-associated differences in the human lung extracellular matrix. *Am J Physiol Lung Cell Mol Physiol* 2023; 324: L799–L814.
195 Ulldemolins A, Narciso M, Sanz-Fraile H, *et al.* Effects of aging on the biomechanical properties of the lung extracellular matrix: dependence on tissular stretch. *Front Cell Dev Biol* 2024; 12: 1381470.
196 Sicard D, Haak AJ, Choi KM, *et al.* Aging and anatomical variations in lung tissue stiffness. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L946–L955.
197 García-Río F, Pino JM, Dorgham A, *et al.* Spirometric reference equations for European females and males aged 65–85 yrs. *Eur Respir J* 2004; 24: 397–405.
198 Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. *Clin Interv Aging* 2006; 1: 253–260.
199 Jaslove JM, Goodwin K, Sundarakrishnan A, *et al.* Transmural pressure signals through retinoic acid to regulate lung branching. *Development* 2022; 149: dev199726.
200 Kim Hye Y, Pang M-F, Varner Victor D, *et al.* Localized smooth muscle differentiation is essential for epithelial bifurcation during branching morphogenesis of the mammalian lung. *Dev Cell* 2015; 34: 719–726.
201 Li R, Li X, Hagood J, *et al.* Myofibroblast contraction is essential for generating and regenerating the gas-exchange surface. *J Clin Invest* 2020; 130: 2859–2871.
202 Leslie MN, Chou J, Young PM, *et al.* How do mechanics guide fibroblast activity? Complex disruptions during emphysema shape cellular responses and limit research. *Bioengineering (Basel)* 2021; 8: 110.
203 Edwards YS. Stretch stimulation: its effects on alveolar type II cell function in the lung. *Comp Biochem Physiol A Mol Integr Physiol* 2001; 129: 245–260.
204 Nawroth JC, Roth D, van Schadewijk A, *et al.* Breathing on chip: dynamic flow and stretch accelerate mucociliary maturation of airway epithelium *in vitro*. *Mater Today Bio* 2023; 21: 100713.
205 Kostyunina DS, Rowan SC, Pakhomov NV, *et al.* Shear stress markedly alters the proteomic response to hypoxia in human pulmonary endothelial cells. *Am J Respir Cell Mol Biol* 2023; 68: 551–565.
206 Mitchel JA, Das A, O'Sullivan MJ, *et al.* In primary airway epithelial cells, the unjamming transition is distinct from the epithelial-to-mesenchymal transition. *Nat Commun* 2020; 11: 5053.
207 Park JA, Atia L, Mitchel JA, *et al.* Collective migration and cell jamming in asthma, cancer and development. *J Cell Sci* 2016; 129: 3375–3383.
208 Swartz MA, Tschumperlin DJ, Kamm RD, *et al.* Mechanical stress is communicated between different cell types to elicit matrix remodeling. *Proc Natl Acad Sci USA* 2001; 98: 6180–6185.
209 Lan B, Mitchel JA, O'Sullivan MJ, *et al.* Airway epithelial compression promotes airway smooth muscle proliferation and contraction. *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L645–L652.
210 Rutting S, Thamrin C, Cross TJ, *et al.* Fixed airflow obstruction in asthma: a problem of the whole lung not of just the airways. *Front Physiol* 2022; 13: 898208.

211 Wang L, Paré PD. Deep inspiration and airway smooth muscle adaptation to length change. *Respir Physiol Neurobiol* 2003; 137: 169–178.
212 Lim SE, Vicente-Munuera P, Mao Y. Forced back into shape: mechanics of epithelial wound repair. *Curr Opin Cell Biol* 2024; 87: 102324.
213 Gomez GA, McLachlan RW, Yap AS. Productive tension: force-sensing and homeostasis of cell–cell junctions. *Trends Cell Biol* 2011; 21: 499–505.
214 Klarlund JK, Block ER. Free edges in epithelia as cues for motility. *Cell Adh Migr* 2011; 5: 106–110.
215 Ghosh MC, Gorantla V, Makena PS, *et al.* Insulin-like growth factor-I stimulates differentiation of ATII cells to ATI-like cells through activation of Wnt5a. *Am J Physiol Lung Cell Mol Physiol* 2013; 305: L222–L228.
216 Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. *Am J Physiol Lung Cell Mol Physiol* 2010; 298: L715–L731.
217 Talbott HE, Mascharak S, Griffin M, *et al.* Wound healing, fibroblast heterogeneity, and fibrosis. *Cell Stem Cell* 2022; 29: 1161–1180.
218 Li B, Wang JH. Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *J Tissue Viability* 2011; 20: 108–120.
219 Epa AP, Thatcher TH, Pollock SJ, *et al.* Normal human lung epithelial cells inhibit transforming growth factor-β induced myofibroblast differentiation *via* prostaglandin E2. *PLoS One* 2015; 10: e0135266.
220 Hopkinson SB, Findlay K, deHart GW, *et al.* Interaction of BP180 (type XVII collagen) and α6 integrin is necessary for stabilization of hemidesmosome structure. *J Invest Dermatol* 1998; 111: 1015–1022.
221 Barczyk M, Carracedo S, Gullberg D. Integrins. *Cell Tissue Res* 2010; 339: 269–280.
222 Schnittert J, Bansal R, Storm G, *et al.* Integrins in wound healing, fibrosis and tumor stroma: high potential targets for therapeutics and drug delivery. *Adv Drug Deliv Rev* 2018; 129: 37–53.
223 Li Y, Peng S, Xu J, *et al.* Integrin signaling in tumor biology: mechanisms of intercellular crosstalk and emerging targeted therapies. *PeerJ* 2025; 13: e19328.
224 Zhang H, Yang M, Kim SH, *et al.* Integrin force loading rate in mechanobiology: from model to molecular measurement. *QRB Discov* 2025; 6: e9.
225 Katoh K. Integrin and its associated proteins as a mediator for mechano-signal transduction. *Biomolecules* 2025; 15: 166.
226 Kechagia JZ, Ivaska J, Roca-Cusachs P. Integrins as biomechanical sensors of the microenvironment. *Nat Rev Mol Cell Bio* 2019; 20: 457–473.
227 Deville SS, Cordes N. The extracellular, cellular, and nuclear stiffness, a trinity in the cancer resistome – a review. *Front Oncol* 2019; 9: 1376.
228 Theocharis AD, Manou D, Karamanos NK. The extracellular matrix as a multitasking player in disease. *FEBS J* 2019; 286: 2830–2869.
229 Martino F, Perestrelo AR, Vinarský V, *et al.* Cellular mechanotransduction: from tension to function. *Front Physiol* 2018; 9: 824.
230 Migulina N, Kelley B, Zhang EY, *et al.* Mechanosensitive channels in lung health and disease. *Compr Physiol* 2023; 13: 5157–5178.
231 Koskimäki S, Tojkander S. TRPV4 – a multifunctional cellular sensor protein with therapeutic potential. *Sensors (Basel)* 2024; 24: 6923.
232 Cheng D, Wang J, Yao M, *et al.* Joining forces: crosstalk between mechanosensitive PIEZO1 ion channels and integrin-mediated focal adhesions. *Biochem Soc Trans* 2023; 51: 1897–1906.
233 Ezzo M, Hinz B. Novel approaches to target fibroblast mechanotransduction in fibroproliferative diseases. *Pharmacol Ther* 2023; 250: 108528.
234 Freeberg MAT, Camus SV, Robila V, *et al.* Piezo2 is a key mechanoreceptor in lung fibrosis that drives myofibroblast differentiation. *Am J Pathol* 2025; 195: 626–638.
235 Ji C, McCulloch CA. TRPV4 integrates matrix mechanosensing with Ca^{2+} signaling to regulate extracellular matrix remodeling. *FEBS J* 2021; 288: 5867–5887.
236 Tatler AL, Jenkins G. TGF-β activation and lung fibrosis. *Proc Am Thorac Soc* 2012; 9: 130–136.
237 Teoh CM, Tan SS, Tran T. Integrins as therapeutic targets for respiratory diseases. *Curr Mol Med* 2015; 15: 714–734.
238 Henderson NC, Sheppard D. Integrin-mediated regulation of TGFβ in fibrosis. *Biochim Biophys Acta Mol Basis Dis* 2013; 1832: 891–896.
239 Sundaram A, Chen C, Khalifeh-Soltani A, *et al.* Targeting integrin α5β1 ameliorates severe airway hyperresponsiveness in experimental asthma. *J Clin Invest* 2017; 127: 365–374.
240 Golovina EL, Kochubey VV, Shabanova MA, *et al.* Therapeutic prospects of αv integrins inhibition in fibrotic lung diseases and carcinogenesis. *Int J Mol Sci* 2025; 26: 6202.
241 Wan Z, Zhu Z, Wang P, *et al.* Targeting focal adhesion kinase in lung diseases: current progress and future directions. *Biomolecules* 2025; 15: 1233.
242 Zheng M, Borkar NA, Yao Y, *et al.* Mechanosensitive channels in lung disease. *Front Physiol* 2023; 14: 1302631.

243 Huang J-Q, Zhang H, Guo X-W, *et al.* Mechanically activated calcium channel PIEZO1 modulates radiation-induced epithelial–mesenchymal transition by forming a positive feedback with TGF-β1. *Front Mol Biosci* 2021; 8: 725275.

244 Rahaman SO, Grove LM, Paruchuri S, *et al.* TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest* 2014; 124: 5225–5238.

245 Saraswathibhatla A, Indana D, Chaudhuri O. Cell–extracellular matrix mechanotransduction in 3D. *Nat Rev Mol Cell Bio* 2023; 24: 495–516.

246 Ma X, Schickel ME, Stevenson MD, *et al.* Fibers in the extracellular matrix enable long-range stress transmission between cells. *Biophys J* 2013; 104: 1410–1418.

247 Sapir L, Tzlil S. Talking over the extracellular matrix: how do cells communicate mechanically? *Semin Cell Dev Biol* 2017; 71: 99–105.

248 Horowitz JC, Rogers DS, Sharma V, *et al.* Combinatorial activation of FAK and AKT by transforming growth factor-β1 confers an anoikis-resistant phenotype to myofibroblasts. *Cell Signal* 2007; 19: 761–771.

249 Ayla S, Karahüseyinogluc S. Cancer stem cells, their microenvironment and anoikis. *Crit Rev Oncog* 2019; 24: 27–34.

250 Fang Y, Liang S, Gao J, *et al.* Extracellular matrix stiffness mediates radiosensitivity in a 3D nasopharyngeal carcinoma model. *Cancer Cell Int* 2022; 22: 364.

251 Cordes N, Meineke V. Cell adhesion-mediated radioresistance (CAM-RR). Extracellular matrix-dependent improvement of cell survival in human tumor and normal cells *in vitro*. *Strahlenther Onkol* 2003; 179: 337–344.

252 Domura R, Sasaki R, Ishikawa Y, *et al.* Cellular morphology-mediated proliferation and drug sensitivity of breast cancer cells. *J Funct Biomater* 2017; 8: 18.

253 Cong X, Hubmayr RD, Li C, *et al.* Plasma membrane wounding and repair in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol* 2017; 312: L371–L391.

254 Suki B, Stamenović D, Hubmayr R. Lung parenchymal mechanics. *Compr Physiol* 2011; 1: 1317–1351.

255 Dolhnikoff M, Morin J, Ludwig MS. Human lung parenchyma responds to contractile stimulation. *Am J Respir Crit Care Med* 1998; 158: 1607–1612.

256 Yuan H, Ingenito EP, Suki B. Dynamic properties of lung parenchyma: mechanical contributions of fiber network and interstitial cells. *J Appl Physiol (1985)* 1997; 83: 1420–1431.

257 Discher DE, Janmey P, Wang Y-l. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005; 310: 1139–1143.

258 Burgstaller G, Sengupta A, Vierkotten S, *et al.* Distinct niches within the extracellular matrix dictate fibroblast function in (cell free) 3D lung tissue cultures. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L708–L723.

259 Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Bio* 2006; 7: 265–275.

260 Guo T, He C, Venado A, *et al.* Extracellular matrix stiffness in lung health and disease. *Compr Physiol* 2022; 12: 3523–3558.

261 Parker MW, Rossi D, Peterson M, *et al.* Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J Clin Invest* 2014; 124: 1622–1635.

262 Zhou Y, Horowitz JC, Naba A, *et al.* Extracellular matrix in lung development, homeostasis and disease. *Matrix Biol* 2018; 73: 77–104.

263 Liu F, Mih JD, Shea BS, *et al.* Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* 2010; 190: 693–706.

264 Shkumatov A, Thompson M, Choi KM, *et al.* Matrix stiffness-modulated proliferation and secretory function of the airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L1125–L1135.

265 Júnior C, Narciso M, Marhuenda E, *et al.* Baseline stiffness modulates the non-linear response to stretch of the extracellular matrix in pulmonary fibrosis. *Int J Mol Sci* 2021; 22: 12928.

266 Elowsson Rendin L, Löfdahl A, Åhrman E, *et al.* Matrisome properties of scaffolds direct fibroblasts in idiopathic pulmonary fibrosis. *Int J Mol Sci* 2019; 20: 4013.

267 Blokland KEC, Nizamoglu M, Habibie H, *et al.* Substrate stiffness engineered to replicate disease conditions influence senescence and fibrotic responses in primary lung fibroblasts. *Front Pharmacol* 2022; 13: 989169.

268 Zha B, Zhang C, Wu C. The stiffness of extracellular matrix (ECM) in regulating cellular metabolism. *Am J Physiol Cell Physiol* 2025; 329: C298–C306.

269 Berhan A, Harris T, Jaffar J, *et al.* Cellular microenvironment stiffness regulates eicosanoid production and signaling pathways. *Am J Respir Cell Mol* 2020; 63: 819–830.

270 Fernandez Davila JG, Singh AK, Moore DW, *et al.* Pulmonary matrix-derived hydrogels from patients with idiopathic pulmonary fibrosis induce a proinflammatory state in lung fibroblasts *in vitro*. *Mol Biol Cell* 2024; 35: ar114.

271 Lei M, Chen G. Integration of mechanics and immunology: perspective for understanding fibrotic disease mechanisms and innovating therapeutic strategies. *Acta Biomater* 2025; 199: 35–49.

272 Tschumperlin DJ, Lagares D. Mechano-therapeutics: targeting mechanical signaling in fibrosis and tumor stroma. *Pharmacol Therapeut* 2020; 212: 107575.
273 Li Y, Randriantsilefisoa R, Chen J, *et al.* Matrix stiffness regulates chemosensitivity, stemness characteristics, and autophagy in breast cancer cells. *ACS Appl Bio Mater* 2020; 3: 4474–4485.
274 Mosquera MJ, Kim S, Bareja R, *et al.* Extracellular matrix in synthetic hydrogel-based prostate cancer organoids regulate therapeutic response to EZH2 and DRD2 inhibitors. *Adv Mater* 2022; 34: 2100096.
275 Pietilä EA, Gonzalez-Molina J, Moyano-Galceran L, *et al.* Co-evolution of matrisome and adaptive adhesion dynamics drives ovarian cancer chemoresistance. *Nat Commun* 2021; 12: 3904.
276 Marinkovic A, Mih JD, Park JA, *et al.* Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-β responsiveness. *Am J Physiol Lung Cell Mol Physiol* 2012; 303: L169–L180.
277 Chen H, Qu J, Huang X, *et al.* Mechanosensing by the α6-integrin confers an invasive fibroblast phenotype and mediates lung fibrosis. *Nat Commun* 2016; 7: 12564.
278 Caliari SR, Perepelyuk M, Soulas EM, *et al.* Gradually softening hydrogels for modeling hepatic stellate cell behavior during fibrosis regression. *Integr Biol (Camb)* 2016; 8: 720–728.
279 Caliari SR, Perepelyuk M, Cosgrove BD, *et al.* Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation. *Sci Rep* 2016; 6: 21387.
280 Hewawasam RS, Blomberg R, Šerbedžija P, *et al.* Chemical modification of human decellularized extracellular matrix for incorporation into phototunable hybrid-hydrogel models of tissue fibrosis. *ACS Appl Mater Interfaces* 2023; 15: 15071–15083.
281 Lee JWN, Holle AW. Engineering approaches for understanding mechanical memory in cancer metastasis. *APL Bioeng* 2024; 8: 021503.
282 Balestrini JL, Chaudhry S, Sarrazy V, *et al.* The mechanical memory of lung myofibroblasts. *Integr Biol (Camb)* 2012; 4: 410–421.
283 Liu Y, Nizamoglu M, Zhao F, *et al.* Contrasting responses of control and fibrotic lung fibroblasts to fibrotic stimuli: the role of osteoprotegerin in extracellular matrix remodeling. *bioRxiv* 2025; preprint [https://doi.org/10.1101/2025.02.20.639137].
284 Wu DT, Jeffreys N, Diba M, *et al.* Viscoelastic biomaterials for tissue regeneration. *Tissue Eng Part C Methods* 2022; 28: 289–300.
285 Chaudhuri O, Cooper-White J, Janmey PA, *et al.* Effects of extracellular matrix viscoelasticity on cellular behaviour. *Nature* 2020; 584: 535–546.
286 Wu DT, Diba M, Yang S, *et al.* Hydrogel viscoelasticity modulates migration and fusion of mesenchymal stem cell spheroids. *Bioeng Transl Med* 2022; 8: e10464.
287 Chaudhuri O, Gu L, Klumpers D, *et al.* Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater* 2016; 15: 326–334.
288 Chaudhuri O, Gu L, Darnell M, *et al.* Substrate stress relaxation regulates cell spreading. *Nat Commun* 2015; 6: 6364.
289 Hazur J, Endrizzi N, Schubert DW, *et al.* Stress relaxation amplitude of hydrogels determines migration, proliferation, and morphology of cells in 3-D culture. *Biomater Sci* 2021; 10: 270–280.
290 Adebowale K, Gong Z, Hou JC, *et al.* Enhanced substrate stress relaxation promotes filopodia-mediated cell migration. *Nat Mater* 2021; 20: 1290–1299.
291 Martinez-Garcia FD, De Hilster RHJ, Sharma PK, *et al.* Architecture and composition dictate viscoelastic properties of organ-derived extracellular matrix hydrogels. *Polymers* 2021; 13: 3113.
292 Janson IA, Putnam AJ. Extracellular matrix elasticity and topography: material-based cues that affect cell function *via* conserved mechanisms. *J Biomed Mater Res A* 2015; 103: 1246–1258.
293 Kim D-H, Provenzano PP, Smith CL, *et al.* Matrix nanotopography as a regulator of cell function. *J Cell Biol* 2012; 197: 351–360.
294 Kim DH, Lipke EA, Kim P, *et al.* Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs. *Proc Natl Acad Sci USA* 2010; 107: 565–570.
295 Jones MG, Andriotis OG, Roberts JJ, *et al.* Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *eLife* 2018; 7: e36354.
296 Kim J, Staunton JR, Tanner K. Independent control of topography for 3D patterning of the ECM microenvironment. *Adv Mater* 2016; 28: 132–137.
297 Asadi Tokmedash M, Kim C, Chavda AP, *et al.* Engineering multifunctional surface topography to regulate multiple biological responses. *Biomaterials* 2025; 319: 123136.
298 Diehl KA, Foley JD, Nealey PF, *et al.* Nanoscale topography modulates corneal epithelial cell migration. *J Biomed Mater Res A* 2005; 75: 603–611.
299 Kim DH, Han K, Gupta K, *et al.* Mechanosensitivity of fibroblast cell shape and movement to anisotropic substratum topography gradients. *Biomaterials* 2009; 30: 5433–5444.
300 Zangi S, Hejazi I, Seyfi J, *et al.* Tuning cell adhesion on polymeric and nanocomposite surfaces: role of topography *versus* superhydrophobicity. *Mater Sci Eng C Mater Biol Appl* 2016; 63: 609–615.

301 Biela SA, Su Y, Spatz JP, *et al.* Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano-micro range. *Acta Biomater* 2009; 5: 2460–2466.
302 Charest JL, Eliason MT, García AJ, *et al.* Combined microscale mechanical topography and chemical patterns on polymer cell culture substrates. *Biomaterials* 2006; 27: 2487–2494.
303 Ge L, Yang L, Bron R, *et al.* Topography-mediated fibroblast cell migration is influenced by direction, wavelength, and amplitude. *ACS Appl Bio Mater* 2020; 3: 2104–2116.
304 Hale NA, Yang Y, Rajagopalan P. Cell migration at the interface of a dual chemical-mechanical gradient. *ACS Appl Mater Interfaces* 2010; 2: 2317–2324.
305 Klymov A, Bronkhorst EM, te Riet J, *et al.* Bone marrow-derived mesenchymal cells feature selective migration behavior on submicro- and nano-dimensional multi-patterned substrates. *Acta Biomater* 2015; 16: 117–125.
306 Mostaco-Guidolin LB, Osei ET, Ullah J, *et al.* Defective fibrillar collagen organization by fibroblasts contributes to airway remodeling in asthma. *Am J Respir Crit Care Med* 2019; 200: 431–443.
307 Tjin G, Xu P, Kable SH, *et al.* Quantification of collagen I in airway tissues using second harmonic generation. *J Biomed Opt* 2014; 19: 36005.
308 Hewitt RJ, Puttur F, Gaboriau DCA, *et al.* Lung extracellular matrix modulates KRT5^{+} basal cell activity in pulmonary fibrosis. *Nat Commun* 2023; 14: 6039.
309 Tino M, Wright J. Interactions of surfactant protein A with epithelial cells and phagocytes. *Biochim Biophys Act* 1998; 1408: 241–263.
310 Han S, Mallampalli RK. The role of surfactant in lung disease and host defense against pulmonary infections. *Ann Am Thorac Soc* 2015; 12: 765–774.
311 Ochs M, Timm S, Elezkurtaj S, *et al.* Collapse induration of alveoli is an ultrastructural finding in a COVID-19 patient. *Eur Respir J* 2021; 57: 2004165.
312 Vladar EK, Nayak JV, Milla CE, *et al.* Airway epithelial homeostasis and planar cell polarity signaling depend on multiciliated cell differentiation. *JCI Insight* 2016; 1: e88027.
313 Walsh D, Bevan J, Harrison F. How does airway surface liquid composition vary in different pulmonary diseases, and how can we use this knowledge to model microbial infections? *Microorganisms* 2024; 12: 732.
314 Chen Z, Zhong M, Luo Y, *et al.* Determination of rheology and surface tension of airway surface liquid: a review of clinical relevance and measurement techniques. *Respir Res* 2019; 20: 274.
315 Button B, Boucher RC. Role of mechanical stress in regulating airway surface hydration and mucus clearance rates. *Respir Physiol Neurobiol* 2008; 163: 189–201.
316 Carpenter J, Lynch SE, Cribb JA, *et al.* Buffer drains and mucus is transported upward in a tilted mucus clearance assay. *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L910–L9l8.
317 Loiseau E, Gsell S, Nommick A, *et al.* Active mucus–cilia hydrodynamic coupling drives self-organization of human bronchial epithelium. *Nature Physics* 2020; 16: 1158–1164.
318 Roth D, Şahin AT, Ling F, *et al.* Structure and function relationships of mucociliary clearance in human and rat airways. *Nat Commun* 2025; 16: 2446.
319 Sone N, Konishi S, Igura K, *et al.* Multicellular modeling of ciliopathy by combining iPS cells and microfluidic airway-on-a-chip technology. *Sci Transl Med* 2021; 13: eabb1298.
320 Gerovac BJ, Valencia M, Baumlin N, *et al.* Submersion and hypoxia inhibit ciliated cell differentiation in a notch-dependent manner. *Am J Respir Cell Mol Biol* 2014; 51: 516–525.
321 Mitchell B, Jacobs R, Li J, *et al.* A positive feedback mechanism governs the polarity and motion of motile cilia. *Nature* 2007; 447: 97–101.
322 Guirao B, Meunier A, Mortaud S, *et al.* Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. *Nat Cell Biol* 2010; 12: 341–350.
323 Oltean A, Schaffer AJ, Bayly PV, *et al.* Quantifying ciliary dynamics during assembly reveals stepwise waveform maturation in airway cells. *Am J Respir Cell Mol* 2018; 59: 511–522.
324 Bowden DH. Cell turnover in the lung. *Am Rev Respir Dis* 1983; 128: S46–S48.
325 Virchow R. Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre [Cellular pathology, based on physiological and pathological histology]. Berlin, Hirschwald, 1858. Available from: https://collections.nlm.nih.gov/ext/dw/61220740R/PDF/61220740R.pdf
326 Plikus MV, Wang X, Sinha S, *et al.* Fibroblasts: origins, definitions, and functions in health and disease. *Cell* 2021; 184: 3852–3872.
327 Zhang X, Shi X, Xie F, *et al.* Dissecting pulmonary fibroblasts heterogeneity in lung development, health and diseases. *Heliyon* 2023; 9: e19428.
328 Travaglini KJ, Nabhan AN, Penland L, *et al.* A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature* 2020; 587: 619–625.
329 Sikkema L, Ramírez-Suástegui C, Strobl DC, *et al.* An integrated cell atlas of the lung in health and disease. *Nat Med* 2023; 29: 1563–1577.
330 Younesi FS, Miller AE, Barker TH, *et al.* Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. *Nat Rev Mol Cell Biol* 2024; 25: 617–638.

331 Liu F, Lagares D, Choi KM, *et al.* Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L344–LL57.

332 van Soldt BJ, Cardoso WV. Hippo-Yap/Taz signaling: complex network interactions and impact in epithelial cell behavior. *Wiley Interdiscip Rev Dev Biol* 2020; 9: e371.

333 Zhu L, Liu L, Wang A, *et al.* Positive feedback loops between fibroblasts and the mechanical environment contribute to dermal fibrosis. *Matrix Biol* 2023; 121: 1–21.

334 Cui Y, Hameed FM, Yang B, *et al.* Cyclic stretching of soft substrates induces spreading and growth. *Nat Commun* 2015; 6: 6333.

335 Dabaghi M, Singer R, Noble A, *et al.* Influence of lung extracellular matrix from non-IPF and IPF donors on primary human lung fibroblast biology. *Biomater Sci* 2025; 13: 1721–1741.

336 Jaffar J, Yang S-H, Kim SY, *et al.* Greater cellular stiffness in fibroblasts from patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L59–L65.

337 Fitzgerald AA, Weiner LM. The role of fibroblast activation protein in health and malignancy. *Cancer Metastasis Rev* 2020; 39: 783–803.

338 Baird SK, Allan L, Renner C, *et al.* Fibroblast activation protein increases metastatic potential of fibrosarcoma line HT1080 through upregulation of integrin-mediated signaling pathways. *Clin Exp Metastasis* 2015; 32: 507–516.

339 Kolanko E, Cargnoni A, Papait A, *et al.* The evolution of *in vitro* models of lung fibrosis: promising prospects for drug discovery. *Eur Respir Rev* 2024; 33: 230127.

340 Dabaghi M, Carpio MB, Saraei N, *et al.* A roadmap for developing and engineering *in vitro* pulmonary fibrosis models. *Biophys Rev* 2023; 4: 021302.

341 Nizamoglu M, de Hilster RHJ, Zhao F, *et al.* An *in vitro* model of fibrosis using crosslinked native extracellular matrix-derived hydrogels to modulate biomechanics without changing composition. *Acta Biomaterialia* 2022; 147: 50–62.

342 Hewawasam RS, Blomberg R, Šerbedžija P, *et al.* Chemical modification of human decellularized extracellular matrix for incorporation into phototunable hybrid-hydrogel models of tissue fibrosis. *ACS Appl Material Interfaces* 2023; 15: 15071–15083.

343 Asano S, Ito S, Takahashi K, *et al.* Matrix stiffness regulates migration of human lung fibroblasts. *Physiol Rep* 2017; 5: e13281.

344 Caracena T, Blomberg R, Hewawasam RS, *et al.* Alveolar epithelial cells and microenvironmental stiffness synergistically drive fibroblast activation in three-dimensional hydrogel lung models. *Biomater Sci* 2022; 10: 7133–7148.

345 Al Yazeedi S, Abokor AF, Brussow J, *et al.* The effect of the mechanodynamic lung environment on fibroblast phenotype *via* the Flexcell. *BMC Pulm Med* 2024; 24: 362.

346 Zheng M, Yao Y, Borkar NA, *et al.* Piezo channels modulate human lung fibroblast function. *Am J Physiol Lung Cell Mol Physiol* 2024; 327: L547–LL56.

347 Wang Q, Herrmann J, Worthington KS, *et al.* Cyclic mechanical loading of photopolymerized methacrylated hydrogels for probing interdependent effects of strain, stiffness, and substrate composition in pulmonary fibrogenesis. *Sci Rep* 2025; 15: 5997.

348 Southern BD, Li H, Mao H, *et al.* A novel mechanoeffector role of fibroblast S100A4 in myofibroblast transdifferentiation and fibrosis. *J Biol Chem* 2024; 300: 105530.

349 Huh D, Matthews BD, Mammoto A, *et al.* Reconstituting organ-level lung functions on a chip. *Science* 2010; 328: 1662–1668.

350 Mandrycky CJ, Howard CC, Rayner SG, *et al.* Organ-on-a-chip systems for vascular biology. *J Mol Cell Cardiol* 2021; 159: 1–13.

351 Park S, Newton J, Hidjir T, *et al.* Bidirectional airflow in lung airway-on-a-chip with matrix-derived membrane elicits epithelial glycocalyx formation. *Lab Chip* 2023; 23: 3671–3682.

352 Zamprogno P, Wüthrich S, Achenbach S, *et al.* Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. *Commun Biol* 2021; 4: 168.

353 Onal S, Alkaisi MM, Nock V. Microdevice-based mechanical compression on living cells. *iScience* 2022; 25: 105518.

354 Sedlak JM, Clyne AM. A modified parallel plate flow chamber to study local endothelial response to recirculating disturbed flow. *J Biomech Eng* 2020; 142: 0410031-04100312.

355 Rosmark O, Ibáñez-Fonseca A, Thorsson J, *et al.* A tunable physiomimetic stretch system evaluated with precision-cut lung slices and recellularized human lung scaffolds. *Front Bioeng Biotechnol* 2022; 10: 995460.

356 Maurat E, Raasch K, Leipold AM, *et al.* A novel *in vitro* tubular model to recapitulate features of distal airways: the bronchioid. *Eur Respir J* 2024; 64: 2400562.

357 Shiwarski DJ, Hudson AR, Tashman JW, *et al.* 3D bioprinting of collagen-based high-resolution internally perfusable scaffolds for engineering fully biologic tissue systems. *Sci Adv* 2025; 11: eadu5905.

358 Tallapragada NP, Cambra HM, Wald T, *et al.* Inflation–collapse dynamics drive patterning and morphogenesis in intestinal organoids. *Cell Stem Cell* 2021; 28: 1516–32.

359 AhmadianKia N, Goli-Malekabadi Z, Pournaghmeh S. Application of cell laden hydrogels with temporally tunable stiffness in biomedical research. *J Biomater Appl* 2023; 38: 179–193.

360 Lorenzo-Martín LF, Hübscher T, Bowler AD, *et al.* Spatiotemporally resolved colorectal oncogenesis in mini-colons *ex vivo*. *Nature* 2024; 629: 450–457.

361 Kalli M, Poskus MD, Stylianopoulos T, *et al.* Beyond matrix stiffness: targeting force-induced cancer drug resistance. *Trends Cancer* 2023; 9: 937–954.

362 Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep* 2014; 15: 1243–1253.

363 Kalli M, Stylianopoulos T. Defining the role of solid stress and matrix stiffness in cancer cell proliferation and metastasis. *Front Oncol* 2018; 8: 55.

364 Kalli M, Papageorgis P, Gkretsi V, *et al.* Solid stress facilitates fibroblasts activation to promote pancreatic cancer cell migration. *Ann Biomed Eng* 2018; 46: 657–669.

365 Delarue M, Montel F, Vignjevic D, *et al.* Compressive stress inhibits proliferation in tumor spheroids through a volume limitation. *Biophys J* 2014; 107: 1821–1828.

366 Desmaison A, Frongia C, Grenier K, *et al.* Mechanical stress impairs mitosis progression in multi-cellular tumor spheroids. *PLoS One* 2013; 8: e80447.

367 Stylianopoulos T. The solid mechanics of cancer and strategies for improved therapy. *J Biomech Eng* 2017; 139: 021004.

368 Jiang Y, Zhang H, Wang J, *et al.* Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J Hematol Oncol* 2022; 15: 34.

369 De Martino D, Bravo-Cordero JJ. Collagens in cancer: structural regulators and guardians of cancer progression. *Cancer Res* 2023; 83: 1386–1392.

370 Jain RK. Antiangiogenic therapy for cancer: current and emerging concepts. *Oncology (Williston Park)* 2005; 19: 7–16.

371 Böckelmann LC, Schumacher U. Targeting tumor interstitial fluid pressure: will it yield novel successful therapies for solid tumors? *Expert Opin Ther Targets* 2019; 23: 1005–1014.

372 Zanotelli MR, Reinhart-King CA. Mechanical forces in tumor angiogenesis. *Adv Exp Med Biol* 2018; 1092: 91–112.

373 Basil MC, Morrisey EE. Lung regeneration: a tale of mice and men. *Semin Cell Dev Biol* 2020; 100: 88–100.

374 Danopoulos S, Shiosaki J, Al Alam D. FGF signaling in lung development and disease: human *versus* mouse. *Front Genet* 2019; 10: 170.

375 Stucki AO, Sauer UG, Allen DG, *et al.* Differences in the anatomy and physiology of the human and rat respiratory tracts and impact on toxicological assessments. *Regul Toxicol Pharmacol* 2024; 150: 105648.

376 Ramirez GO, Mariano CA, Carter D, *et al.* Visceral pleura mechanics: characterization of human, pig, and rat lung material properties. *Acta Biomater* 2024; 189: 388–398.

377 Li X-N, Lin Y-P, Han M-M, *et al.* Modulating fibrotic mechanical microenvironment for idiopathic pulmonary fibrosis therapy. *Adv Mater* 2024; 36: 2407661.

378 Leask A, Asmaa F, Naik A. A modest proposal: targeting αv integrin-mediated activation of latent TGFβ as a novel therapeutic approach to treat scleroderma fibrosis. *Expert Opin Investig Drugs* 2024; 33: 279–285.

379 Decaris ML, Schaub JR, Chen C, *et al.* Dual inhibition of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ reduces fibrogenesis in lung tissue explants from patients with IPF. *Respir Res* 2021; 22: 265.

380 Wilkinson AL, John AE, Barrett JW, *et al.* Pharmacological characterisation of GSK3335103, an oral $\alpha_v\beta_6$ integrin small molecule RGD-mimetic inhibitor for the treatment of fibrotic disease. *Eur J Pharmacol* 2021; 913: 174618.

381 Raghu G, Mouded M, Chambers DC, *et al.* A Phase IIb randomized clinical study of an anti-$\alpha_v\beta_6$ monoclonal antibody in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2022; 206: 1128–1139.

382 Teicher BA. TGFβ-directed therapeutics: 2020. *Pharmacol Ther* 2021; 217: 107666.

383 Lukey PT, Harrison SA, Yang S, *et al.* A randomised, placebo-controlled study of omipalisib (PI3K/mTOR) in idiopathic pulmonary fibrosis. *Eur Respir J* 2019; 53: 1801992.

384 Mercer PF, Woodcock HV, Eley JD, *et al.* Exploration of a potent PI3 kinase/mTOR inhibitor as a novel anti-fibrotic agent in IPF. *Thorax* 2016; 71: 701–711.

385 Setargew YFI, Wyllie K, Grant RD, *et al.* Targeting lysyl oxidase family meditated matrix cross-linking as an anti-stromal therapy in solid tumours. *Cancers (Basel)* 2021; 13: 491.

386 Montesi SB, Cosgrove GP, Turner SM, *et al.* Dual $\alpha_v\beta_6$ and $\alpha_v\beta_1$ inhibition over 12 weeks reduces active type I collagen deposition in individuals with idiopathic pulmonary fibrosis: a phase 2, double-blind, placebo-controlled clinical trial. *Am J Respir Crit Care Med* 2025; 211: 1229–1240.

387 Lancaster L, Cottin V, Ramaswamy M, *et al.* Bexotegrast in patients with idiopathic pulmonary fibrosis: the INTEGRIS-IPF clinical trial. *Am J Respir Crit Care Med* 2024; 210: 424–434.

388 Khalil N, Manganas H, Ryerson CJ, *et al.* Phase 2 clinical trial of PBI-4050 in patients with idiopathic pulmonary fibrosis. *Eur Respir J* 2019; 53: 1800663.

389 Raghu G, Brown KK, Collard HR, *et al.* Efficacy of simtuzumab *versus* placebo in patients with idiopathic pulmonary fibrosis: a randomised, double-blind, controlled, phase 2 trial. *Lancet Respir Med* 2017; 5: 22–32.

390 Daniels CE, Lasky JA, Limper AH, *et al.* Imatinib treatment for idiopathic pulmonary fibrosis: randomized placebo-controlled trial results. *Am J Respir Crit Care Med* 2010; 181: 604–610.

391 Richeldi L, Azuma A, Cottin V, *et al.* Nerandomilast in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2025; 392: 2193–2202.

392 Richeldi L, Azuma A, Cottin V, *et al.* Design of a phase III, double-blind, randomised, placebo-controlled trial of BI 1015550 in patients with idiopathic pulmonary fibrosis (FIBRONEER-IPF). *BMJ Open Respir Res* 2023; 10: e001563.

393 Kim GH, Zhang X, Brown MS, *et al.* Minimum clinically important difference in Quantitative Lung Fibrosis score associated with all-cause mortality in idiopathic pulmonary fibrosis: subanalysis from two phase II trials of pamrevlumab. *BMJ Open* 2025; 15: e094559.

394 Raghu G, Hamblin MJ, Brown AW, *et al.* Long-term evaluation of the safety and efficacy of recombinant human pentraxin-2 (rhPTX-2) in patients with idiopathic pulmonary fibrosis (IPF): an open-label extension study. *Respir Res* 2022; 23: 129.

395 Raghu G, van den Blink B, Hamblin MJ, *et al.* Long-term treatment with recombinant human pentraxin 2 protein in patients with idiopathic pulmonary fibrosis: an open-label extension study. *Lancet Respir Med* 2019; 7: 657–664.

396 Raghu G, van den Blink B, Hamblin MJ, *et al.* Effect of recombinant human pentraxin 2 *vs* placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. *JAMA* 2018; 319: 2299–2307.

397 Miller CE, Franco V, Smith JS, *et al.* Parenteral treprostinil induction for rapid attainment of therapeutic doses of oral treprostinil. *Respir Med* 2023; 218: 107374.

398 Saggar R, Khanna D, Vaidya A, *et al.* Changes in right heart haemodynamics and echocardiographic function in an advanced phenotype of pulmonary hypertension and right heart dysfunction associated with pulmonary fibrosis. *Thorax* 2014; 69: 123–129.

399 Liu L, Song W, Zeng J, *et al.* Evaluating a specific dual ROCK inhibitor against bleomycin-induced idiopathic pulmonary fibrosis in rats. *ACS Pharmacol Transl Sci* 2022; 5: 819–828.

400 Jiang C, Huang H, Liu J, *et al.* Fasudil, a Rho-kinase inhibitor, attenuates bleomycin-induced pulmonary fibrosis in mice. *Int J Mol Sci* 2012; 13: 8293–8307.

401 Zhou Y, Huang X, Hecker L, *et al.* Inhibition of mechanosensitive signaling in myofibroblasts ameliorates experimental pulmonary fibrosis. *J Clin Invest* 2013; 123: 1096–1108.

402 Bei Y, Hua-Huy T, Duong-Quy S, *et al.* Long-term treatment with fasudil improves bleomycin-induced pulmonary fibrosis and pulmonary hypertension *via* inhibition of Smad2/3 phosphorylation. *Pulm Pharmacol Ther* 2013; 26: 635–643.

403 Wagner DE, Alsafadi HN, Mitash N, *et al.* Inhibition of epithelial cell YAP-TEAD/LOX signaling attenuates pulmonary fibrosis in preclinical models. *Nat Commun* 2025; 16: 7099.

404 Kimura RH, Wang L, Shen B, *et al.* Evaluation of integrin $\alpha_v\beta_6$ cystine knot PET tracers to detect cancer and idiopathic pulmonary fibrosis. *Nat Commun* 2019; 10: 4673.

405 Mori Y, Kramer V, Novruzov E, *et al.* Initial results with [^{18}F]FAPI-74 PET/CT in idiopathic pulmonary fibrosis. *Eur J Nucl Med Mol Imaging* 2024; 51: 1605–1611.

406 van Leer B, Londema M, Kasalak Ö, *et al.* [^{68}Ga]FAPI PET/CT reveals increased pulmonary fibroblast activation protein expression in long COVID patients after ICU discharge. *Eur J Nucl Med Mol Imaging* 2025; 53: 565–573.

407 Xie J, Shi D, Tang G, *et al.* Optimal image-derived input function models for multi-parameter analysis and acceptably reduced acquisition time in [^{18}F]F-FAPI-42 PET total-body dynamic imaging for lung cancer. *EJNMMI Phys* 2025; 12: 90.

408 Li Y, Jing X, Liu Q, *et al.* Theranostics targeting fibroblast activation protein in the pan-cancer field. *Int J Cancer* 2025; 157: 2209–2223.

409 Vizier R, Garnier AR, Dias A, *et al.* SPECT imaging of lysyl oxidase-like 2 in a model of idiopathic pulmonary fibrosis. *Mol Pharm* 2023; 20: 3613–3622.