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Extracellular Matrix Stiffness in Lung Health and Disease

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Abstract

The extracellular matrix (ECM) provides structural support and imparts a wide variety of environmental cues to cells. In the past decade, a growing body of work revealed that the mechanical properties of the ECM, commonly known as matrix stiffness, regulate the fundamental cellular processes of the lung. There is growing appreciation that mechanical interplays between cells and associated ECM are essential to maintain lung homeostasis. Dysregulation of ECM-derived mechanical signaling via altered mechanosensing and mechanotransduction pathways is associated with many common lung diseases. Matrix stiffening is a hallmark of lung fibrosis. The stiffened ECM is not merely a sequelae of lung fibrosis but can actively drive the progression of fibrotic lung disease. In this article, we provide a comprehensive view on the role of matrix stiffness in lung health and disease. We begin by summarizing the effects of matrix stiffness on the function and behavior of various lung cell types and on regulation of biomolecule activity and key physiological processes, including host immune response and cellular metabolism. We discuss the potential mechanisms by which cells probe matrix stiffness and convert mechanical signals to regulate gene expression. We highlight the factors that govern matrix stiffness and outline the role of matrix stiffness in lung development and the pathogenesis of pulmonary fibrosis, pulmonary hypertension, asthma, chronic obstructive pulmonary disease (COPD), and lung cancer. We envision targeting of deleterious matrix mechanical cues for treatment of fibrotic lung disease. Advances in technologies for matrix stiffness measurements and design of stiffness-tunable matrix substrates are also explored.

Introduction

The extracellular matrix (ECM) is a cell-secreted, well-organized meshwork composed of various fibrous proteins and polysaccharides. It provides structural support to the cell and imparts crucial biochemical and mechanical cues that regulate cellular phenotype and function (63, 174). Studies in the past decade have established that the mechanical properties or stiffness of the ECM, defined as the extent to which the ECM resists deformation in

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response to mechanical stress, is of fundamental importance in lung health and disease. An optimal ECM rigidity is essential for lung development and the maintenance of proper lung function throughout adulthood (392, 450), whereas abnormal matrix stiffness is associated with many pathological conditions, including fibrosis of both the airways and parenchyma, pulmonary hypertension (PH), chronic obstructive pulmonary disease (COPD), asthma, and lung cancer (70, 370, 393, 405). Compelling evidence indicates that ECM stiffening, a hallmark of tissue fibrosis, is not merely an epiphenomenon of lung fibrosis but can actively drive dysregulation of cellular function, leading to persistent and/or progressive pulmonary fibrosis (158, 226, 451).

Cells can convert mechanical stimuli into chemical signals, a process that involves mechanosensing and mechanotransduction. Cells sense mechanical cues through transmembrane receptors and intracellular proteins that link them to the cytoskeleton. Mechanotransduction of matrix mechanical cues initiates intracellular signaling pathways, leading to the activation of gene transcription and protein production that alter the cell phenotype. Cell-based studies are routinely performed on plastic or glass surfaces that are many orders of magnitude stiffer than the native lung ECM. A variety of biocompatible materials with tunable stiffness have been developed, which has enabled the study of cellular responses to substrate stiffness. While 2D matrix substrates have provided a simple model to study the effects of matrix stiffness on cellular function, novel 3D platforms that better mimic the physiological lung environment will provide greater insights into the pathogenesis of lung diseases. Assessing the micromechanical properties of lung tissue has become possible by the advent of high-resolution techniques, such as atomic force microscopy (AFM). Ongoing development of noninvasive modalities is expected to provide experimental and clinical assessments of the efficacy of therapeutic strategies targeting lung ECM mechanics.

The goal of this article is to present an overview of the functional role of ECM stiffness in lung health and disease. We begin by summarizing the effects of matrix stiffness on cellular behavior and phenotype in various lung cell types, activities of key biomolecules (e.g., TGF-β, microRNAs, epigenetic modifiers, and chromatin), and important physiological processes (e.g., host immune response and cellular metabolism). We discuss the potential mechanisms by which cells probe matrix stiffness and transduce physical signals to chemical signals that control gene expression. We examine the factors that govern the stiffness of the ECM, focusing on enzymatic and nonenzymatic crosslinking of the ECM, specific ECM components, and cellular forces. We review the role of ECM stiffness in lung development and discuss the changes in ECM mechanics during the progression of common lung diseases, including pulmonary fibrosis, PH, asthma, COPD, and lung cancer. For investigators interested in conducting matrix stiffness studies, we describe the available technologies for properly quantifying ECM stiffness and methods for fabricating stiffness-tunable matrix substrates. Finally, we envision targeting matrix stiffness as a promising novel strategy for treating lung diseases, in particular persistent/progressive pulmonary fibrosis.

Physiological Function of Matrix Stiffness

Regulation of phenotype and behavior of various cell types

Matrix rigidity plays an important role in regulating cell phenotype and behavior. A variety of lung cell types can sense and respond to the substrate stiffness on which they are adhered to (as in 2D culture) or surrounded by (as in 3D culture). By doing so, cells can modulate their morphology, growth, differentiation, migration/invasion, metabolism, immune response, and other processes.

Fibroblasts—Fibroblasts are particularly sensitive to mechanical cues. Matrix stiffness within the pathophysiological range exerts profound effects on the key aspects of fibroblast biology. It has been shown that stiffening of the ECM promotes lung fibroblast proliferation, enhances collagen synthesis, and suppresses transcription of genes encoding matrix-degrading proteases (226), suggestive of lung fibroblast activation. Matrix stiffness-dependent fibroblast activation occurs at least in part by suppression of the endogenous COX-2/PGE2 inhibitory pathway (226). The capacity of lung fibroblasts to generate force and deform the ECM is associated with myofibroblast differentiation and the fibrogenic function. We have shown that lung fibroblasts cultured on stiff matrices express higher levels of α-smooth muscle actin (αSMA), a marker of myofibroblast differentiation, and phosphorylated myosin light chain (MLC), which regulates the contractility of actin cytoskeleton, than those cultured on soft matrices (170, 356). Taken together, these findings indicate that matrix stiffening promotes lung myofibroblast differentiation. Others have shown that both baseline and TGF-β1-stimulated contraction of lung fibroblasts depended on matrix stiffness (251). Stiff matrix enhances lung fibroblast migration, as demonstrated by both platelet-derived growth factor (PDGF)-induced chemotaxis assay and a random walk assay (10). We have found that stiff matrix upregulates expression of α6-integrin subunit, a major cellular receptor of laminin in the basement membrane (BM). Interactions of α6β1 integrins with laminin bring idiopathic pulmonary fibrosis (IPF) lung fibroblasts into the close proximity to the BM, facilitating MMP-2-dependent pericellular proteolysis of collagen IV and fibroblast invasion into the BM (64). These findings provide a potential mechanism underlying stiff matrix-dependent fibrogenic fibroblast invasion into the ECM. Consistent with our findings, it has been shown that primary human fibroblasts spontaneously form invadosome-like protrusions (ILPs) in 3D soft ECM (143). Soft tissues with low levels of collagen matrix and larger pores are expected to be more permissive to cell invasion. In contrast, invasion through small pores in stiff matrices causes high nuclear stress, which can potentially disrupt nuclear lamina and increase DNA damage, leading to cell death (154).

Lung fibroblasts primed on soft matrices and subsequently transferred to stiff matrices maintained lower cell numbers, αSMA stress fiber expression, collagen production, and active TGF-β1 compared to those cultured exclusively on stiff matrices. On the other hand, lung fibroblasts primed under pathologically stiff conditions exhibited continued high levels of αSMA expression in stress fibers, contractility, proliferation, collagen production, and active TGF-β1 when returned to healthy soft conditions (16). These findings suggest that fibroblasts maintain a “mechanical memory” that mediates their long-term behavior.

Though, there is also evidence of IPF and normal lung fibroblasts being equally responsive to changes in matrix stiffness, as the proliferative and contractile differences between them were ablated when cultured on physiologically soft matrices (250).

Endothelial cells—Endothelial cells (ECs) lining the vessel wall control vascular permeability and homeostasis. Maintenance of endothelial barrier integrity is essential to prevent vascular leak and edema associated with acute lung injury and acute respiratory distress syndrome (ARDS) (197). Either decreasing or increasing ECM stiffness relative to normal levels has been shown to disrupt junctional integrity and increase pulmonary vascular leakage (245), indicating that ECM mechanics plays a key role in control of vascular permeability. *In vitro*, human pulmonary micro- and macrovascular ECs grown on soft substrates developed few actin stress fibers, whereas the amount of stress fibers increased with increasing matrix stiffness (37). ECM stiffness also modulates the localization of vascular endothelial (VE) cadherin on endothelial monolayers (43). Furthermore, pharmaceutical inhibition of focal adhesion kinase (FAK) activity prevents matrix stiffness-dependent disruption of endothelial integrity and vascular leak both *in vitro* and *ex ovo* (408), suggesting that matrix stiffness regulates endothelial barrier integrity through activation of FAK. Further, alterations of the ECM stiffness regulate EC proliferation (434). Increased ECM stiffness inhibits EC branching morphogenesis by both blocking pseudopodium initiation and retracting branches after their successful initiation (121). Endothelial response to mechanical cues of the ECM is heterogeneous, as vascular EC populations respond differentially to changes in substrate stiffness (175, 420).

Epithelial cells—Increased matrix stiffness upregulates expression of α3 laminin in rat type II alveolar epithelial cells (AT2), leading to enhanced laminin and fibronectin (Fn) assembly into fibrils (106). Matrix stiffness also regulates the organizations of focal adhesion (FA) structure and actin cytoskeleton but affects neither differentiation of AT2 into AT1 nor epithelial-mesenchymal transition (EMT) *in vitro* (106). Desmosomes within intercellular junctions of adjacent epithelial cells lay a crucial role in maintaining the mechanical integrity of epithelium. We have recently found that desmoplakin (DSP), the most abundant component of desmosomes, is a matrix stiffness-regulated mechanosensitive protein (313). Patients with IPF express significantly higher levels of DSP in lung tissues than healthy subjects (118). Genome-wide association studies (GWAS) identified a strong association of the *DSP* gene locus with the susceptibility to IPF (3, 118, 164, 254). Our findings point to a potential mechanism of DSP expression dysregulation by matrix stiffness in fibrotic conditions. Further studies are needed to understand the functional role of DSP in the pathogenesis of lung fibrosis. In epithelial cells of marsupial kidney, gene transcription and nuclear recruitment of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), histone H3 hyperacetylation, and chromatin decondensation occurred above 50 kPa, whereas DNA replication became efficient above 200 kPa (205). These findings suggest that matrix stiffness regulates gene transcription and DNA replication in epithelial cells. Corneal epithelial cells exhibited greater and more rapid movement on substrates of 30 kPa than substrates of 8 kPa, similar to that of the corneal BM (288). These findings suggest that the rigidity of the BM plays a critical role in migration of the corneal epithelial sheet.

Macrophages—The stiffness of tissues where macrophages reside varies significantly from compliant organs such as lung (in the magnitude of <1 kPa) to extremely hard tissues such as bone (in the magnitude of MPa). Macrophages respond to the mechanical cues and adapt to the environment by adjusting their shape and function. It has been shown that stiff matrix increases the macrophage contact area with the ECM compared with soft matrix (66, 161, 298). Human peripheral blood monocyte-derived macrophages show enhanced migration on stiff matrix compared with soft matrix (1, 161). This increased ability of migration is partially regulated by both PI3K-Akt1 and Rac pathways (161). Alveolar macrophages cultured on stiff matrix have increased phagocytosis than those cultured on soft matrix (298). Phorbol 12-myristate 13-acetate (PMA)-primed THP-1 human monocytes cultured in the presence of IFN-γ and lipopolysaccharide (LPS) display a higher level of pro-inflammatory cytokine TNF-α when cultured on a stiff matrix compared with those on a soft matrix (285, 358), suggesting that matrix stiffness modulates macrophage polarization in response to soluble simulants. Whether or not matrix stiffness alone is sufficient to regulate macrophage polarization remains to be determined. A recent study showed increased arginase-1 expression in bone marrow-derived macrophages (BMDMs) cultured on stiff compared with soft matrices. In contrast, expression of inducible nitric oxide synthase (iNOS) a marker of classically activated macrophages, was reduced in BMDMs cultured on stiff *versus* soft matrix (188). A separate study also showed increased arginase-1 and TGF-β1 gene expression in BMDMs cultured on stiff compared with soft matrix. Together, these findings suggest that BMDMs acquire an alternatively activated phenotype when exposed to matrix substrates with stiffness comparable to the fibrotic lungs. Furthermore, the authors showed that when hydrogels of different stiffness are implanted subcutaneously, more profibrotic $CD206^{+}$ macrophages were recruited to the site of implantation of stiff hydrogels than soft hydrogels (188). Stiff matrix-dependent macrophage polarization is likely linked to inhibition of the NF-κb pathway. In addition to the NF-κb pathway, mechanosensitive transcriptional factor Yes-associated protein (YAP) along with epigenetic modulation of histone methylation and acetylation have been found to contribute to stiffness-induced macrophage polarization (255).

Neutrophils—Polymorphonuclear neutrophils are capable of sensing differences in substrate stiffness. Neutrophils on soft gels are less adhesive, preventing the production of traction forces, while neutrophils on stiff gels adhere firmly (277, 365). Neutrophil transmigration is an important immune process in response to acute lung injury and infection. Substrate stiffness dictates the ability and efficiency of neutrophils to migrate *in vitro* (278, 367). Neutrophils migrate more slowly but more persistently on stiffer substrates, resulting in neutrophils moving greater distances over time (277). Neutrophil response to stiffness during chemokinesis is biphasic with the optimum stiffness for motility depending on the concentration of ECM coating on the gel (365). Neutrophil extravasation requires a coordinated effort between neutrophils and endothelial cells. The fraction of transmigrating neutrophils correlates with increasing stiffness below the endothelium. Suppression of EC contraction through inhibition of myosin light-chain kinase (MLCK) decreases neutrophil transmigration on stiff substrates, while transmigration on soft substrates is unaffected (366). Neutrophil transmigration promotes EC retraction and subsequent pore formation

in endothelium on stiff substrates (366), indicating that stiffness-enhanced neutrophil transmigration causes serious endothelial damage.

Smooth muscle cells—Airway smooth muscle cells (ASMCs) are important in both regulations of airway tone and caliber under normal conditions and airway hyperresponsiveness (AHR). ASMCs cultured on collagen-conjugated polyacrylamide (PA) hydrogels stiffer than normal airway display faster proliferation, reduced reactivity to histamine, and lower VEGF secretion than those cultured at stiffness equivalent to normal airway (347, 406). The extent of ASMC spreading is proportional to the elastic modulus of matrix substrates (347). Stiffening of the vascular wall leads to increased proliferation of vascular smooth muscle cells (VSMCs) due to upregulation of platelet-derived growth factor receptor (PDGFR) signaling (49). Both FA and actin cytoskeletal assembly in VSMCs depend on the mechanical properties of the underlying ECM (110, 301). It has been found that the maximum speed of VSMC migration can be achieved by optimizing substrate stiffness (304).

Stem cells—The mechanical properties of the ECM trigger cellular responses essential for many aspects of stem cell functions. Embryonic stem cells (ESCs) differentiate and deposit the ECM early in development. As the ECM content increases during development, there is increased connection between the ESCs and matrix stiffness. *In vitro* studies have shown that the contact area and proliferation of mouse ESCs increase as a function of substrate stiffness (114). Stiff substrates downregulate expression of inner cell mass-associated genes while promoting expression of genes in the late epiblast stage, suggesting that a lower stiffness is associated with the maintenance of pluripotency (39). Matching cell and substrate stiffness to the intrinsic mouse embryonic environment promotes self-renewal and the pluripotent state of mouse ESCs (73). Culturing mouse embryonic fibroblasts (MEFs) on soft substrates promotes expression of stem cell markers and enhances expression of two of the transfected Yamanaka factors, Oct3/4 and Nanog, suggesting that soft matrix plays a role in the initiation of reprogramming of somatic cells into induced pluripotent stem (iPS) cells (160).

Matrix stiffness also acts as a potent regulator of adult stem cells. In a pioneer study, Engler and colleagues observed that human mesenchymal stem cells (MSCs) adapt to the stiffness of their matrix substrates by differentiating toward lineages that reflect the substrate stiffness (112). Culturing adipose-derived stem cells (ASCs) on gels with the stiffness of native adipose tissue significantly upregulates adipogenic markers in the absence of exogenous adipogenic growth factors. With increased substrate stiffness, ASCs lose their original morphology and fail to upregulate adipogenic markers (437). Unipotent muscle progenitor cells cultured on soft substrates self-renew and contribute extensively to muscle regeneration when subsequently transplanted into mice. In contrast, muscle stem cells cultured on rigid plastic dishes do not self-renew and lose “stemness,” leading to diminished regenerative potential (134).

Stem cells tend to spread more and form stronger actin-myosin stress fibers on stiffer substrates (95, 96, 297). The level of matrix adhesion strength correlates with the commitment of MSCs to specific cell lineages (112). MSCs acquire a spheroid shape

on matrix resembling adipose tissue and a spindle shape on stiffer matrix mimicking the elasticity of striated muscle (412). Suppression of MSC adhesion strength on rigid substrates replicates cell behavior on soft substrates, as evidenced by expression of lineage-specific markers (95). MSCs migrate faster on soft matrix compared with stiff matrix under an identical EGF chemokine gradient, indicating that soft matrix enhances chemotaxis of MSCs (334). In contrast, MSCs seeded on hydrogels with a gradient stiffness of 1 kPa/mm in the presence of standard cell culture medium migrated toward the stiffer area. Moreover, the presence of gradient stiffness was associated with higher migration rates in the range of 1 to 100 kPa (395). In contrast, a reduction in migration speed at higher stiffness conditions has been observed on uniform hydrogels (151, 176, 233, 277).

In sum, the studies highlight the critical role of matrix stiffness in directing stem cell fate and therefore the importance of mechano-niche in stem cell biology.

Regulation of immune response

Matrix stiffness plays a significant role in regulating both innate and adaptive immune cell function. T and B cells sense the mechanical microenvironment either directly by mechanosensitive T-cell receptors (TCRs) and B-cell receptors (BCRs) or through their interactions with antigen-presenting cells (APCs) (173, 446). The stiffness of lymphoid tissues increases during immune activation (86, 159). Increasing matrix stiffness has been shown to enhance $CD4^+$ T-cell migration and its binding with APCs (244). Stiff matrix-conditioned $CD4^+$ T cells have increased CD25 expression and production of IL-2 and IFN-γ compared with $CD4^+$ T cells cultured on soft matrix (192, 244). In contrast, it has been reported that the activity of human naïve $CD4^+$ and $CD8^+$ cells is inversely correlated with matrix stiffness (279). In the latter study, the authors used a range of stiffness from 100 kPa to 2 MPa, which is much higher than the studies used by others. It is possible that T-cell response to matrix stiffness could be a bell-shaped normal distribution where an extremely stiff matrix might inhibit T-cell activation. Furthermore, a minimal matrix stiffness may be required to initiate immune activation as there was no difference in IL-2 secretion when $CD4^+$ T cells were cultured on matrix with stiffness less than 10 kPa (192). T-cell activation requires the formation of immunological synapses (IS) between APCs and T cells. It has been found that the size of IS increases on stiff matrix by Wiskott-Aldrich syndrome protein (WASP)-dependent and lymphocyte function-associated antigen 1 (LFA-1)-independent mechanisms (244). Mechanotransduction of the stiffness signal in $CD4^+$ T cell is associated with activation of zeta chain of TCR associated protein kinase 70 (ZAP70), proto-oncogene tyrosine-protein kinase Src, and YAP (192, 258). Stiffness-induced YAP activation suppresses T-cell proliferation via metabolic reprogramming (258). The cytotoxic effects of $CD8^+$ cells are enhanced on stiff matrix compared to soft matrix, and the enhanced cytotoxicity is related to stiffness-mediated perforin release (21). Increased BCR recruitment to the IS is augmented on stiff (22 kPa) than intermediate (7 kPa) or soft (2 kPa) matrix. BCR activation is related to tyrosine kinase phosphorylation but is inversely linked to PI3K activation (442). B cells' lack of FAK expression or function does not respond to different matrix stiffness (346), suggesting that mechanosensing of matrix stiffness by B cells depends on FAK.

In addition to neutrophils and macrophages described in previous sections, primary natural killer (NK) cells have a bell-shaped response to various matrix stiffness with maximal activation at 150 kPa (267). Stiffness-mediated NK cell activation appears to require the presence of biochemical signaling such as major histocompatibility complex (MHC) class I polypeptide-related sequence A (MICA). While greater stiffness increases NK cell adhesion, it has no impact on the cell contact area. Mechanistically, NK cell activation is associated with stiffness-dependent interaction between β-actin and the SH2-domain-containing protein tyrosine phosphatase-1 (SHP-1) (253). Matrix stiffness also modulates dendritic cell (DC) function. It was found that stiff matrix-conditioned bone marrow-derived DCs (BMDCs) have increased pro-inflammatory cytokine TNF-α production compared to BMDCs cultured on soft matrix (60). This stiffness-dependent pro-inflammatory status is closely related to an increment in glucose uptake and glycolysis. Stiffness activates mechanosensitive transcription factor PDZ-binding motif (TAZ) in DCs. Genetic ablation of TAZ in DCs attenuates pro-inflammatory activation even in the presence of the extracellular stiff signal. PIEZO1, a surface Ca^{2+}-dependent mechanosensitive channel, also contributes activation of mouse splenic DCs and human peripheral blood monocyte-derived DCs in response to stiff matrix signals (60). DCs cultured on 12-kPa matrix substrates express lower levels of CD206, CD209, and CCR7 than DCs cultured on either 2 or 50 kPa matrix substrates (260).

Regulation of cellular metabolism

Metabolism provides energy for cell proliferation and function. Transfer of human bronchial epithelial cells from stiff to soft substrates causes a downregulation of glycolysis via proteasomal degradation of the rate-limiting glycolytic enzyme phosphofructokinase (PFK) (296). In contrast, transformed non-small cell lung cancer (NSCLC) cells maintain high glycolytic rates and retain PFK expression regardless of changing matrix stiffness. These data reveal a connection between cell metabolism and the mechanical properties of the ECM. The changes of metabolic pathways enable normal lung epithelial cells to tune energy production in variable microenvironments. Matrix stiffening upregulates expression of glutaminase (GLS1) in pulmonary arterial endothelial cells (PAECs), resulting in enhanced glutaminolysis crucial for sustained proliferation and migration of PAECs (30). Proline metabolism is important for energy production, redox balance, and protein (particularly collagen) synthesis. Increased matrix stiffness upregulates proline production, which in turn promotes proliferation and apoptosis resistance of lung cancer cells (145). Mechanical stimuli from the tumor microenvironment provide crucial molecular signals to guide both tumor cells and carcinoma-associated fibroblasts (CAFs) in capturing nutrients to support their metabolic needs. The mechanical response of lung epithelial cancer cells results in glutamate accumulation accompanied by an impaired TCA cycle and a reduced production of aspartate (31). Mechanically regulated accumulation of glutamate in CAFs leads to production of aspartate and control of actomyosin-dependent ECM remodeling and matrix stiffening. Furthermore, aspartate produced by CAFs is released within the tumor microenvironment and uptaken by the tumor cells to supply their proliferative needs (31). These results indicate that matrix stiffening coordinates cancer cell proliferation and stromal cell-dependent ECM remodeling with their energetic and biosynthetic requirements to promote metastasis.

Regulation of biomolecule activity

TGF-β1—TGF-β signaling controls tissue growth and morphogenesis during embryonic development and maintains tissue homeostasis throughout adulthood. Dysregulation of TGF-β signaling contributes to the pathogenesis of a variety of diseases including cancer, fibrosis, autoimmune, and vascular diseases. TGF-β1 is considered the central profibrotic cytokine that causes excessive ECM production and promotes fibrogenic myofibroblast differentiation (163, 386). TGF-β1 is synthesized as a homodimer together with a latency-associated peptide (LAP). The association with LAP prevents the binding of TGF-β1 to cell receptors. The majority of cell types secrete TGF-β1 as a large latent complex (LLC), consisting of TGF-β1, LAP, and the latent TGF-β1-binding protein (LTBP-1). LTBP-1 is an ECM protein that binds to several other ECM components, including fibrillin-1, Fn, and vitronectin, thereby depositing latent TGF-β1 in the ECM (7, 385). Activation of TGF-β1 by its dissociation from LAP and/or the ECM-bound LLC can occur through various mechanisms, including mechanical tension-induced conformational change of the latent complex. Induced contraction of cultured lung fibroblasts activates latent TGF-β1 from a preformed ECM (418, 449). Similar results were obtained by stimulating the contraction of epithelial and smooth muscle cells (133, 378, 426). The level of latent TGF-β1 activated by cell contraction increases with augmented substrate stiffness (133, 418), suggesting that the efficiency of latent TGF-β1 activation depends on the mechanical properties of the ECM. Elevated cell remodeling strain under the fibrotic conditions stiffens LTBP-1-containing ECM and thereby primes latent TGF-β1 for subsequent activation (203). Together, stiff ECM provides mechanical resistance to allow activation of latent TGF-β1.

mRNA—Alternative polyadenylation (APA) at the 3′-end of pre-mRNAs is a widespread and important mechanism involved in the regulation of gene expression. APA considerably affects mRNA stability, cellular localization, and protein translation efficiency (381). Dysregulation of APA is associated with multiple disease states, including lung fibrosis (413). We have found that matrix stiffening downregulates expression of mammalian cleavage factor I and promotes APA in favor of the proximal poly(A) site usage in the 3′-UTRs of type I collagen (COL1A1) in primary human lung fibroblasts. Selectively targeting mRNA transcripts for 3′-UTR shortening results in increased type I collagen biogenesis, contributing to lung fibrogenesis (452). Alternative splicing of pre-mRNAs gives rise to different protein isoforms that play a crucial role in regulating cellular function. The stiffness of the microenvironment modulates intracellular splicing events. For example, stiff matrix increases the amount of extra domain-B splice variant of fibronectin (EDB-FN), a hallmark of tumor angiogenesis, *in vitro* and within mouse mammary tumors through mechanisms involving phosphorylation of serine/arginine-rich (SR) splicing factors, Rho-associated protein kinase (ROCK)-mediated contractility, and activation of the PI3K/AKT pathway (42). Changes in mechanical microenvironment also instruct alternative splicing of CD44 pre-mRNA, which encodes a membrane receptor for hyaluronic acid (HA), in gastric cancer cells (46). Asymmetric localization of mRNAs contributes to the formation and maintenance of polarized cells mainly through local translation of protein factors (32). Stiff substrates promote the formation of a network of detyrosinated microtubules. This posttranslationally modified microtubule network is required to support adenomatous polyposis coli (APC)-dependent RNA localization at the leading edge and protrusions

of migrating cells (407). The findings provide a link between mechanosensitive RNA localization and cell migration.

Epigenetic modifiers—Emerging evidence indicates a key role of epigenetic mechanisms, including DNA methylation, histone modifications, and noncoding RNA-dependent mechanisms, in mediating matrix mechanical signal-induced modulation of gene transcription programs and chromatin structure. We have found that stiff matrix regulates *DSP*, a risk allele of IPF (3, 118, 164, 254), by induction of demethylation of the *DSP* promoter. Targeted DNA methylation by CRISPR/dCas9/Dnmt3A-mediated epigenome editing reverses stiff matrix-dependent DSP expression and restores the epigenetic control of DSP expression (313). Lung fibroblasts cultured on stiff matrices display elevated global histone 3 lysine 9 (H3K9) methylation compared with fibroblasts cultured on physiologically soft substrates. H3K9 methylation contributes to fibroblast activation by activating profibrotic gene transcription (218). In NSCLC, stiff ECM upregulates expression of histone acetyltransferase (HAT) p300, which increases the binding of c-Myb and LEF to the *DDR2* promoter and upregulates DDR2 expression. Increased DDR2 expression contributes to EMT and lung cancer cell invasion (201). In vascular SMCs, matrix stiffening induces a contractile-to-synthetic transition through downregulation of DNA methyltransferase 1 (DNMT1) and subsequent global DNA methylation (425). MicroRNAs (miRNAs) are noncoding RNAs that regulate gene expression by affecting mRNA stability and translation into protein. A recent study showed that ECM stiffening downregulates miR-7 levels in PAECs. A mechanosensitive QKI-miR-7-SRSF1 signal axis controls endothelial migration across the pulmonary vasculature (421). In PAH, vascular matrix stiffness controls mechanoactivation of transcriptional coactivators YAP and TAZ. Mechanosensitive YAP/TAZ signaling activates miR-130/301, which regulate lysyl oxidase (LOX) activity. The study identifies a feedback loop that coordinates ECM remodeling and sustains matrix stiffening in PAH (29). Fibrocytes produce miRNAs and package them into extracellular vesicles (EVs). Human circulating fibrocytes and fibrocytes in bronchoalveolar lavage fluid (BALF) express higher levels of miR-21-5p, a regulator of tissue fibrosis, on rigid substrates than soft substrates (332).

Regulation of nuclear stability, chromatin accessibility, and genomic integrity

Lamins are intermediate filament proteins found in cell nuclei. They contribute to nuclear stability, chromatin regulation, and various signaling pathways that affect gene expression. Lamins sense mechanical signals from outside and respond by reinforcing the cytoskeleton. It has been shown that the protein levels of A-type lamins (lamin A/C) are regulated by matrix stiffness, whereas expression of B-type lamins is not subject to matrix stiffness-mediated regulation. High lamin A/C levels promote osteogenic differentiation of bone marrow-derived MSCs, whereas low lamin-A levels are associated with lipogenic differentiation of MSCs (374). Soft matrix couples with myosin-II activity to promote lamin A/C phosphorylation at Ser22. Phosphorylation of lamin A/C promotes nuclear softening and proteolysis (55). Stiffness-associated increase in lamin A/C is accompanied by decreases in lamin B receptor (LBR), which contribute to lipid biosynthesis (54). Furthermore, mammary epithelial cells cultured on stiff matrix display more accessible chromatin sites. This facilitates the binding of Sp1 transcription factor and correlates with

the regulation of tumorigenicity. In contrast, cells cultured on soft environments recapitulate the chromatin state observed *in vivo* (363). A recent study showed that matrix rigidity alters chromatin accessibility of naive lung fibroblasts. The sites with altered chromatin accessibility are in close proximity to fibroblast activation genes such as Col1A1 and Acta2 (189). Increased ECM stiffness promotes activation of histone deacetylase, resulting in fibroblast differentiation into myofibroblasts (403). Finally, low ECM stiffness activates MAP4K4/6/7 kinases, resulting in phosphorylation of ubiquitin in cells. Phosphorylated ubiquitin impairs RNF8-mediated ubiquitin signaling at DNA double-strand break (DSB) sites, leading to DSB repair deficiency and increased cellular susceptibility to genotoxic agents (91).

Mechanisms of Matrix Stiffness Sensing and Mechanotransduction

Matrix stiffness-induced changes in cell behavior generally involve two major processes, mechanosensing and mechanotransduction. Mechanosensing refers to the ability of cells to perceive mechanical stimuli from the ECM and remodel their cytoskeleton. Mechanotransduction is the ability of cells to respond to mechanical signals with changes in intracellular biochemical signals, ultimately leading to the regulation of gene expression and cellular function (see Figure 1).

Cellular mechanosensors

Cells employ a variety of mechanosensors to probe the mechanical properties of the ECM. Integrin-based FA complexes are the main cellular components responsible for detecting mechanical stimuli exerted by the ECM (95). FA-mediated cellular responses are inherently associated with the reorganization of the cytoskeleton and the initiation of complex signaling cascades (165). Mechanosensing by αv integrins within the FAs is associated with the fibrotic responses in tissue remodeling (108, 336). Thy-1, a GPI-anchored glycoprotein, attenuates FA sensitivity to matrix stiffness by modifying αvβ3 and αvβ5 integrin interactions with the ECM (119, 449). Integrins dynamically associate with adaptor proteins including talin and vinculin (371). Talin is essential for coupling cell adhesion with integrin mechanosensing and is thus required for cells to detect tissue stiffness (13). Talin itself changes its conformation in response to mechanical tension. This conformational change exposes a cryptic domain of talin, leading to increased recruitment of actin-binding protein vinculin (89, 137, 429). The actin cytoskeleton mediates stiffness sensing at spatial scales larger than FAs, which depends on the interactions between myosin II molecular motors and actin filaments (146). A motor-clutch model has been proposed for mechanosensing mechanisms. In this model, nonmuscle myosin II acts as a cellular motor, and FAs as a clutch; myosin II pulling on FA-anchored actin filaments coordinately probes substrate stiffness and regulates actin polymerization (61). In addition to an increase in their number, actin stress fibers become aligned as substrate stiffness increases (441), suggesting that the reorganization of actin cytoskeleton could play a role in sensing matrix stiffness at a larger scale. Mechanosensing can also occur through nonintegrin membrane receptors, including mechanically activated ion channels, growth factor receptors, and G-protein-coupled receptors (GPCRs) (214). Previous studies have shown that matrix stiffening activates transient receptor potential vanilloid 4 (TRPV4) channels in lung fibroblasts.

Inhibition of TRPV4 blocks matrix stiffness-dependent lung myofibroblast differentiation (315). The role of FAK in sensing mechanical force among other cues has been well characterized and reviewed elsewhere (377, 398).

Intracellular mechanotransduction pathways

The RhoA/Rho kinase (ROCK)/actin cytoskeleton/MKL1 pathway—Cells convert ECM-derived mechanical signals into biochemical signals through mechanotransduction signaling pathways, of which the small Rho GTPase ROCK-mediated mechanotransduction pathway has been extensively studied (283). Upon activation by matrix mechanical signals, RhoA/ROCK elicit phosphorylation of the downstream targets, many of which are associated with the regulation of FA assembly, actin cytoskeletal organization, stress fiber formation, and cell contractility. The net outcome of RhoA/ROCK signaling is the shifting of actin dynamics toward enhanced actin polymerization (319). Our previous studies have shown that matrix stiffening promotes the production and activation of RhoA in normal human lung fibroblasts, resulting in increased ROCK activity and changes in actin dynamics in favor of filamentous actin polymerization (170). Megakaryoblastic leukemia 1 protein (MKL1; also known as MRTF-A, MAL) is a serum response factor (SRF) cofactor. It binds to monomeric G-actin via the N-terminal RPEL motifs, effectively sequestering itself in the cytoplasm. Actin polymerization releases MKL1 from G-actin, resulting in its nuclear translocation, where MKL1 associates with SRF to drive gene transcription (262). We found that matrix stiffening promotes RhoA/ROCK-dependent nuclear translocation of MKL1 and activation of αSMA gene expression, a marker of myofibroblast differentiation. Mouse lung fibroblasts deficient in Mkl1 do not respond to matrix stiffening with increased αSMA expression, whereas ectopic expression of human MKL1 cDNA restores the ability of Mkl1 null lung fibroblasts to express αSMA (170). We also showed that inhibition of RhoA/ROCK not only abrogates stiff matrix-induced lung fibroblast-to-myofibroblast differentiation but also promotes the existing lung myofibroblasts to undergo mitochondria-dependent intrinsic apoptosis *in vitro* and *in vivo* by downregulation of MKL1-dependent BCL-2 expression (451). These findings strongly support the role of the RhoA/ROCK/actin cytoskeleton/MKL1-mediated mechanotransduction pathway in sustained lung myofibroblast phenotype. In addition to its interaction with SRF, MKL1 also coordinates with histone H3 lysine 4 (H3K4) methylation on the MMP9 promoter to drive lung cancer cell migration and invasion (68, 382), suggesting that nuclear MKL1 can regulate gene transcription epigenetically. Furthermore, cyclic strain upregulation of tenascin-C (TNC) expression was found to occur by SAP domain-dependent, SRF-independent interactions of MKL1 with the TNC promoter (11).

The Hippo signaling/YAP/TAZ pathway—The Hippo signaling pathway is a complex network of proteins that control organ size via cellular proliferation, survival, and differentiation. The canonical Hippo pathway starts with a kinase cascade of MST1/2 and LATS1/2, which phosphorylate two effectors, YAP and its close paralog, transcriptional coactivator with PDZ-binding motif (TAZ, also known as WWTR1), at serine residues, resulting in cytoplasmic retention of YAP and TAZ. YAP/TAZ are potent transcriptional coactivators that associate with various transcription factors, predominantly members of the TEAD family. In the absence of the activation of Hippo signaling, YAP/TAZ are

imported into the nucleus where they promote TEAD-mediated gene transcription, whereas activation of the Hippo signaling inhibits YAP/TAZ activity. On stiff substrates YAP/TAZ are predominantly nuclear and become increasingly cytoplasmic on softer substrates (102), suggesting that YAP/TAZ localization and activity are controlled by the stiffness of the ECM. Earlier studies have shown that LATS activation downstream of the Hippo cascade is not required for the regulation of YAP/TAZ by mechanical signals (8, 102, 117, 388, 447), indicating that mechanoregulation of YAP/TAZ could be independent of the canonical Hippo pathway. However, more recent studies reported that deletion of LATS1 and LATS2 abrogates the cytoplasmic localization of YAP on soft substrates (75, 259). Moreover, transcriptomic analysis demonstrated that transcriptional changes in gene expression in response to altered matrix stiffness are dependent on both LATS1/2 and YAP/TAZ (259). Thus, mechanoregulation of YAP/TAZ likely involves both Hippo signaling-dependent and -independent mechanisms. Matrix stiffness-dependent nuclear accumulation of TAZ/YAP is similar to that of MKL1. It has been shown that inhibition of Rho or the cytoskeletal tension blocks YAP/TAZ transcriptional activity, whereas triggering F-actin polymerization and stress fiber formation promotes YAP/TAZ activation (102). Despite that actin cytoskeletal reorganization is a dominant regulator of YAP/TAZ, YAP and TAZ are not known to directly bind to actin. Previous studies have shown that loss of CapZ or Cofilin potently rescues YAP/TAZ nuclear localization, transcriptional activity, and cell proliferation under soft matrix conditions (8). These findings suggest that YAP/TAZ are regulated, at least in part, by F-actin-capping and -severing proteins. In human IPF lung tissues, YAP and TAZ were found to be highly expressed in spindle-shaped fibroblastic cells, with prominent nuclear localization of TAZ. *In vitro* studies showed that increasing matrix stiffness drives nuclear localization of YAP and TAZ, whereas knockdown of YAP/TAZ attenuates proliferation, contraction, and matrix production in human lung fibroblasts. Mechanistic studies suggested that matrix stiffness-dependent fibroblast activation is driven in part by TGF-β-independent expression of the YAP/TAZ target plasminogen activator inhibitor (PAI)-1 (225). Increased YAP/TAZ activity is found in pulmonary artery smooth muscle cells (PASMCs) derived from human pulmonary arterial hypertension (PAH) and experimental PH. Stiffness-dependent YAP/TAZ activation promotes cell growth, actin polymerization, and LOX expression via reduced COX-2 and prostaglandin activity in PASMCs (93).

Cross talk between MKL1 and YAP/TAZ—Coimmunoprecipitation analysis has shown that MKL1 associates with YAP in binding of the CCN1 (Cyr61) promoter. Knockdown of either YAP or MKL1 can block CCN1 expression by S1P-mediated activation of GPCRs. Functionally, YAP/MKL1-dependent CCN expression drives glioblastoma cell proliferation in response to S1P stimulation (438). Knockdown of either MKL1 or YAP blocks mechanical strain-induced spreading and proliferation of primary MEFs on soft matrices (82). TAZ also associates with MKL1 by a WW domain/PPxY-dependent mechanism (65). Deletion of TAZ or MKL1 increases the cytosolic-nuclear shuttling and nuclear uptake of the other factor (357), suggesting that MKL1 and TAZ reciprocally mitigate nuclear accumulation of each other. Furthermore, MKL1/SRF signaling promotes TAZ gene expression in breast cancer cells (223). Knockdown of MKL1 downregulates TAZ mRNA and protein in a kidney epithelial cell line (357). On the other hand, MKL1 and TAZ

antagonize each another on the stimulatory effect on the αSMA promoter which harbors *cis*-elements for both transcription factors in close proximity (223). Together, YAP/TAZ and MKL1 exhibit complex cross talk at the multiple levels, which may contribute to fine-tuning mechanical signals that regulate gene transcriptional programs.

Additional mechanosensitive transcription factors—While MKL1 and YAP/TAZ are the most studied mechanosensitive transcription factors, additional mechanically regulated transcription factors have been identified. Lung adenocarcinoma cells exhibit lower NF-κB activity on soft substrates than on stiff substrates. Matrix stiffness-dependent NF-κB activation requires phosphorylation of myosin regulatory light chain and actomyosin contractile activity (177). Our studies demonstrated that stiff matrix upregulates α_6-integrin expression by ROCK-dependent phosphorylation and activation of the c-Fos/c-Jun transcription factor complex in profibrotic lung fibroblasts, which mediates fibroblast invasion into the BM (64). STAT3 is an essential mediator of lung myofibroblast activation and function. Studies have shown that STAT3 phosphorylation is constitutively active on stiff matrix in a ligand/receptor-independent and ROCK- and JAK2-dependent fashion (281). Zinc Finger Protein 416 (ZNF416) has been recently identified as a matrix stiffness-regulated transcriptional regulator that controls various lung fibroblast function, including proliferation, matrix synthesis, and contractility (189). Human lung fibroblasts cultured on stiff matrix express higher levels of phosphorylated ELK1 than cells cultured on soft matrix. Stiff matrix increases nuclear translocation of ELK1 and its binding to immobilized oligonucleotides containing the ELK1 transcription factor-binding motifs. Knockdown of ELK1 by siRNAs blocks stiff matrix-dependent expression of MDM4, an endogenous inhibitor of p53 (312). Furthermore, stiff ECM activates β-catenin in MSCs, which binds to the Wnt1 promoter region and upregulates the Wnt gene transcription (99). This finding reveals a mechanical feedforward mechanism that controls and maintains stem cell differentiation.

Modulation of Matrix Stiffness

The mechanical properties of tissues are influenced by the degree of enzymatic and/or nonenzymatic crosslinking of the ECM, specific ECM components, and cell-ECM interactions.

Enzymatic crosslinking of the ECM

Enzymatic crosslinking is typically mediated by the LOX family of enzymes. The LOX family comprises LOX and four lysyl oxidase-like proteins (LOXL1-4). LOX and LOXLs are secreted copper-dependent amine oxidases, which catalyze the oxidative deamination of primary amine groups into reactive aldehydes in collagen and elastin (345). These reactive aldehydes spontaneously condense with other aldehydes or ε-amino groups of selected lysine and hydroxylysine residues to form covalent intra- and intermolecular crosslinks. The covalent crosslinking between telopeptides and triple-helical domains of the neighboring collagen molecules stiffens collagen fibrils by resisting intermolecular sliding (104). Highly crosslinked collagens are resistant to proteolytic degradation by matrix metalloproteinases (MMPs) (178). Previous studies found that expression of LOX, LOXL1, and LOXL2 are

increased in IPF vs. non-IPF lung tissues (12, 19, 384). Inhibition of LOX activity limits fibrillar collagen crosslinking and interferes ECM stiffening in a decellularized matrix model (384). *Loxl1*-deficient mice have less crosslinked lung collagen and reduced lung tissue stiffness compared to the wild-type control animals (23). Pharmacological targeting of LOXL2 is efficacious in treating experimental lung fibrosis in mice (19) and reverses profibrotic markers in lung tissue samples from IPF subjects (411). In elastase-induced emphysema model, hamsters exposed to cigarette smoke show a decrease in lung LOX compared to uninjured control animals, whereas lung LOX activity is restored in recovering animals under standard atmospheric conditions (290). This study suggests that impairment of the production of LOX and the synthesis of crosslinked elastin by smoke inhalation exacerbates alveolar destruction. Dysregulation of LOXs has been found in both human idiopathic PAH and experimental PH (271). Downregulation of LOX attenuates hypoxia-induced PH in rats (424). Thus, alterations of lung matrix crosslinking can affect pulmonary vascular remodeling associated with PH. Studies have shown that higher expression of LOX is an independent predictor of poor prognosis in patients with early-stage lung adenocarcinoma (415). Metastatic lung adenocarcinomas produce a stable type of collagen crosslinks driven by high expression of lysyl hydroxylase 2 (LH2), and LH2 depletion in tumor cells leads to a reduction in tumor stiffness and metastasis (67).

The family of transglutaminases (TGs) consists of eight family members (TG1-7 and factor XIII7), of which TG2 is the most abundant and ubiquitously expressed enzyme. TG2 and most other family members are calcium-dependent enzymes that catalyze covalent crosslinking between the γ-carboxy-amine group of glutamine residues and the ε-amino group of lysine residues, generating ε-(γ-glutamyl)lysine isopeptide crosslinks (141). TG2-mediated crosslinking of collagen increases ECM stiffness (123, 360). TG2 crosslinking provides a covalent linkage of fibrillin to tropoelastin, which stabilizes the elastin precursor and facilitates the elastic fiber assembly (234). TG2 crosslinking plays an essential role in the maturation of lung parenchyma during development (337). TG2 expression and TG2-generated crosslinks are increased in lung biopsy sections in human subjects with IPF compared with normal control subjects (287, 306). Inhibition of TG2, but not of LOX, activity enhances the turnover of ECM *in vitro* (306). TG2-deficient mice develop significantly reduced lung fibrosis compared with wild-type mice (280, 287). These data suggest a role of TG2 in both human and experimental pulmonary fibrosis. It has been found that the levels of TG2 in sputum and plasma are elevated in patients with COPD and correlated with lung function reduction (284). Furthermore, TG2 expression is upregulated in the alveolar septa of preterm infants with bronchopulmonary dysplasia (BPD) and in hyperoxia-exposed mouse pups (419), suggesting a role of perturbed TG2 activity in arrested alveolarization associated with BPD. Inhibition of TG2 activity restores normal dihydroxylysinonorleucine and hydroxylysylpiridinoline collagen crosslink abundance under hyperoxic conditions, indicating that TG2 contributes to disordered lung alveolarization by interruption of normal crosslinking of collagen (265). Finally, TG2 is overexpressed in NSCLC as compared with histologically normal lung epithelium, where TG2 expression is minimal (443). TG2 expression significantly correlates with the recurrence and shorter disease-free survival (DFS) in NSCLC (71).

Nonenzymatic crosslinking of the ECM

Long-lived structural ECM proteins, such as collagen and elastin, undergo continuous nonenzymatic glycation crosslinking during aging and in certain disease conditions such as diabetes. This abnormal protein crosslinking is mainly mediated by advanced glycation end products (AGEs). Glycation differs from the enzymatically regulated glycosylation process. It is a nonenzymatic chemical addition of the carbonyl group of free reducing sugars (primarily glucose) to reactive amino groups of proteins. This generates a reversible Schiff base. The Schiff base is then rearranged to form a more stable Amadori product. Over the time, the Amadori products dehydrate, condense, fragment, and finally form a complex array of AGE products (397). AGEs slowly accumulate *in vivo* during aging, and the rate and extent of accumulation are accelerated particularly in individuals with diabetes (397). In contrast to normal collagen crosslinks that occur only at two discrete sites at the N- and C-terminal ends of the molecule, AGEs can form crosslinks throughout the collagen molecule (50). Accumulation of AGEs induces abnormal matrix crosslinking that ultimately leads to a progressive increase in tissue stiffness. Lung is rich in collagen and is influenced by nonenzymatic glycation. Pentosidine, a well-characterized AGE crosslink, has been found to accumulate with aging in the lungs of humans, mice, and rats (24, 312). Accumulation of AGE crosslinks contributes to aging-associated changes in lung stiffness and function, such as loss of elastic recoil and subsequently reduced total lung capacity (312, 348). Patients with IPF and aged mice subjected to bleomycin-induced lung injury present higher levels of AGEs in the lung and/or blood than the control subjects (208, 242, 312), indicating that AGE crosslinking is increased in aged lungs and accelerates in lung fibrosis. Diabetes is identified as a risk factor of IPF (105, 113, 130, 140, 202), and hyperglycemia could further augment nonenzymatic AGE crosslinking in patients with IPF.

Specific ECM components

Collagen, elastin, and fibronectin—In normal aging or as a result of tissue injury, the elastic fibers become fragmented and discontinuous. The damaged elastic fibers are generally not regenerated because elastin expression is turned off in adult tissues. Instead, more collagen is produced to replace elastin, subsequently decreasing the amount of elastin compared to collagen. This results in a shift of the mechanical properties of the ECM to the stiffer range of collagen fibers (401). Fn is a large dimeric glycoprotein that exists in its insoluble form as a part of the ECM. Fn-null murine livers develop more extensive cirrhosis, which is accompanied by increased liver tissue stiffness and deteriorated hepatic function. Mechanistically, mutant livers show elevated TGF-β bioavailability and TGF-β-dependent LOX expression, which in turn modulate ECM remodeling and stiffening (180). These findings provide a functional link between Fn-mediated control of TGF-β bioavailability and collagen fibril stiffness regulated by LOX.

Glycosaminoglycans and proteoglycans—Glycosaminoglycans (GAGs) are long, unbranched chains of repeating disaccharide units that have important roles in retaining water in tissue (341). Removal of GAGs affects ECM fiber hydration, leading to altered tissue mechanics. GAGs also directly interact with collagen fibrils via electrostatic interaction to regulate collagen fibril spacing (340). AFM has shown that depletion of GAGs changes the stiffness of both adventitia and media of the porcine aorta (22), suggesting

that GAGs contribute to the mechanical properties of arteries. HA (also named hyaluronan) is a unique type of GAGs. HA does not contain any sulfate and is not found covalently attached to proteins. Enzymatic disruption of HA increases ECM stiffness in adult bovine cartilage (427). Targeted expression of HA synthase (HAS)-2 in VSMCs promotes HA accumulation throughout the tunica media, resulting in increased vessel stiffness in mice (59). In contrast to the matrix stiffening function of HA in vessels, increased HA content lowers the stiffness of inflamed pancreatic islets in autoimmune pancreatitis, which was attributed to the hygroscopic properties of HA (269). HA-containing soft substrates promote proliferation and migration of cardiac myocytes and glioma cells to the levels seen under the rigid matrix conditions (72, 307). These findings suggest that matrices composed of crosslinked HA may change local stiffness, or the HA-receptor signaling might be an important element in mechanical control of cell behavior.

Proteoglycans (PGs) are formed by GAGs bound to a core protein. The crosslinking of PGs to other matrix proteins such as the collagen network results in the formation of supermolecular structures that function to increase tissue stiffness. The class I small leucine-rich proteoglycans (SLRPs), decorin and biglycan, are key regulators of collagen fibrillogenesis and matrix assembly. They have overlapping functions that permit functional compensation in mice deficient of the individual PG (77). Acute ablation of both *Dcn* (decorin) and *Bgn* (biglycan) genes in mature tendons results in decreased tendon stiffness and a significant increase in percent relaxation and tissue viscosity compared to wild-type controls (320), indicating a critical role for decorin and biglycan in maintaining mechanical homeostasis of adult tendon. The class II collagen-binding SLRPs, fibromodulin, and lumican are widely distributed in interstitial connective tissues. Lumican and fibromodulin compete for the same binding site on collagen type I (100). In tissues where both PGs are present, fibromodulin is required early in collagen fibrillogenesis and stabilizes small fibril intermediates, whereas lumican is needed at a later stage and primarily limits lateral growth of fibrils (320). Fibromodulin deficiency leads to significant reduction in tendon stiffness in mice, whereas lumican deficiency does not affect tendon stiffness. Interestingly, tendon stiffness is reduced in a lumican-dependent manner on the fibromodulin-deficient background (320).

Perlecan is a large heparin sulfate (HS) PG that presents in all BMs as well as the pericellular matrix (PCM) of mature cartilage. In perlecan knockout mice, BMs are degraded in regions with increased mechanical loading (79), suggesting that perlecan contributes to the mechanical stability of the BMs. In a murine model of Schwartz-Jampel syndrome, disruption of perlecan incorporation significantly decreases the stiffness of both the interstitial matrix and chondrocytes during cartilage development (428). Heparinase III-mediated enzymatic removal of HS chains from perlecan increases PCM elastic moduli in the PCM, specifically at the interior region expressing both perlecan and type VI collagen (417). In contrast, heparinase III digestion has no effects on ECM elasticity. These findings suggest that heparinase III could be utilized to selectively manipulate the mechanical properties of perlecan-rich matrix such as the PCM.

Stiffening of the large arteries (arteriosclerosis) is associated with severe adverse hemodynamic consequences, including widening of the pulse pressure and altered shear

stress. Single-nucleotide polymorphisms (SNPs) in the genes for aggrecan (rs2882676 and rs2293087) and fibulin-1 (rs2018279 and rs2238823) predict aortic stiffness in young healthy subjects (432). Aggrecan transcription declines with age and is significantly lower in individuals with stiffer aortas. Loss and degradation of aggrecan and fibulin-1 appear to be important drivers for age-related aortic stiffening (432). However, upregulation of fibulin-1 has been linked with increased stiffness (153) and kidney disease (338). Taken together, these data suggest that aggrecan and fibulin-1 are key regulators of the arterial wall stiffness (240).

Cell contractility and other cellular factors

Physical interactions between cells and the microenvironment actively alter the mechanical properties of the ECM. Active microrheology (AMR) analysis showed that cells grown in 3D type 1 collagen gels modulate the mechanical microenvironment in a cellular contraction-dependent manner. Inhibition of cellular contractility instantaneously softens the pericellular space and reduces stiffness heterogeneity (198). Nonlinear stress inference microscopy (NSIM) demonstrated that cell contraction induces large stresses, which generate a massive stiffness gradient over an extended region in 3D matrices of collagen, fibrin, and Matrigel (152). K14 promoter-driven expression of ROCK increases actomyosin contractile activity in mouse skin. This results in increased collagen deposition and skin tissue stiffening (329). Together, these data suggest that cellular contractility significantly modifies the mechanical properties of the surrounding ECM.

The binding of αvβ3 integrin to matrix Fn is restrained by cell surface protein Thy-1. Augmentation of αvβ3 integrin engagement through loss of Thy-1 stiffens the provisional ECM, leading to the progression of mouse lung fibrosis in a bleomycin injury model (120). This study suggests a central role for αvβ3 integrin in physical stiffening of the fibrotic niche. Epigenetic silencing of RASSF1A, a tumor suppressor, correlates with metastatic potential and adverse prognosis of advanced lung cancer. RASSF1A promoter methylation is characterized by increased collagen deposition and stiffened ECM in NSCLC. Furthermore, changes in ECM mechanics trigger cancer stem-like programming and are associated with metastasis *in vivo* (293). α11 Integrin subunit is overexpressed in NSCLC-associated stromal fibroblasts (453). In immune-compromised α11-deficient mice, loss of tumor stromal α11 expression correlates with decreased collagen crosslinking and reduced stiffness of fibrillar collagen matrices (270). These findings suggest that both RASSF1A and α11 are crucial stiffness regulators associated with the risk of metastatic progression in lung adenocarcinoma.

Matrix Stiffness in Lung Development

Mechanical forces are involved in tissue patterning and organogenesis (207). The growth, differentiation, and morphogenesis of a developing embryo are dependent on intrinsic and extrinsic mechanical forces that drive the assembly of cells and promote growth into higher-order structures (359, 400). In *Xenopus laevis*, the head mesoderm underlying the cephalic neural crest stiffens during morphogenesis. This stiffening is necessary and sufficient to initiate an epithelial-to-mesenchymal transition of the neural crest cells and trigger their

collective migration (18). It has been observed that capillary morphogenesis is modulated by the relative magnitudes of apparent matrix stiffness and cellular traction forces *in vitro*, and this balance was implicated in regulating angiogenesis *in vivo* (349). Furthermore, a matrix stiffness gradient in the *Drosophila* egg chamber instructs tissue elongation (80). Terminal differentiation of ESCs is regulated by substrate stiffness. Increasing substrate stiffness promotes the spreading, cell proliferation, mesendodermal gene expression, and terminal differentiation of ESCs (114, 216).

The lung is formed by epithelial branching, which involves the repetitive formation of epithelial clefts and buds that invade the surrounding ECM during embryonic development. Lung branching morphogenesis requires Fn assembly at the nascent cleft (87, 327). Although FN does not appear to contribute to bulk lung stiffness, it may contribute to other mechanical aspects of tissue development by serving as a medium for force transmission (325, 448). Elastin and collagen deposition increases ECM stiffness in the neonatal lung by facilitating signaling through the endothelial lipoprotein receptor-related protein 5 (LRP5)-Tie2 pathway (231). β-Aminopropionitrile, an inhibitor of LOX crosslinking enzyme, downregulates LRP5 and TIE2 expression and softens neonatal mouse lung tissues, resulting in inhibition of the vascular and alveolar morphogenesis in neonatal mice (246). Stiffening of neonatal mouse lungs by hypoxia-induced LOX activation deregulates alveolar morphogenesis (246). These findings demonstrated a crucial role of LOX-modulated matrix stiffness in regulating postnatal lung development. Drosophila tracheal tubes undergo axial elongation through epithelial membrane growth (155). The apical ECM (aECM) of the trachea is mechanically coupled to epithelial cells. The mechanical properties of aECM play an important role to counter the tube elongation “stretching” forces originated from epithelial apical membrane growth (98).

Matrix Stiffness in the Pathogenesis of Lung Diseases

Pulmonary fibrosis

IPF is a devastating, progressive fibrosing disease with limited treatment options. It is characterized by extensive and disorganized accumulation of abnormal ECM in the lung parenchyma. IPF is thought to result from repetitive microinjuries to the lung epithelium, promoting fibroblast activation and differentiation into ECM-producing contractile myofibroblasts. The fibroblastic focus is a key histopathological component of the usual interstitial pneumonia pattern that characterizes IPF. It is a dynamic structure in which an active cellular fibrotic front of proliferating mesenchymal progenitor cells (MPCS) and activated macrophages embedded in a hyaluronan-rich ECM invades the adjacent normal alveolar interstitium. As the front advances, the progeny cells behind differentiate into a myofibroblast core synthetizing a fibrotic ECM composed of collagen, Fn, tenascin C, hyaluronan, and latent TGF-β (158). Persistent myofibroblast activation leads to progressive ECM deposition, which eventually destroys normal alveolar architecture and severely impairs lung function.

Measurements of stiffness at the local microscale level revealed that the fibrotic lesions in both human IPF lung and fibrotic mouse lung exhibit median stiffness higher than the surrounding normal lung parenchyma (41, 226). The increases in lung stiffness far exceed

the previous macroscale measurements of fibrotic stiffening of the lung (103). Changes in tissue mechanics at the organ level have been traditionally viewed as the sequelae of fibrosis. Accumulating evidence indicates that matrix stiffening at the cellular level can induce many of the same functional changes seen in IPF fibroblasts, including collagen production, proliferation, myofibroblast differentiation, apoptosis resistance, migration, and invasion (described in previous sections). Furthermore, a stiffened environment is required for mechanical tension-induced activation of latent TGF-β, a potent fibrotic cytokine (418). Thus, stiffened fibrotic ECM contributes to the progression of lung fibrosis through the formation of a pathological feedback loop.

Excessive deposition of fibrillar collagen is the hallmark of tissue fibrosis. LOX/LOXLs and TG2 crosslinking enzymes are responsible for posttranslational modifications of collagen. The crosslinking reactions are essential to stabilize the supramolecular assembly of collagen and produce stable collagen fibrils. In IPF, increased tissue stiffness is caused by dysregulated posttranslational collagen crosslinking rather than increased collagen concentration (190). Immunohistochemical analysis demonstrated strong induction of LOX and LOXL2 in bronchial and alveolar epithelium as well as fibroblastic foci in IPF versus non-IPF subjects (12). Loxl1 was found to be upregulated in active fibrotic regions of bleomycin-treated mice. Genetic ablation of *Loxl1* prevents accumulation of insoluble crosslinked collagen and lung stiffing and protects mice from AdTGF-β1-induced pulmonary fibrosis (23). It has been reported that dual inhibition of Loxl2 and Loxl3 is sufficient to normalize collagen fibrillogenesis, reduce tissue stiffness, and improve lung function in animal models of lung fibrosis (190). Previous studies have shown that TG2 and TG2-generated crosslinks are strongly expressed in IPF lung tissues. During the fibrotic phase of injury, inhibition of TG activity attenuates collagen deposition in a bleomycin mouse model (306).

Aging is a strong risk factor and an independent prognostic factor of IPF. Aged mice develop nonresolving pulmonary fibrosis after bleomycin-induced lung injury (156). We and others have shown that aging lungs are characterized by increased formation of AGEs and enhanced glycation crosslinking, measured by pentosidine crosslinks (24, 312). Accumulation of glycation crosslinks occurs at an accelerated rate in both IPF and experimental lung fibrosis (208, 242, 312, 348). We found that inhibition of glycation crosslinking reduces stiffness in IPF lung tissue and renders IPF lung collagen more susceptible to MMP1-mediated degradation, presumably by exposure of hidden protease cryptic sites within the highly crosslinked triple helical structure. Destiffening of the fibrotic lungs by targeting AGE crosslinking promotes lung fibrosis resolution in aged mice (312).

Pulmonary hypertension

PH is associated with structural and mechanical changes in the pulmonary vascular bed, characterized by narrowing and stiffening of the proximal and distal pulmonary arteries. Increased pulmonary vascular stiffness is an important indicator of disease progression in PH and is a key component in the pathogenesis of PAH, a severe subtype of PH (129, 243). The mechanical properties of pulmonary arteries are intimately linked with the composition and structure of the ECM proteins (172). Pulmonary arterial remodeling during PH

leads to degradation of elastin and collagen accumulation, which correlates with collagen-predominant stiff mechanics (209). Various pathogenic factors can trigger pulmonary arterial remodeling and stiffening. Inflammation contributes to vascular remodeling and stiffening through induction of the breakdown of elastin, proliferation of smooth muscle cells and fibroblasts, and changes in the composition of the ECM (335). Hypoxia exposure induces a significant increase in tissue stiffness in freshly isolated proximal pulmonary arteries *ex vivo* (209). MMPs play an important role in the remodeling of ECM in pulmonary vessels in both patients and animal models of PAH (215, 414). It has been found that increased MMP activity promotes vascular stiffening (237). However, the underlying mechanisms remain to be determined. A potential mechanism contributing to PH associated with IPF is deposition of hyaluronan. Hyaluronan fragments increase stiffness of PASMCs, inhibit their migration, and stimulate their proliferation in a RhoA-dependent manner (76). Vascular calcification drives distal pulmonary vasculature stiffening through activation of a miR-204/RUNX2/HIF-1α-dependent pathway (326).

Emerging evidence indicates that vascular stiffening can precede PH and promote pulmonary vascular remodeling (28, 224). These findings suggest a causative role of vascular stiffening in the pathogenesis of PH. Vascular stiffness-derived mechanical signals enhance proliferation and migration of vascular cells and promote excessive matrix production (30, 224). These in turn perpetuate vascular stiffness, thus giving rise to a self-sustaining loop that amplifies vascular remodeling in PH. Vascular stiffening also increases glycolysis, decreases mitochondrial oxidative phosphorylation, and enhances anaplerotic replenishment of amino acids to meet the metabolic demands of the disease state (28, 30). The results demonstrated a link between matrix mechanotransduction and vascular metabolic reprogramming in the initiation and development of PH. Understanding the molecular mechanisms by which pulmonary vascular ECM stiffens and how vascular cells sense ECM stiffening is necessary for developing novel interventions to prevent, treat, and even reverse PH.

Chronic obstructive pulmonary disease

The levels of ECM proteins are changed in subjects with mild or moderate COPD in comparison to control subjects, and these changes progress to more significant pathology in severe COPD (166). The most notable changes are reductions in expression and/or the functional organization of elastic fibers and small molecule PGs such as biglycan and decorin in the airway and parenchyma. The ECM makes an important contribution to the mechanical properties of the airway smooth muscle (ASM) and the airway wall under both healthy and diseased conditions (40). The contractile and noncontractile functions of ASM are strongly regulated by its interaction with the surrounding ECM through cell surface signaling and specific receptors (295). In patients with mild to moderate COPD, fibrosis of the small airways contributes to the stiffening of the overall airway wall and impairs the ability of ASM cells to relax in the presence of bronchodilators (34). Both increases and decreases in total amount of lung collagen have been reported in emphysema (210, 343, 361). In a porcine pancreatic elastase (PPE)-induced mouse model of emphysema, lung stiffness decreased despite an increase in lung collagen (179), suggesting that the total

amount of lung collagen may not be the primary determinant of the mechanical properties in the emphysematous state.

Asthma

Asthma is the result of chronic inflammation of the airway that leads to exaggerated airway narrowing and stiffening. Sustained inflammatory signals acting on the ASM play a central role in the development of hypercontractile airways. Airway stiffness is determined by the property and structural organization of the various elements in the airway wall. It can be divided into active and passive components (344). Active stiffness is associated with activation of ASM, which generates contractile forces that stiffen the airway. Passive stiffness is primarily derived from nonmuscle compartments of the airway wall, in particular the ECM. Collagen deposition results in an increase in matrix stiffness in asthma (9, 416). The mechano-interactions between the ASM and a stiff environment promote ASM hypercontractility (5, 308) and hyperplasia (347) and airway remodeling (45). A recent study has shown that ECM stiffening in the airway, induced by riboflavin and ultraviolet-A radiation-mediated crosslinking of collagen fibers, is sufficient to drive excessive airway constriction even in the absence of inflammatory signals (182). The finding suggests that ECM remodeling may be a core pathological change in driving airway hyperreactivity in asthma.

Lung cancer

Stromal stiffening is a cardinal feature of many malignant tumors, including lung cancer (67). Rigid matrix promotes proliferation of various lung cancer cell lines by stimulating expression of programmed death-ligand 1 (PD-L1) (264). Targeting PD-L1 has been shown effective in treating patients with NSCLC (379). Stiff matrix upregulates spindle pole body component 25 homolog (SPC25), a component of the heterotetrametric NDC80 complex, which mediates lung cancer cell proliferation (187). ECM-derived mechanical signals promote NSCLC growth by upregulation of CTGF, AREG, and survivin through a Hippo/Yap-dependent pathway (439).

Epithelial-mesenchymal transition (EMT) is a vital process in cancer dissemination and metastasis (194). Increasing matrix stiffness promotes the recruitment of c-Myb to the *DDR2* promoter, leading to activation of the *DDR2* gene in lung cancer cells. Increased DDR2 expression promotes EMT and mediates lung cancer cell invasion (201). In lung carcinoma, ubiquitin domain-containing protein 1 (UBTD1) is highly correlated with both the disease progression and patient survival. UBTD1 localization is drastically modified by the ECM stiffness. It has been found that UBTD1 accumulates at cell junctions on stiff ECM, whereas it remains mostly diffuse in the cytoplasm on soft ECM (387). Furthermore, UBTD1 controls a YAP-dependent EMT program that dramatically increases invasion and migration of lung cancer cells (387). Matrix stiffness can also regulate migration and invasion of lung cancer cells through EMT-independent mechanisms. It has been shown that stiff matrix triggers stem cell-like reprogramming by regulation of stem cell transcription factor, NANOG, which promotes lung cancer metastasis (294). It has also been found that stiff ECM-induced activation of integrin β1 is associated with the motility of lung

cancer cells (6). Additionally, stiff matrix-dependent NF-κB upregulation participates in lung cancer cell spreading via regulation of the actomyosin contractile apparatus (177).

Mechanical interactions between cancer-associated fibroblasts (CAFs) and the microenvironment contribute to lung tumorigenesis. Stiffened stroma promotes accumulation of CAFs in lung squamous cell carcinoma (311). CAFs influence lung cancer cell proliferation and migration through secretion of growth factors, regulation of tumor angiogenesis, and remodeling of the ECM (33). Neuropilin-1 expressed by CAFs promotes assembly of Fn fibrils, leading to matrix stiffening and lung tumor growth (431). Additionally, CAFs increase matrix stiffness by promoting integrin α11-dependent collagen crosslinking, and stiffened matrix initiates signals to promote the growth and metastasis of lung cancer cells (270).

Measurements of Lung Matrix Stiffness

Matrix mechanical properties are typically reported as elastic modulus (*E*, also known as Young's modulus) or shear modulus (*G*). Elastic modulus and shear modulus are related to each other as a function of the material's Poisson's ratio. Elastic modulus corresponds to the slope of the uniaxial strain-stress relationship and is approximately three times the shear modulus for isotropic and incompressible materials. Several mechanical test methods have been used to test the mechanical properties of lung matrix, which are summarized in the following sections (see Table 1).

Atomic force microscopy

AFM is the most widely used technique for direct measurements of the mechanical properties of the ECM at the microscale, the same scale that cells use to sense their environment (36). AFM is based on a sample displacement by applying loads as low as few nN (even pN) through a microfabricated cantilever with a sharp tip at its end (2). The loading force compresses the surface of the sample, producing an indentation that bends the cantilever. By calibrating the stiffness of the cantilever, the force on it can be estimated by measuring the vertical deflection of the cantilever with optical techniques. From the deflection and the force, a force-distance curve is obtained. These curves carry information about the sample's mechanical properties (191, 227, 369). The unique aspects of AFM-based measurements include the ability to perform mechanical testing in a liquid environment, which is important in studying biological samples and mapping local heterogeneities in the matrix environment. In cell and tissue experiments, AFM is placed on the stage of an inverted optical microscope to visualize the sample with transmitted light, which enables accurate placement of the tip at the point of interest for measurements (see Figure 2).

AFM microindentation has been used to quantitatively measure the lung tissue elasticity on the micrometer length scale (see Table 2). Using AFM measurements, an average elastic modulus of mouse bronchus is 23.1 ± 14.0 kPa, with a broad distribution ranging from 2 to 45 kPa, indicating substantial heterogeneity across large and small airways (226, 256, 347). The fact that different sites within the lung scaffold exhibit marked geographic differences in stiffness has also been reported in healthy rat lungs (257). Regional mechanical differences

were also demonstrated in decellularized lungs using AFM (41, 238, 257). The local stiffness in fibrotic lungs increases according to the degree of the structural fibrotic lesion (226). Mice with lung fibrosis exhibit local stiffness that is approximately twofold higher than that in the control mice: from 27.2 ± 1.6 to 64.8 ± 7.1 kPa in the alveolar septa, from 56.6 ± 4.6 to 99.9 ± 11.7 kPa in the visceral pleura, from 41.1 ± 8.0 to 105.2 ± 13.6 kPa in the tunica adventitia, and from 79.3 ± 7.2 to 146.6 ± 28.8 kPa in the tunica intima (256). Native normal human lung has a mean Young's modulus of 1.96 ± 0.13 kPa (53). In contrast, IPF lung has eight-fold increase in stiffness (16.52 ± 2.25 kPa) in the area of fibrosis (41). The deposition of collagen and other matrix components in the lung structure increases with aging (169, 196, 354). An AFM-based study indeed indicates that acellular native lung stiffness in aged mice is, on average, 29% higher than that in the young animals (256).

There are some important considerations when using AFM. Cryotome of biological tissues can alter bulk ECM mechanics (38, 399). Significant differences in tissue mechanics have been observed in cryotomed versus vibratomed sections (427, 428). Measurements on cryosections likely do not reflect the true material properties and need to be avoided. Because fresh, unfixed tissue is required for AFM measurements, the time elapsed from tissue harvest to measurement should be minimized, and samples should be stored at 4°C to avoid changes in mechanical properties (227). The spring constant of cantilevers determines the sensitivity of AFM measurements; typically, cantilevers with spring constants in the range 10 to 200 pN/nm are used for biological samples (111). A standard cantilever with a 5-μm diameter spherical tip is sufficient to characterize mouse lungs ranging from 100 Pa to 50 kPa (shear modulus) (227). Pyramidal tips provide smaller contact areas and thus increase spatial resolution. However, data fitting is more complex for these types of tips (15, 27, 318, 402). Indentation speeds must be carefully chosen to minimize both hysteresis and cantilever drift. The elastic modulus derived from the force-distance curve is sensitive to the postprocessing methods (373). Hence, the methods by which the mechanics of the probe-tissue contact is modeled need to be determined appropriately to increase the reproducibility among different researchers.

There are several limitations of the AFM methodology. The lung is one of the organs that are under a preexisting stress in physiological conditions. AFM measures stiffness in compression, whereas physiological lungs are under tensile prestress. If the material is nonlinear, the stiffness from the compression test can be different from that during the tensile stretch (369). AFM measurements neglect this factor, precluding the application of physiological prestress and air-liquid interface variations in stretch. AFM scans only tissue surface and is not suitable for scanning an entire lung. Consequently, the stiffness of internal lung tissues can only be accessed if the 3D organ is destroyed. Finally, the Hertz model assumes homogeneity and absolute elastic behavior of the sample, whereas biological materials including the lung typically display time-dependent viscoelastic behaviors. Nonetheless, AFM microindentation can provide valuable insights into the changes in tissue stiffness during the development of lung fibrosis and fibrosis progression.

Elastography

Elastography measures the shear wave propagation in tissues from which elastic modulus is reported. Magnetic resonance and ultrasound-based elastography have been developed to enable noninvasive visualization and quantification of tissue stiffness (88, 268, 331). Both techniques have been validated clinically for liver fibrosis and breast cancer staging (26, 351, 396, 436).

Ultrasound elastography—Ultrasound elastography is the most frequently used technique to assess tumor tissue stiffness due to its ease of use and relatively low cost. Numerous ultrasound-based shear wave elastography techniques have been described to measure the propagation speed and amplitude decay of mechanical shear waves inside tumor (25, 274, 330). In conventional ultrasound elasticity imaging (UEI), an external static load is applied to the surface of tissues to generate compression, usually via a mechanical device such as platen. A sequence of pre- and postcompression ultrasound images are processed using a cross-correlation algorithm or speckle tracking scheme to detect the displacement at each location within the sample to infer the elasticity inside the sample (35, 92, 236, 289). This technique has a particular advantage in imaging spatial variations of the mechanical properties within a tissue volume. Acoustic radiation force (ARF) elasticity imaging uses the force associated with an ultrasound beam to achieve deformation in a material body in a noncontact fashion (97). Unlike UEI, ARF is a body force produced within a material sample generated by momentum transfer from the ultrasound wave to the medium (90, 97). ARF impulse imaging has been investigated for multiple clinical applications, including changes in cardiac tissue stiffness (168).

Magnetic resonance elastography—Magnetic resonance elastography (MRE) is originally developed as a noninvasive method to measure internal organ stiffness by visualizing mechanically induced low-frequency spin motion (268). MRE is a dynamic technique that utilizes small shear displacements (~μm) to enable direct assessment of shear properties of the solid ECM (135, 249, 268). The MRE technique has three essential components: (i) generation of shear waves in the tissue evoked by the MRI system; (ii) acquisition of MR images showing the propagation of the induced shear waves; and (iii) conversion of the MRI acquired wave motion into elastic maps known as elastograms (249). MRE offers the advantage of 3D stiffness and viscosity measurements over a wide range of mechanical actuation frequencies with low variability (261). MRE is limited by (i) the fact that only accessible parts of the human body can be screened; (ii) inability to distinguish tissue stiffening between fibrosis and inflammation or altered perfusion, such as portal venous hypertension in cirrhosis (328); and (iii) substantially longer imaging times and higher costs compared to ultrasound elastography.

Fluorescent microscopy

Microscopic indentation methods have been developed for convenient and rapid measurements of the elastic modulus of hydrogels (83, 124, 233, 235). Most conventional fluorescent microscopic indentation methods consist of the following steps: (i) fluorescent beads are embedded in the hydrogel to visualize its top surface; (ii) a sphere or ball indentor is placed on the hydrogel where the indentation force from the indentor deforms the gel

surface locally; (iii) after the indentor is removed and the hydrogel is allowed to recover elastically, the fluorescent beads identified by a fluorescence microscope are tracked by raising the focal plane; and (iv) the indentation depth is measured from the displacement of the focal plane, and the E of the hydrogel is calculated using a proper indentation model (20, 211). The fluorescent microscopy method is cost-effective and does not require special equipment. Hence, this method can be widely utilized in laboratories with a conventional fluorescent microscope.

In Vitro Model Systems—Hydrogels as Engineered Stiffness-Tunable Matrix Substrates

Hydrogels are water-swollen crosslinked polymeric networks with structural similarity to native ECM (323). These polymer networks can be fabricated from various materials, including natural, synthetic, and semisynthetic/hybrid polymers (454). The transformation of the polymer network into a gel (gelation) requires the introduction of crosslinks between polymer chains (200). In general, hydrogel stiffness is controlled by adjusting the concentration of polymers and/or crosslinkers. Hydrogels are functionalized with collagen, Fn, or other ECM proteins to provide the binding sites for cell adhesion. A growing body of research suggests that cells cultured on 2D substrates differ greatly from those grown *in vivo* (44, 181, 292, 299, 409). In this regard, cell-laden hydrogels are increasingly being explored for studying the molecular basis by which ECM stiffness influences cellular function in the context of a 3D ECM.

Natural hydrogels

Naturally derived hydrogels are mainly made of ECM components, such as collagen, fibrin, HA, and Matrigel or materials derived from other biological sources, such as alginate, agarose, and pectin (17, 383). These polymers have advantages of inherent biocompatibility and are abundant in source (4, 380). Natural gel stiffness has been routinely modulated by altering the concentration or composition of the gel constituents or by varying crosslink density. However, such approaches may simultaneously alter gel pore size, fiber architecture, and/or the number or availability of adhesion sites as well (132, 321).

Collagen—Collagen is a major component of the ECM proteins in mammalian tissues. Collagen hydrogels are conventionally prepared via physical crosslinking by pH and temperature adjustments. They provide 3D fibrillar matrices recapitulating the ECM *in vivo* (193). A major disadvantage of this approach is the weak mechanical properties of collagen gels. Many chemical crosslinking methods have been developed to generate stiffer and more stable collagen hydrogels. For example, genipin, an excellent natural protein crosslinker with low cytotoxicity, was used to fabricate collagen gels with 2 to 50 kPa of compressive strength without changing the overall collagen density (252, 445). Other chemical crosslinking methods include treatments of collagen gels with glutaraldehyde, carbodiimide, or hexamethylene diisocyanate (389). These crosslinking agents target primary amines and carboxylates on the collagen backbone, which might involve specific adhesion receptor-binding sequences. Therefore, such crosslinking agents

could potentially alter cell adhesion. It has been found that addition of soluble elastin improves the mechanical properties of collagen hydrogels (101).

Hyaluronic acid—HA is a nonsulfated GAG composing of repeating disaccharide units of glucuronate and *N*-acetylglucosamine. The advantages of HA as a hydrogel platform include the biological relevance and the ability to be modified to present functional groups enabling a range of crosslinking chemistry (52). A standard method to make HA gels is crosslinking of thiolated HA (HA-SH) with acrylated PEG by Michael addition (109, 217). The stiffness of HA hydrogels can be readily tuned from 1 Pa to 4 kPa with magnitude increments by varying the amount of PEG crosslinker (131). This type of HA hydrogel offers a flexible biochemical composition, biomechanical compliance, and simplicity of use. However, the crosslinking schemes have major drawbacks. At physiological pH, HA gelation by Michael addition is slow, making the handling difficult (217). Additionally, thiolated HA readily oxidizes to disulfides when in solution at physiological pH, resulting in a significant loss of the crosslinker. It has been reported that HA modified with methacrylates (HA-MA) and photo-crosslinked with a radical initiator has the advantage of higher stability and fast gelation (342). To achieve spatial control of matrix stiffness precisely, a two-step crosslinking process has been developed to fabricate HA hydrogels. The initial gelation fabricates hydrogels with stiffness (E~2 kPa) mimicking that of healthy tissue. The hydrogels are then stiffened by secondary crosslinking via UV light-mediated radical polymerization, mimicking the stiffness of fibrotic tissue (E~25 kPa) (149). The two-step approach generates HA hydrogels with stiffness levels equivalent to normal and fibrotic lungs. Thus, it is beneficial for lung-related studies. High molecular weight HA crosslinked by TG has a gelling speed tunable from seconds to hours depending on the amount of enzyme added (47). Unlike thiol or (meth)acrylate derivatives, HA-TG derivative is chemically inert and resistant to oxidation and hydrolysis, guaranteeing high stability and long-term storage of HA hydrogels.

Alginate—Alginate is a brown algae-derived polysaccharide with many favorable properties, including biocompatibility, no mammalian receptors, low toxicity, low protein adsorption, and ease of gelation (213). Unlike collagen and HA, alginate must be modified with an adhesive ligand to enable cell attachment. Alginate can rapidly form stable hydrogels in the presence of divalent ions such as magnesium and calcium (455). The mechanical properties of alginate hydrogels can be fine-tuned for specific applications either using alginates with different average molecular weights or by varying the ratio of M/G blocks and the ion concentration used for crosslinking (456). Alginate gels quickly dissolve in the absence of divalent ions or in the presence of acids or other ions (e.g., phosphate), limiting their usage for long-term applications (456). Silk fibroin, a macromolecular protein derived from silk worms, has been added into alginate gels to keep them stable over long periods of time (456). The silk-alginate gels have biocompatibility characteristics, tunable mechanical and morphological properties, and fast gelation.

Agarose—Agarose forms hydrogels by physical crosslinking (128). In comparison with chemical crosslinking, physical crosslinking offers several advantages including the absence of reactive chemistry and ease of implementation. However, increasing the polymer

concentration beyond the gelation concentration in agarose gels yields only modest changes in stiffness. A method for promoting the association of agarose polymer chains in physical gelation has been developed previously (122). In this method, induction of a switch in the secondary structure from α-helix to β-sheet through carboxylation enables the agarose gel modulus to be tuned over four orders of magnitude (G' 6 Pa to 3.6 × 10^4 Pa) independently of the polymer concentration and its molecular weight.

Pectin—Pectin is a structural polysaccharide extracted from the primary cell wall of plants. The branched polymer comprises multiple polysaccharide domains, providing a multitude of target sites for chemical modification. This feature, together with the lack of endogenous cell-adhesive and cell-proteolytic sites, allows for the precise introduction of specific biochemical moieties onto the inert polymer backbone (302).

Decellularized extracellular matrix

Decellularized ECM (dECM) has physicochemical properties more representative of the native extracellular microenvironment, thereby providing numerous benefits over other hydrogels. dECM derived from human pulmonary fibroblasts has an average Young's modulus of 105 ± 14 Pa (355). Crosslinking of this dECM by glutaraldehyde demonstrates a 67% increase in the stiffness (355). This fibroblast-derived dECM model provides a unique platform for the study of the physical and biochemical properties of cell-produced matrices as well as interactions between lung fibroblasts and their natural microenvironment. A unique lung-specific, stiffness-tunable hydrogel system has been developed recently (222). Briefly, normal pig, rat, and mouse lungs are decellularized, lyophilized, and cryomilled into powder. The dECM powder is digested by acidic pepsin digestion and neutralized to form pregel solution. Cells are suspended in the pregel solution, and gelation is induced by incubation at 37°C to achieve a 3D culture. The stiffness of lung dECM hydrogels can be tailored by addition of different concentrations of genipin. Decellularization of the whole lung scaffolds recapitulates lung-specific ECM structure and composition (41, 78, 273, 291, 303, 310, 404). Acellular normal lung matrices exhibit an elastic modulus similar to that expected *in vivo*, whereas IPF acellular matrices demonstrate increased but heterogeneous stiffness (41). Interestingly, despite a variation in the lung ECM stiffness observed during the decellularization process, the resistance and elastance of the lungs return to basal level at the final decellularization step (275). It has also been reported that the conventional decellularization procedures do not result in substantially different local stiffness in the acellular lung (257). The recently developed tissue-specific dECM bioink can be mechanically tailored by sequential vitamin B2-induced UVA crosslinking (183). The printable dECM bioink enables 3D fabrication of tissues that are more functionally matched than prototypic natural hydrogels.

Synthetic hydrogels

The chemical and mechanical properties of synthetic hydrogels can be more precisely controlled than those of natural hydrogels. Synthetic hydrogels allow to examine how the magnitude of matrix stiffness directs cell behavior *in vitro*. The mechanical properties of synthetic hydrogels can be modified by altering the density of crosslinks between the polymers.

Polyacrylamide—PA hydrogels are produced by reacting acrylamide monomer and bisacrylamide crosslinker in the presence of ammonium persulfate (APS) and tetramethylethylenediamine (TEMED). APS provides free radicals, while TEMED catalyzes redox radical polymerization of the PA. Stiffness ranging between 0.5 and 100 kPa, comparable to the stiffness of most tissues in nature, can be prepared by adjusting the monomer and crosslinker concentrations (394). PA hydrogels are biologically inert and require surface functionalization through coating ECM proteins or peptides. Protein conjugation to the PA hydrogel surface is achieved using a bifunctional crosslinker such as sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate (sulfo-SANPAH) (139). Sulfo-SANPAH has limited stability and shows variations in crosslinking that result in heterogeneous ECM coating (195, 394). 3,4-Dihydroxy-L-phenylalanine (L-DOPA) can functionalize PA gels more stably and more homogeneously compared to sulfo-SANPAH (422). Incorporation of hydroxyl groups in acrylamide monomers overcomes the intrinsic anti-adhesive property of PA hydrogels (138). Furthermore, the presence of hydroxyl groups facilitates hydrogen-bonding interactions between PA gels and peptides, thus increasing the affinity of hydrogels and biomolecules (139). PA gels have a number of advantages, including cost-effectiveness, ease of preparation, constant surface chemistry, reproducibility, and availability in a wide range of stiffness (81, 199, 300, 394). A major limitation of PA gels is that PA hydrogels can only be used as a 2D scaffold and cannot encapsulate cells due to the cytotoxicity of soluble precursor components.

Polyethylene glycol—Polyethylene glycol (PEG) has relatively low protein adsorption and is amenable to user-defined crosslinking chemistry. The mechanical properties of PEG hydrogels can be controlled by changing the network crosslinking density, which may be tuned by changing the concentration, molecular weight, or the number of arms of PEG (115, 136). Unlike PA, unpolymerized components of PEG are cytocompatible. Therefore, PEG hydrogels can encapsulate cells and study stiffness in 3D (51, 247). The utility of PEG has been demonstrated in a diverse set of cell culture applications, including studies of stem cell differentiation, mechanobiology, and angiogenesis (14, 204, 239, 266, 305).

Polydimethylsiloxane microposts—Polydimethylsiloxane (PDMS) hydrogels can be simply prepared by mixing base and curing agent, where a platinum catalyst facilitates hydrosilylation to crosslink vinyl-terminated PDMS base polymer with methyl hydrogen siloxane units from the curing agent (433). The modulus of PDMS (from kPa to MPa) fabricated through this mechanism can be controlled by changing the base-to-curing agent mixing ratio, curing temperature, and reaction time (127, 147, 171, 390). PDMS microposts bend laterally when a cell attaches and exerts force to the substrates (127, 376). The micropost stiffness is tuned solely by the height of the post, while keeping adhesive and other material bulk and surface properties constant (127). This micropost system provides an approach to decouple the effect of the substrate stiffness from alterations in the surface chemical properties. An advantage of PDMS hydrogels is the unlimited shelf life under ambient conditions, as compared to several days or at best several weeks under refrigeration in the case of PA hydrogels.

Polyisocyanopeptide-based hydrogels—Polyisocyanopeptide (PIC) hydrogels are derived from helical oligo ethylene glycol PICs (84). These hydrogels exhibit a nonlinear stress response (stress stiffening) (206). When the stress is increased beyond a threshold value, the matrices become stiffer with increasing applied stress. PIC hydrogels provide a platform for investigating the effect of stress stiffening on cells encapsulated in a 3D microenvironment. This 3D physiological microenvironment can be tuned independently of matrix stiffness, ligand density, and matrix porosity, thereby improving the traditional synthetic matrix scaffolds.

Self-assembling peptide hydrogels—Self-assembling peptides (SAPs) are a family of 8 to 32 amino acid peptides. SAP hydrogels undergo multihierarchical self-assembly when exposed to physiological salt solutions (58, 276, 286, 444). The synthetic hydrogels exhibit many advantages of naturally derived polymers, including interactions with cells and assembly into hierarchical structures, mimicking the native proteins (410). Unlike natural hydrogels, which change architecture, pore size, and ligand density when matrix stiffness is altered, SAP gels offer a tractable system with alterations of stiffness in a dynamic range without significantly affecting any of these variables.

Hybrid hydrogels

Hybrid hydrogels are developed by combining the strengths of natural and synthetic polymers to better mimic the complex *in vivo* environments. An example of hybrid hydrogels, called the minimal matrix model of scars (MMMS), involves mixing soluble collagen I with acrylamide monomers and bis-acrylamide crosslinker and then polymerizing the mix into a hybrid hydrogel (94). Upon initiation of polymerization, collagen I fibers separate from pregelation clusters of PA to form highly branched fractal fiber bundles that segregate as islands heterogeneously entrapped at the subsurface of the hydrogel. Since collagen in the subsurface fiber bundles is not accessible for cell adhesion, a uniform overcoating of matrix ligand is provided for cell attachment. Studies indicate that MMMS is a promising system to study cellular mechanotransduction in scar/fibrosis (70).

Mechanically tunable gelatin-PEG composite hydrogels are made by conjugating thiolated gelatin to one end of PEG diacrylate (PEGDA) by Michael-type addition reaction. The hybrid hydrogels combine the benefits of natural gelatin and synthetic PEG hydrogels and can encapsulate fibroblasts *in situ* with high cell viability. The stiffness of the hydrogels is manipulated by alternating the concentration of PEGDA, ranging from 1.3 ± 0.5 kPa to 23.0 ± 1.0 kPa (57).

PIC hydrogels, as described previously, resemble both strain-stiffening response and the fibrillar structure of native ECM (184, 185). PIC composite hydrogels are constructed by combining PIC hydrogels with fibrin (semiflexible), PA (flexible), or carbon nanotubes (rigid) polymers (186). The combination of polymers with different mechanical properties into hybrid hydrogels may help the design of novel responsive materials and tunable matrix substrates for tissue engineering applications.

Layer-by-layer (LbL)-modified hydrogels consist of LbL-assembled films of native matrix proteins on top of mechanically tunable PA hydrogels to form chemically and mechanically

relevant cell culture substrates. LbL hydrogels have been utilized to demonstrate a role of matrix substrate stiffness in hepatic stellate cell myofibroblastic differentiation (333).

Gradient stiffness matrices

ECM stiffness gradients exist in physiological and perhaps more commonly in pathological conditions, such as fibrosis and cancer. Stiffness gradients of the ECM influence cellular function. Diverse technologies including physical crosslinking, photopolymerization, and microengineering structuralization have been utilized to create gradient stiffness matrix substrates (423). Cylindrical polyvinyl alcohol (PVA)/HA hydrogels produce a wide-range stiffness gradient (between ~20 and ~200 kPa) using a gradual freezing-thawing approach (282). Photopolymerization employs light or high-energy radiation to crosslink polymers. By moving a photo mask at controlled speed to progressively uncover the gel solution, it produces a linear gradient stiffness hydrogel of approximate 115 kPa/mm, extending from 1 to 240 kPa in Young's modulus (372). Soft lithography creates a wide range of stiffness values with diversified patterns through changing the geometric structure of lower layer substrates and the height and type of upper layer substrates (62, 219). Mechanically tunable 3D printed microstructures are programmable and have gradient variations in the apparent interfacial stiffness over a macroscale lateral span (322, 435).

Mechanically dynamic matrices

The ECM undergoes dynamic remodeling in normal and disease processes. While static hydrogels are unable to capture the dynamic landscape of ECM, mechanically dynamic hydrogels mimic the dynamic nature of the cellular microenvironment. Degradation through a photocleavable moiety incorporated within the gel backbone results in temporal softening of the hydrogels. For example, PA hydrogels are incorporated photocleavable 2-nitrobenzyl-derived crosslinkers. Irradiation with near-ultraviolet light (~365 nm) results in the cleavage of the linkers and dynamic softening of PA gels (125, 430). By contrast, activation of photocrosslinkable norbornene and 4-hydroxyphenylacetic acid causes dynamic crosslinking and stiffening of HA gels (148, 229). Furthermore, HA hydrogels bearing both photocleavable crosslinkers and methacrylates are able to soften on crosslinker cleavage and stiffen on methacrylate polymerization (324). Visible light-mediated radical polymerization represents a scheme of safer stiffening of hydrogels compared to UV light-initiated photocrosslinking (56). The use of near-infrared (NIR) light allows for transdermal gelation and stiffness tuning (362), providing an avenue for *in vivo* translation of this technology.

Tyrosinase-triggered postgelation crosslinking provides a platform for on-demand stiffening of cell-laden hydrogels through enzymatic reactions (228). Enzymatic stiffening of hydrogels utilizes a peptide linker with additional tyrosine residues that are susceptible to tyrosinase-mediated crosslinking. Tyrosinase catalyzes the oxidation of tyrosine residues into dihydroxyphenylalanine (DOPA) dimer. The formation of DOPA dimer promotes crosslinking and thus stiffening of cell-laden hydrogels (228). In hydrogels prepared by 8-arm PEG-norbornene (PEG8NB) and a simple peptide linker (i.e., KCYGPQGIWGQYCK) sensitive to tyrosinase-triggered dityrosine crosslinking, tyrosine residues in the primary network serve as substrates for exogenously added tyrosinase. Tyrosinase catalyzes dityrosine crosslinking and increases PEG hydrogel crosslinking density and stiffness (228).

The mechanism in DNA-crosslinked hydrogels is based on DNA-powered molecular machines (440). In the DNA-crosslinked gel system, two DNA strands are covalently attached to polymer chains, and a third crosslinker DNA strand base pairs with the first two strands. By inclusion of a toehold at the end of the crosslinker DNA, one can introduce complementary strand to the crosslinkers and displace the crosslinker DNA out of the gel network, thereby reducing the crosslinking density and gel stiffness (220).

In summary, hydrogels are useful tools for understanding the fundamental mechanisms involved in matrix mechanobiology, providing essential means for the expansion and directed differentiation of various cell types in ways not possible with conventional culture. Nevertheless, it is important to note that both 2D and 3D natural hydrogels and synthetic polymers have various strengths and drawbacks (see Table 3). Natural hydrogels have low stability, relatively poor mechanical properties, and rapid degradation rate (144, 272, 368, 454). By comparison, synthetic polymers lack adhesion sites and biocompatibility (241). Decellularized ECM often has poorly defined composition and may contain many different biochemical and physical signals, making it difficult to test specific hypotheses. Under most circumstances, modulation of matrix substrate stiffness also influences other physicochemical factors which could impact cell function (263). Additional efforts will need to develop more tractable *in vitro* systems with well-matched physicochemical properties for a specific microenvironment to investigate cell-matrix interactions in the context of disease modeling and drug testing.

Mechanotherapy: A Promising Novel Strategy for the Treatment of Lung Diseases

Mechanotherapy is an emerging field focused on therapeutically targeting the mechanical cues by directly manipulating matrix stiffness or disrupting the cellular response to aberrant tissue mechanics.

Targeting ECM stiffness

Crosslinking of the ECM is the principal determinant of ECM stiffness. Targeting matrix crosslinking to decrease lung tissue stiffness represents a promising antifibrotic strategy. Increasing serum LOXL2 levels is associated with IPF severity and rapid progression (69). Given that LOX family enzymes are vital mediators of ECM crosslinking, inhibition of LOX proteins may alleviate or even halt fibrotic lung disease progression. Studies in animal models of lung fibrosis pointed to a prominent role of LOXL2 in fibrotic lung remodeling and demonstrated the therapeutic efficacy of a LOXL2-specific antibody (AB0023) in experimental lung fibrosis (19). However, a randomized, double-blinded, phase II clinical trial using the LOXL2 specific antibody Simtuzumab failed to improve progression-free survival in IPF subjects (314). This result suggests that ECM crosslinking and stiffening in IPF may involve complex mechanisms.

Plasminogen activation (PA) promotes matrix degradation *in vitro*, and enhanced PA limits the severity of lung fibrosis in several different animal models (339, 353). In a bleomycin injury model of lung fibrosis, transgenic mice overexpressing lung-specific urokinase

plasminogen activator (uPA) displayed an enhanced resolution of established lung fibrosis compared with the littermate controls. This resolution was accompanied by a reduction in lung parenchymal stiffness and an increase in fibroblast apoptosis. These findings suggest that the role of PA in fibrosis extends beyond fibrinolysis and correlates with not only the development but also the resolution of lung fibrosis (167).

In a recent study, we found that nonenzymatic AGE crosslinking is increased in aged human and mouse lungs, and it occurs at an accelerated rate in lung fibrosis. Pharmacological targeting of nonenzymatic AGE crosslinking with chebulic acid, a potent breaker and inhibitor of AGE crosslinking, destiffens fibrotic lungs and promotes lung fibrosis resolution in aged mice. Importantly, treatment of IPF lung tissue slices with chebulic acid increases MMP1-mediated degradation of lung collagen *ex vivo*, presumably by exposing the cryptic protease sites within the highly crosslinked triple helical structures. Mechanistic studies showed that reducing matrix stiffness activates p53 in lung myofibroblasts by suppressing mechanosensitive MDM4 (an endogenous inhibitor of p53) expression. MDM4-dependent p53 activation sensitizes lung myofibroblasts to apoptosis by upregulation of Fas expression. p53 activation induces an immunogenic conversion of lung myofibroblasts which recruit macrophages to engulf apoptotic myofibroblasts, resulting in the clearance of myofibroblasts and the reversal of lung fibrosis (312). These findings suggest that targeting nonenzymatic AGE crosslinking provides a potential new therapeutic approach to treat aging-associated persistent lung fibrosis. It is noteworthy that LOX/LOXLs are copper-dependent enzymes. Most of the AGE inhibitors, including chebulic acid, have chelating activities on copper-catalyzed oxidative reactions (212). Thus, AGE inhibitors may inhibit not only nonenzymatic glycation crosslinking but LOX enzyme-mediated crosslinking as well.

Mild hyperthermia generated by magnetic nanoparticles under an alternating magnetic field can reorganize collagen fibers and modulate tissue stiffness. It has been shown that magnetic carbon nanotube-generated heat stress induces a remodeling of the tumor microenvironment through local denaturation of the ECM, particularly on collagen fibers. Following photothermal therapy, the treated tumors exhibit a significant decrease in the stiffness together with volume reduction, whereas nontreated tumors show an increase in tumor rigidity, which correlates with tumor growth (248). Insights on whether stiffened fibrotic lung ECM could be modulated by hyperthermia would guide the development of novel approaches utilizing thermal energy at the nanoscale as an antifibrotic therapy.

Targeting mechanosensing and mechanotransduction pathways

Myofibroblast resistance to apoptosis is associated with nonresolving and progressive fibrosis including IPF. Using *ex vivo* and *in vivo* approaches, we have defined a mechanotransduction pathway involving Rho/ROCK, actin cytoskeleton, and a mechanosensitive transcription coactivator MKL1 that coordinately regulate myofibroblast differentiation and apoptosis resistance in response to matrix stiffening (170, 451). Fasudil is a clinically approved small-molecule inhibitor of ROCK. Disruption of the Rho/ROCK/actin cytoskeleton/MKL1-mediated mechanotransduction pathway by fasudil promotes lung myofibroblast apoptosis by downregulation of BCL-2 and protects mice from experimental

lung fibrosis (451). Other studies have shown that small-molecule inhibitors of MKL1 prevent experimental pulmonary fibrosis *in vivo* (352). It would be interesting to see whether targeting the Rho/ROCK/actin cytoskeleton/MKL1 mechanotransduction pathway could be effective for treatment of IPF.

G protein-coupled receptors (GPCRs) that bind to G-protein α subunits ($G\alpha_{s)}$ can inhibit YAP/TAZ activity via cyclic adenosine monophosphate (cAMP). It has been shown that dihydrexidine (DHX), an agonist of $G\alpha_s$-coupled dopamine receptor D1 (DRD1), selectively inhibits YAP/TAZ function in lung mesenchymal cells, shifting cell phenotype from profibrotic to fibrosis resolving. DHX reverses ECM stiffening *in vitro* and in experimental mouse lung fibrosis *in vivo* (150). Verteporfin, an α1β3 integrin inhibitor, is a drug that has been used to treat certain types of eye diseases. Verteporfin inhibits YAP and ameliorates TGF-β-induced renal fibrosis (232, 375). It would be interesting to repurpose this drug for the treatment of lung fibrosis.

Stiffened ECM provides a permissive environment for activation of latent TGF-β1. Blocking mechanical activation of latent TGF-β1 could potentially be accomplished by inhibiting the binding of latent TGF-β complex to αv integrins (126, 157) and/or by inhibiting myofibroblast contraction using peptides that prevent the incorporation of αSMA into stress fibers (162). Targeting integrin-mediated mechanoactivation of TGF-β1 represents a potential strategy for treatments of fibrotic lung diseases.

Airway fibrosis in orthotopic tracheal transplantation (OTT) is persistent and unresponsive to all immunomodulating agents tested to date. It has been reported that simultaneously targeting myofibroblast contractility by relaxin and ECM stiffness by LOX inhibitors reverses the established airway fibrosis in an OTT mouse model (221). This study reveals the synergistic roles of cellular contractility and tissue stiffness in propagation of airway fibrosis and suggests a novel therapeutic strategy for the treatment of chronic rejection in tracheal transplantation and airway fibrosis.

Airway stiffness is a factor that determines the degree of airway obstruction in asthmatic subjects. Active airway stiffness can be inhibited by inhibiting the ASM contractility. However, passive airway stiffness cannot be readily modulated without altering the airway wall structure. Previous studies have shown that the removal of intracellular calcium abolishes a substantial portion of passive airway stiffness. Furthermore, calcium-sensitive stiffness originates from intracellular sources and is sensitive to pharmacological ROCK inhibitor Y-27632 (316). These findings may benefit the development of new drugs that decrease airway stiffness in asthmatic subjects.

Mechanoresponsive cell system for targeted lung therapy

Systemically infused MSCs preferentially home to and integrate with tumors in primary breast tumors and lung metastases (317). This selective and active homing ability makes MSCs an appealing vector for localized delivery of therapeutics in cancer treatment. In an elegant study, MSCs are utilized to generate a mechanoresponsive cell system (MRCS) with genetically engineered YAP/TAZ stiffness-sensing promoter. The MRCS senses the mechanosignaling from breast cancer and lung metastases and deliver cytosine deaminase to

target the prodrug 5-fluorocytosine (5-FC) for local activation and tumoricidal effects (230). MRCS may serve as a platform for future therapies targeting aberrant tissue stiffness in conditions such as pulmonary fibrosis.

Conclusions and Future Perspectives

Mechanical cues from the extracellular environment regulate the phenotype and function of various lung cell types and guide both lung development and tissue repair following injury. Disruption of normal mechanical environment perturbs lung cell function and is associated with many types of lung diseases. Currently, our understanding of the mechanisms by which lung cells sense matrix stiffness and transduce matrix mechanical signals remains limited. The Rho/ROCK/actin cytoskeleton/MKL1 pathway and the Hippo/YAP/TAZ pathway are well-characterized intracellular pathways that translate matrix mechanical cues into the cellular responses. A better understanding of mechanosensing and mechanotransduction mechanisms can provide us with many opportunities to develop novel therapeutic strategies targeting altered ECM mechanics in lung diseases. The lung has the capacity to effectively resolve a significant fibrotic burden. The mechanisms underlying destiffening of the fibrotic ECM and matrix turnover during lung fibrosis resolution remain poorly understood. Matrix stiffness influences the response of many types of cancer cells to drug treatments (457). It is currently unknown whether changes in matrix stiffness could sensitize lung cells to the existing therapeutics of lung diseases. Furthermore, drug screens on regular tissue culture plates can give many false positives (116). Drug screens on hydrogel-based platforms with physiologically relevant stiffness could potentially avoid the false positives from tissue culture plate screens.

There is an increasing availability of experimental tools to investigate the mechanical properties of lung tissues. AFM allows mechanical testing of lung ECM at the microscale level, providing important insights into mechanical cross talks between cells and the ECM. The development of new imaging modalities capable of noninvasively monitoring lung tissue mechanics *in vivo* will benefit earlier diagnoses and potentially lead to better patient outcomes. Stiffness-tunable hydrogel systems currently available are much more complex than standard tissue culture plastic or glass. However, they are still oversimplified when compared to the dynamic *in vivo* cell microenvironment and, hence, need to be further improved to match the complexity of native extracellular environment. Physiological tissues and ECMs are viscoelastic, exhibiting stress relaxation or creep over time (63). Cells sense and respond to the viscoelastic properties of ECMs. Changes in matrix viscoelasticity drive broad changes in cell proliferation, gene expression, migration, and fate (107). It is likely that changes in tissue viscoelasticity are associated with many types of diseases including cancer and fibrosis (350, 364). Advances in microphysiological systems (e.g., tissue-on-chip) (74, 309) and 3D printing with viscoelastic materials (142, 391) allow tissue and organ structure and properties to be more faithfully recapitulated. Understanding lung mechanopathobiology may open the door to treatment avenues for various lung diseases.

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List of Abbreviations

αSMA	α-smooth muscle actin
5-FC	5-fluorocytosine
aECM	apical extracellular matrix
AFM	atomic force microscopy
AGEs	advanced glycation end products
AHR	airway responsiveness
AMR	active microrheology
APA	alternative polyadenylation
APC	adenomatous polyposis coli
APCs	antigen-presenting cells
APS	ammonium persulfate
ARAs	age-related aortic stiffening
ARDS	acute respiratory distress syndrome
ARF	acoustic radiation force
ASCs	adipose-derived stem cells
ASM	airway smooth muscle
ASMCs	airway smooth muscle cells
AT2	type II alveolar epithelial cells
BALF	bronchoalveolar lavage fluid
BCRs	B-cell receptors
Bgn	biglycan
BMDCs	bone marrow-derived dendritic cells
BMDMs	bone marrow-derived macrophages
BPD	bronchopulmonary dysplasia
CAFs	cancer-associated fibroblasts

cAMP	cyclic adenosine monophosphate
COL1A1	type I collagen
COPD	chronic obstructive pulmonary disease
DC	dendritic cell
Dcn	decorin
dECM	decellularized extracellular matrix
DFS	disease-free survival
DHX	dihydrexidine
DOPA	dihydroxyphenylalanine
DRD1	dopamine receptor D1
DSB	double-strand break
DSP	desmoplakin
ECM	extracellular matrix
ECs	endothelial cells
EDB-FN	extra domain-B splice variant of fibronectin
EGF	epidermal growth factor
EMT	epithelial-mesenchymal transition
ESCs	embryonic stem cells
EVs	extracellular vesicles
FA	focal adhesion
FAK	focal adhesion kinase
Fn	fibronectin
GAGs	glycosaminoglycans
GLS1	glutaminase
GPCRs	GTP-binding protein (G protein)-coupled receptors
GWAS	Genome-wide association studies
H3K4	histone 3 lysine 4
H3K9	histone 3 lysine 9
HA	hyaluronic acid

HAS	HA synthase
HAT	histone acetyltransferase
hnRNP A1	heterogeneous nuclear ribonucleoprotein A1
HS	heparin sulfate
ILP	invadosome-like protrusions
IPF	idiopathic pulmonary fibrosis
iPS	induced pluripotent stem cells
IS	immunological synapses
LAP	latency associated propeptide
LbL	layer by layer
LBR	lamin B receptor
L-DOPA	3,4-dihydroxy-L-phenylalanine
LFA-1	lymphocyte function-associated antigen 1
LH2	lysyl hydroxylase 2
LLC	large latent complex
LOX	lysyl oxidase
LOXL	lysyl oxidase-like proteins
LRP5	lipoprotein receptor-related protein 5
LTBP-1	latent TGF-b1-binding protein
MDMD4	mouse double minute 4 homolog
MEFs	mouse embryonic fibroblasts
MHC	major histocompatibility complex
MICA	major histocompatibility complex class I polypeptide-related sequence A
miRNAs	MicroRNAs
MKL1	Megakaryoblastic leukemia 1
MLC	myosin light chain
MLCK	myosin light-chain kinase
MMMS	minimal matrix model of scars

MMPs	matrix metalloproteinases
MRE	magnetic resonance elastography
MSCs	mesenchymal stem cells
NIR	near-infrared
NK	natural killer
NSCLC	non-small cell lung cancer
NSIM	nonlinear stress inference microscopy
OTT	orthotopic tracheal transplantation
PA	polyacrylamide
PAECs	pulmonary arterial endothelial cells
PAH	pulmonary arterial hypertension
PAI	plasminogen activator inhibitor
PASMC	spulmonary artery smooth muscle cells
PCM	pericellular matrix
PDGFR	platelet-derived growth factor receptor
PDL-1	programmed death-ligand 1
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
PEGDA	PEG diacrylate
PEG8NB	8-arm PEG-norbornene
PFK	phosphofructokinase
PGs	proteoglycans
PH	pulmonary hypertension
PIC	polyisocyanopeptide
PMA	phorbol 12-myristate 13-acetate
PPE	porcine pancreatic elastase
PVA	polyvinyl alcohol
ROCK	Rho-associated protein kinase
SAPs	self-assembling peptides

SHP-1	SH2-domain-containing protein tyrosine phosphatase-1
SJS	Schwartz-Jampel syndrome
SLRPs	small leucine-rich proteoglycans
SNPs	single-nucleotide polymorphisms
SPC25	spindle pole body component 25 homolog
SR	serine/arginine rich
SRF	serum response factor
sulfo-SANPAH	sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate
TAZ	PDZ-binding motif
TCRs	T-cell receptors
TEMED	tetramethylethylenediamine
TGs	transglutaminases
TNC	tenascin-C
TRPV4	transient receptor potential vanilloid 4
UBTD1	ubiquitin domain-containing protein 1
UEI	ultrasound elasticity imaging
VE-cadherin	vascular endothelial cadherin
VSMCs	vascular smooth muscle cells
WASP	Wiskott-Aldrich syndrome protein
YAP	Yes-associated protein
ZAP70	zeta chain of T-cell receptor-associated protein kinase 70
ZNF416	Zinc Finger Protein 416

References

1. Adlerz KM, Aranda-Espinoza H, Hayenga HN. Substrate elasticity regulates the behavior of human monocyte-derived macrophages. Eur Biophys J 45: 301–309, 2016. [PubMed: 26613613]
2. Alcaraz J, Buscemi L, Grabulosa M, Trepat X, Fabry B, Farré R, Navajas D. Microrheology of human lung epithelial cells measured by atomic force microscopy. Biophys J 84: 2071–2079, 2003. [PubMed: 12609908]
3. Allen RJ, Porte J, Braybrooke R, Flores C, Fingerlin TE, Oldham JM, Guillen-Guio B, Ma SF, Okamoto T, John AE, Obeidat M, Yang IV, Henry A, Hubbard RB, Navaratnam V, Saini G, Thompson N, Booth HL, Hart SP, Hill MR, Hirani N, Maher TM, McAnulty RJ, Millar AB, Molyneaux PL, Parfrey H, Rassl DM, Whyte MKB, Fahy WA, Marshall RP, Oballa E, Bossé Y,

Nickle DC, Sin DD, Timens W, Shrine N, Sayers I, Hall IP, Noth I, Schwartz DA, Tobin MD, Wain LV, Jenkins RG. Genetic variants associated with susceptibility to idiopathic pulmonary fibrosis in people of European ancestry: A genome-wide association study. Lancet Respir Med 5: 869–880, 2017. [PubMed: 29066090]

4. Allison DD, Grande-Allen KJ. Review. Hyaluronan: A powerful tissue engineering tool. Tissue Eng 12: 2131–2140, 2006. [PubMed: 16968154]
5. An SS, Mitzner W, Tang WY, Ahn K, Yoon AR, Huang J, Kilic O, Yong HM, Fahey JW, Kumar S, Biswal S, Holgate ST, Panettieri RA, Solway J, Liggett SB. An inflammation-independent contraction mechanophenotype of airway smooth muscle in asthma. J Allergy Clin Immunol 138: 294–297.e294, 2016. [PubMed: 26936804]
6. Anguiano M, Morales X, Castilla C, Pena AR, Ederra C, Martínez M, Ariz M, Esparza M, Amaveda H, Mora M. The use of mixed collagen-Matrigel matrices of increasing complexity recapitulates the biphasic role of cell adhesion in cancer cell migration: ECM sensing, remodeling and forces at the leading edge of cancer invasion. PloS one 15: e0220019, 2020. [PubMed: 31945053]
7. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. J Cell Sci 116: 217–224, 2003. [PubMed: 12482908]
8. Aragona M, Panciera T, Manfrin A, Giulitti S, Michielin F, Elvassore N, Dupont S, Piccolo S. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. Cell 154: 1047–1059, 2013. [PubMed: 23954413]
9. Araujo BB, Dolhnikoff M, Silva LF, Elliot J, Lindeman JH, Ferreira DS, Mulder A, Gomes HA, Fernezlian SM, James A, Mauad T. Extracellular matrix components and regulators in the airway smooth muscle in asthma. Eur Respir J 32: 61–69, 2008. [PubMed: 18321931]
10. Asano S, Ito S, Takahashi K, Furuya K, Kondo M, Sokabe M, Hasegawa Y. Matrix stiffness regulates migration of human lung fibroblasts. Physiol Rep 5, 5 (9): e13281, 2017.
11. Asparuhova MB, Ferralli J, Chiquet M, Chiquet-Ehrismann R. The transcriptional regulator megakaryoblastic leukemia-1 mediates serum response factor-independent activation of tenascin-C transcription by mechanical stress. FASEB J 25: 3477–3488, 2011. [PubMed: 21705668]
12. Aumiller V, Strobel B, Romeike M, Schuler M, Stierstorfer BE, Kreuz S. Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. Sci Rep 7: 149, 2017. [PubMed: 28273952]
13. Austen K, Ringer P, Mehlich A, Chrostek-Grashoff A, Kluger C, Klingner C, Sabass B, Zent R, Rief M, Grashoff C. Extracellular rigidity sensing by talin isoform-specific mechanical linkages. Nat Cell Biol 17: 1597–1606, 2015. [PubMed: 26523364]
14. Aydin D, Louban I, Perschmann N, Blümmel J, Lohmüller T, Cavalcanti-Adam EA, Haas TL, Walczak H, Kessler H, Fiammengo R, Spatz JP. Polymeric substrates with tunable elasticity and nanoscopically controlled biomolecule presentation. Langmuir 26: 15472–15480, 2010. [PubMed: 20831282]
15. Azeloglu EU, Bhattacharya J, Costa KD. Atomic force microscope elastography reveals phenotypic differences in alveolar cell stiffness. J Appl Physiol 105 (652-661): 2008, 1985.
16. Balestrini JL, Chaudhry S, Sarrazy V, Koehler A, Hinz B. The mechanical memory of lung myofibroblasts. Integr Biol (Camb) 4: 410–421, 2012. [PubMed: 22410748]
17. Bao M, Xie J, Huck WTS. Recent advances in engineering the stem cell microniche in 3D. Adv Sci (Weinh) 5: 1800448, 2018. [PubMed: 30128252]
18. Barriga EH, Franze K, Charras G, Mayor R. Tissue stiffening coordinates morphogenesis by triggering collective cell migration in vivo. Nature 554: 523–527, 2018. [PubMed: 29443958]
19. Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, Mikels A, Vaysberg M, Ghermazien H, Wai C, Garcia CA, Velayo AC, Jorgensen B, Biermann D, Tsai D, Green J, Zaffryar-Eilot S, Holzer A, Ogg S, Thai D, Neufeld G, Van Vlasselaer P, Smith V. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. Nat Med 16: 1009–1017, 2010. [PubMed: 20818376]
20. Bashirzadeh Y, Chatterji S, Palmer D, Dumbali S, Qian S, Maruthamuthu V. Stiffness measurement of soft silicone substrates for mechanobiology studies using a widefield fluorescence microscope. J Vis Exp, 2018.

21. Basu R, Whitlock BM, Husson J, Le Floc'h A, Jin W, Oyler-Yaniv A, Dotiwala F, Giannone G, Hivroz C, Biais N, Lieberman J, Kam LC, Huse M. Cytotoxic T Cells Use Mechanical Force to Potentiate Target Cell Killing. Cell 165: 100–110, 2016. [PubMed: 26924577]
22. Beenakker JW, Ashcroft BA, Lindeman JH, Oosterkamp TH. Mechanical properties of the extracellular matrix of the aorta studied by enzymatic treatments. Biophys J 102: 1731–1737, 2012. [PubMed: 22768928]
23. Bellaye PS, Shimbori C, Upagupta C, Sato S, Shi W, Gauldie J, Ask K, Kolb M. Lysyl Oxidase-like 1 protein deficiency protects mice from adenoviral transforming growth factor-β1-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 58: 461–470, 2018. [PubMed: 29115860]
24. Bellmunt MJ, Portero M, Pamplona R, Cosso L, Odetti P, Prat J. Evidence for the Maillard reaction in rat lung collagen and its relationship with solubility and age. Biochim Biophys Acta 1272: 53–60, 1995. [PubMed: 7662720]
25. Bercoff J, Tanter M, Fink M. Supersonic shear imaging: A new technique for soft tissue elasticity mapping. IEEE Trans Ultrason Ferroelectr Freq Control 51: 396–409, 2004. [PubMed: 15139541]
26. Berg WA, Cosgrove DO, Doré CJ, Schäfer FK, Svensson WE, Hooley RJ, Ohlinger R, Mendelson EB, Balu-Maestro C, Locatelli M, Tourasse C, Cavanaugh BC, Juhan V, Stavros AT, Tardivon A, Gay J, Henry JP, Cohen-Bacrie C, Investigators B. Shear-wave elastography improves the specificity of breast US: The BE1 multinational study of 939 masses. Radiology 262: 435–449, 2012. [PubMed: 22282182]
27. Berry MF, Engler AJ, Woo YJ, Pirolli TJ, Bish LT, Jayasankar V, Morine KJ, Gardner TJ, Discher DE, Sweeney HL. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. Am J Physiol Heart Circ Physiol 290: H2196–H2203, 2006. [PubMed: 16473959]
28. Bertero T, Cottrill KA, Lu Y, Haeger CM, Dieffenbach P, Annis S, Hale A, Bhat B, Kaimal V, Zhang YY, Graham BB, Kumar R, Saggar R, Wallace WD, Ross DJ, Black SM, Fratz S, Fineman JR, Vargas SO, Haley KJ, Waxman AB, Chau BN, Fredenburgh LE, Chan SY. Matrix remodeling promotes pulmonary hypertension through feedback mechanoactivation of the YAP/TAZ-miR-130/301 circuit. Cell Rep 13: 1016–1032, 2015. [PubMed: 26565914]
29. Bertero T, Handen AL, Chan SY. Factors associated with heritable pulmonary arterial hypertension exert convergent actions on the miR-130/301-vascular matrix feedback loop. Int J Mol Sci 19: 2289, 2018. [PubMed: 30081553]
30. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, Zhao J, Tai Y, Tang Y, Zhang YY, Rehman S, Sugahara M, Qi Z, Gorcsan J, Vargas SO, Saggar R, Wallace WD, Ross DJ, Haley KJ, Waxman AB, Parikh VN, De Marco T, Hsue PY, Morris A, Simon MA, Norris KA, Gaggioli C, Loscalzo J, Fessel J, Chan SY. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. J Clin Invest 126: 3313–3335, 2016. [PubMed: 27548520]
31. Bertero T, Oldham WM, Grasset EM, Bourget I, Boulter E, Pisano S, Hofman P, Bellvert F, Meneguzzi G, Bulavin DV, Estrach S, Feral CC, Chan SY, Bozec A, Gaggioli C. Tumor-stroma mechanics coordinate amino acid availability to sustain tumor growth and malignancy. Cell Metab 29: 124–140.e110, 2019. [PubMed: 30293773]
32. Besse F, Ephrussi A. Translational control of localized mRNAs: Restricting protein synthesis in space and time. Nat Rev Mol Cell Biol 9: 971–980, 2008. [PubMed: 19023284]
33. Bhowmick NA, Moses HL. Tumor–stroma interactions. Curr Opin Genet Develop 15: 97–101, 2005.
34. Bidan CM, Veldsink AC, Meurs H, Gosens R. Airway and extracellular matrix mechanics in COPD. Front Physiol 6: 346, 2015. [PubMed: 26696894]
35. Bilgen M, Insana MF. Deformation models and correlation analysis in elastography. J Acoust Soc Am 99: 3212–3224, 1996. [PubMed: 8642127]
36. Binnig G, Quate CF, Gerber C. Atomic force microscope. Phys Rev Lett 56: 930–933, 1986. [PubMed: 10033323]
37. Birukova AA, Tian X, Cokic I, Beckham Y, Gardel ML, Birukov KG. Endothelial barrier disruption and recovery is controlled by substrate stiffness. Microvasc Res 87: 50–57, 2013. [PubMed: 23296034]

38. Bischof JC, He X. Thermal stability of proteins. Ann N Y Acad Sci 1066: 12–33, 2005. [PubMed: 16533916]
39. Blin G, Lablack N, Louis-Tisserand M, Nicolas C, Picart C, Pucéat M. Nano-scale control of cellular environment to drive embryonic stem cells selfrenewal and fate. Biomaterials 31: 1742–1750, 2010. [PubMed: 19962191]
40. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 15: 786–801, 2014. [PubMed: 25415508]
41. Booth AJ, Hadley R, Cornett AM, Dreffs AA, Matthes SA, Tsui JL, Weiss K, Horowitz JC, Fiore VF, Barker TH, Moore BB, Martinez FJ, Niklason LE, White ES. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. Am J Respir Crit Care Med, 2012.
42. Bordeleau F, Califano JP, Negrón Abril YL, Mason BN, LaValley DJ, Shin SJ, Weiss RS, Reinhart-King CA. Tissue stiffness regulates serine/arginine-rich protein-mediated splicing of the extra domain B-fibronectin isoform in tumors. Proc Natl Acad Sci U S A 112: 8314–8319, 2015. [PubMed: 26106154]
43. Bordeleau F, Mason BN, Lollis EM, Mazzola M, Zanotelli MR, Somasegar S, Califano JP, Montague C, LaValley DJ, Huynh J, Mencia-Trinchant N, Negrón Abril YL, Hassane DC, Bonassar LJ, Butcher JT, Weiss RS, Reinhart-King CA. Matrix stiffening promotes a tumor vasculature phenotype. Proc Natl Acad Sci U S A 114: 492–497, 2017. [PubMed: 28034921]
44. Boudreau N, Werb Z, Bissell MJ. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. Proc Natl Acad Sci U S A 93: 3509–3513, 1996. [PubMed: 8622967]
45. Bourke JE, Li X, Foster SR, Wee E, Dagher H, Ziogas J, Harris T, Bonacci JV, Stewart AG. Collagen remodelling by airway smooth muscle is resistant to steroids and β_2-agonists. Eur Respir J 37: 173–182, 2011. [PubMed: 20595143]
46. Branco da Cunha C, Klumpers DD, Koshy ST, Weaver JC, Chaudhuri O, Seruca R, Carneiro F, Granja PL, Mooney DJ. CD44 alternative splicing in gastric cancer cells is regulated by culture dimensionality and matrix stiffness. Biomaterials 98: 152–162, 2016. [PubMed: 27187279]
47. Broguiere N, Isenmann L, Zenobi-Wong M. Novel enzymatically cross-linked hyaluronan hydrogels support the formation of 3D neuronal networks. Biomaterials 99: 47–55, 2016. [PubMed: 27209262]
48. Brown AC, Fiore VF, Sulchek TA, Barker TH. Physical and chemical microenvironmental cues orthogonally control the degree and duration of fibrosis-associated epithelial-to-mesenchymal transitions. J Pathol 229: 25–35, 2013. [PubMed: 23018598]
49. Brown XQ, Bartolak-Suki E, Williams C, Walker ML, Weaver VM, Wong JY. Effect of substrate stiffness and PDGF on the behavior of vascular smooth muscle cells: Implications for atherosclerosis. J Cell Physiol 225: 115–122, 2010. [PubMed: 20648629]
50. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 318: 1315–1321, 1988. [PubMed: 3283558]
51. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. Biomaterials 23: 4315–4323, 2002. [PubMed: 12219821]
52. Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. Adv Mater 23: H41–H56, 2011. [PubMed: 21394792]
53. Butcher DT, Alliston T, Weaver VM. A tense situation: Forcing tumour progression. Nat Rev Cancer 9: 108–122, 2009. [PubMed: 19165226]
54. Buxboim A, Irianto J, Swift J, Athirasala A, Shin JW, Rehfeldt F, Discher DE. Coordinated increase of nuclear tension and lamin-A with matrix stiffness outcompetes lamin-B receptor that favors soft tissue phenotypes. Mol Biol Cell 28: 3333–3348, 2017. [PubMed: 28931598]
55. Buxboim A, Swift J, Irianto J, Spinler KR, Dingal PC, Athirasala A, Kao YR, Cho S, Harada T, Shin JW, Discher DE. Matrix elasticity regulates lamin-A,C phosphorylation and turnover with feedback to actomyosin. Curr Biol 24: 1909–1917, 2014. [PubMed: 25127216]

56. Caliari SR, Perepelyuk M, Cosgrove BD, Tsai SJ, Lee GY, Mauck RL, Wells RG, Burdick JA. Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation. Sci Rep 6: 21387, 2016. [PubMed: 26906177]
57. Cao Y, Lee BH, Peled HB, Venkatraman SS. Synthesis of stiffness-tunable and cell-responsive Gelatin-poly(ethylene glycol) hydrogel for three-dimensional cell encapsulation. J Biomed Mater Res A 104: 2401–2411, 2016. [PubMed: 27170015]
58. Caplan MR, Schwartzfarb EM, Zhang S, Kamm RD, Lauffenburger DA. Control of self-assembling oligopeptide matrix formation through systematic variation of amino acid sequence. Biomaterials 23: 219–227, 2002. [PubMed: 11762841]
59. Chai S, Chai Q, Danielsen CC, Hjorth P, Nyengaard JR, Ledet T, Yamaguchi Y, Rasmussen LM, Wogensen L. Overexpression of hyaluronan in the tunica media promotes the development of atherosclerosis. Circ Res 96: 583–591, 2005. [PubMed: 15705963]
60. Chakraborty M, Chu K, Shrestha A, Revelo XS, Zhang X, Gold MJ, Khan S, Lee M, Huang C, Akbari M, Barrow F, Chan YT, Lei H, Kotoulas NK, Jovel J, Pastrello C, Kotlyar M, Goh C, Michelakis E, Clemente-Casares X, Ohashi PS, Engleman EG, Winer S, Jurisica I, Tsai S, Winer DA. Mechanical stiffness controls dendritic cell metabolism and function. Cell Rep 34: 108609, 2021. [PubMed: 33440149]
61. Chan CE, Odde DJ. Traction dynamics of filopodia on compliant substrates. Science 322: 1687–1691, 2008. [PubMed: 19074349]
62. Chao PH, Sheng SC, Chang WR. Micro-composite substrates for the study of cell-matrix mechanical interactions. J Mech Behav Biomed Mater 38: 232–241, 2014. [PubMed: 24556044]
63. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB. Effects of extracellular matrix viscoelasticity on cellular behaviour. Nature 584: 535–546, 2020. [PubMed: 32848221]
64. Chen H, Qu J, Huang X, Kurundkar A, Zhu L, Yang N, Venado A, Ding Q, Liu G, Antony VB, Thannickal VJ, Zhou Y. Mechanosensing by the α6-integrin confers an invasive fibroblast phenotype and mediates lung fibrosis. Nat Commun 7: 12564, 2016. [PubMed: 27535718]
65. Chen HI, Sudol M. The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. Proc Natl Acad Sci U S A 92: 7819–7823, 1995. [PubMed: 7644498]
66. Chen M, Zhang Y, Zhou P, Liu X, Zhao H, Zhou X, Gu Q, Li B, Zhu X, Shi Q. Substrate stiffness modulates bone marrow-derived macrophage polarization through NF-kappaB signaling pathway. Bioact Mater 5: 880–890, 2020. [PubMed: 32637751]
67. Chen Y, Terajima M, Yang Y, Sun L, Ahn YH, Pankova D, Puperi DS, Watanabe T, Kim MP, Blackmon SH, Rodriguez J, Liu H, Behrens C, Wistuba II, Minelli R, Scott KL, Sanchez-Adams J, Guilak F, Pati D, Thilaganathan N, Burns AR, Creighton CJ, Martinez ED, Zal T, Grande-Allen KJ, Yamauchi M, Kurie JM. Lysyl hydroxylase 2 induces a collagen cross-link switch in tumor stroma. J Clin Invest 125: 1147–1162, 2015. [PubMed: 25664850]
68. Cheng X, Yang Y, Fan Z, Yu L, Bai H, Zhou B, Wu X, Xu H, Fang M, Shen A, Chen Q, Xu Y. MKL1 potentiates lung cancer cell migration and invasion by epigenetically activating MMP9 transcription. Oncogene 34: 5570–5581, 2015. [PubMed: 25746000]
69. Chien JW, Richards TJ, Gibson KF, Zhang Y, Lindell KO, Shao L, Lyman SK, Adamkewicz JI, Smith V, Kaminski N, O'Riordan T. Serum lysyl oxidase-like 2 levels and idiopathic pulmonary fibrosis disease progression. Eur Respir J 43: 1430–1438, 2014. [PubMed: 24177001]
70. Chin L, Xia Y, Discher DE, Janmey PA. Mechanotransduction in cancer. Curr Opin Chem Eng 11: 77–84, 2016. [PubMed: 28344926]
71. Choi CM, Jang SJ, Park SY, Choi YB, Jeong JH, Kim DS, Kim HK, Park KS, Nam BH, Kim HR, Kim SY, Hong KM, KTORG. Transglutaminase 2 as an independent prognostic marker for survival of patients with non-adenocarcinoma subtype of non-small cell lung cancer. Mol Cancer 10: 119, 2011. [PubMed: 21943122]
72. Chopra A, Lin V, McCollough A, Atzet S, Prestwich GD, Wechsler AS, Murray ME, Oake SA, Kresh JY, Janmey PA. Reprogramming cardiomyocyte mechanosensing by crosstalk between integrins and hyaluronic acid receptors. J Biomech 45: 824–831, 2012. [PubMed: 22196970]

73. Chowdhury F, Li Y, Poh YC, Yokohama-Tamaki T, Wang N, Tanaka TS. Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions. PLoS One 5: e15655, 2010. [PubMed: 21179449]
74. Clevers H. Modeling development and disease with organoids. Cell 165: 1586–1597, 2016. [PubMed: 27315476]
75. Codelia VA, Sun G, Irvine KD. Regulation of YAP by mechanical strain through Jnk and Hippo signaling. Curr Biol 24: 2012–2017, 2014. [PubMed: 25127217]
76. Collum SD, Chen NY, Hernandez AM, Hanmandlu A, Sweeney H, Mertens TCJ, Weng T, Luo F, Molina JG, Davies J, Horan IP, Morrell NW, Amione-Guerra J, Al-Jabbari O, Youker K, Sun W, Rajadas J, Bollyky PL, Akkanti BH, Jyothula S, Sinha N, Guha A, Karmouty-Quintana H. Inhibition of hyaluronan synthesis attenuates pulmonary hypertension associated with lung fibrosis. Br J Pharmacol 174: 3284–3301, 2017. [PubMed: 28688167]
77. Corsi A, Xu T, Chen XD, Boyde A, Liang J, Mankani M, Sommer B, Iozzo RV, Eichstetter I, Robey PG, Bianco P, Young MF. Phenotypic effects of biglycan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, and mimic Ehlers-Danlos-like changes in bone and other connective tissues. J Bone Miner Res 17: 1180–1189, 2002. [PubMed: 12102052]
78. Cortiella J, Niles J, Cantu A, Brettler A, Pham A, Vargas G, Winston S, Wang J, Walls S, Nichols JE. Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. Tissue Eng Part A 16: 2565–2580, 2010. [PubMed: 20408765]
79. Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R. Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 147: 1109–1122, 1999. [PubMed: 10579729]
80. Crest J, Diz-Muñoz A, Chen DY, Fletcher DA, Bilder D. Organ sculpting by patterned extracellular matrix stiffness. Elife 6: e24958, 2017. [PubMed: 28653906]
81. Cretu A, Castagnino P, Assoian R. Studying the effects of matrix stiffness on cellular function using acrylamide-based hydrogels. J Vis Exp, 2010.
82. Cui Y, Hameed FM, Yang B, Lee K, Pan CQ, Park S, Sheetz M. Cyclic stretching of soft substrates induces spreading and growth. Nat Commun 6: 6333, 2015. [PubMed: 25704457]
83. Damljanović V, Lagerholm BC, Jacobson K. Bulk and micropatterned conjugation of extracellular matrix proteins to characterized polyacrylamide substrates for cell mechanotransduction assays. Biotechniques 39: 847–851, 2005. [PubMed: 16382902]
84. Das RK, Gocheva V, Hammink R, Zouani OF, Rowan AE. Stress-stiffening-mediated stem-cell commitment switch in soft responsive hydrogels. Nat Mater 15: 318–325, 2016. [PubMed: 26618883]
85. de Hilster RHJ, Sharma PK, Jonker MR, White ES, Gercama EA, Roobeek M, Timens W, Harmsen MC, Hylkema MN, Burgess JK. Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue. Am J Physiol Lung Cell Mol Physiol 318: L698–L704, 2020. [PubMed: 32048864]
86. de la Zerda A, Kratochvil MJ, Suhar NA, Heilshorn SC. Review: Bioengineering strategies to probe T cell mechanobiology. APL Bioeng 2: 021501, 2018. [PubMed: 31069295]
87. De Langhe SP, Sala FG, Del Moral PM, Fairbanks TJ, Yamada KM, Warburton D, Burns RC, Bellusci S. Dickkopf-1 (DKK1) reveals that fibronectin is a major target of Wnt signaling in branching morphogenesis of the mouse embryonic lung. Dev Biol 277: 316–331, 2005. [PubMed: 15617677]
88. Degos F, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P, Group Fs. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: A multicenter prospective study (the FIBROSTIC study). J Hepatol 53: 1013–1021, 2010. [PubMed: 20850886]
89. del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz MP. Stretching single talin rod molecules activates vinculin binding. Science 323: 638–641, 2009. [PubMed: 19179532]
90. Deng CX, Hong X, Stegemann JP. Ultrasound imaging techniques for spatiotemporal characterization of composition, microstructure, and mechanical properties in tissue engineering. Tissue Eng Part B Rev 22: 311–321, 2016. [PubMed: 26771992]

91. Deng M, Lin J, Nowsheen S, Liu T, Zhao Y, Villalta PW, Sicard D, Tschumperlin DJ, Lee S, Kim J, Lou Z. Extracellular matrix stiffness determines DNA repair efficiency and cellular sensitivity to genotoxic agents. Sci Adv 6: eabb2630, 2020. [PubMed: 32917705]
92. Dewall RJ. Ultrasound elastography: Principles, techniques, and clinical applications. Crit Rev Biomed Eng 41: 1–19, 2013. [PubMed: 23510006]
93. Dieffenbach PB, Haeger CM, Coronata AMF, Choi KM, Varelas X, Tschumperlin DJ, Fredenburgh LE. Arterial stiffness induces remodeling phenotypes in pulmonary artery smooth muscle cells via YAP/TAZ-mediated repression of cyclooxygenase-2. Am J Physiol Lung Cell Mol Physiol 313: L628–L647, 2017. [PubMed: 28642262]
94. Dingal PC, Bradshaw AM, Cho S, Raab M, Buxboim A, Swift J, Discher DE. Fractal heterogeneity in minimal matrix models of scars modulates stiff-niche stem-cell responses via nuclear exit of a mechanorepressor. Nat Mater 14: 951–960, 2015. [PubMed: 26168347]
95. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 310: 1139–1143, 2005. [PubMed: 16293750]
96. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science 324: 1673–1677, 2009. [PubMed: 19556500]
97. Doherty JR, Trahey GE, Nightingale KR, Palmeri ML. Acoustic radiation force elasticity imaging in diagnostic ultrasound. IEEE Trans Ultrason Ferroelectr Freq Control 60: 685–701, 2013. [PubMed: 23549529]
98. Dong B, Hannezo E, Hayashi S. Balance between apical membrane growth and luminal matrix resistance determines epithelial tubule shape. Cell Rep 7: 941–950, 2014. [PubMed: 24794438]
99. Du J, Zu Y, Li J, Du S, Xu Y, Zhang L, Jiang L, Wang Z, Chien S, Yang C. Extracellular matrix stiffness dictates Wnt expression through integrin pathway. Sci Rep 6: 20395, 2016. [PubMed: 26854061]
100. Dunkman AA, Buckley MR, Mienaltowski MJ, Adams SM, Thomas SJ, Satchell L, Kumar A, Pathmanathan L, Beason DP, Iozzo RV, Birk DE, Soslowsky LJ. Decorin expression is important for age-related changes in tendon structure and mechanical properties. Matrix Biol 32: 3–13, 2013. [PubMed: 23178232]
101. Dunphy SE, Bratt JA, Akram KM, Forsyth NR, El Haj AJ. Hydrogels for lung tissue engineering: Biomechanical properties of thin collagen-elastin constructs. J Mech Behav Biomed Mater 38: 251–259, 2014. [PubMed: 24809968]
102. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S. Role of YAP/TAZ in mechanotransduction. Nature 474: 179–183, 2011. [PubMed: 21654799]
103. Ebihara T, Venkatesan N, Tanaka R, Ludwig MS. Changes in extracellular matrix and tissue viscoelasticity in bleomycin-induced lung fibrosis. Temporal aspects. Am J Respir Crit Care Med 162: 1569–1576, 2000. [PubMed: 11029378]
104. Eekhoff JD, Fang F, Lake SP. Multiscale mechanical effects of native collagen cross-linking in tendon. Connect Tissue Res 59: 410–422, 2018. [PubMed: 29873266]
105. Ehrlich SF, Quesenberry CP, Van Den Eeden SK, Shan J, Ferrara A. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. Diabetes Care 33: 55–60, 2010. [PubMed: 19808918]
106. Eisenberg JL, Safi A, Wei X, Espinosa HD, Budinger GS, Takawira D, Hopkinson SB, Jones JC. Substrate stiffness regulates extracellular matrix deposition by alveolar epithelial cells. Res Rep Biol 1–12: 2011, 2011.
107. Elosegui-Artola A. The extracellular matrix viscoelasticity as a regulator of cell and tissue dynamics. Curr Opin Cell Biol 72: 10–18, 2021. [PubMed: 33993058]
108. Elosegui-Artola A, Bazellières E, Allen MD, Andreu I, Oria R, Sunyer R, Gomm JJ, Marshall JF, Jones JL, Trepat X, Roca-Cusachs P. Rigidity sensing and adaptation through regulation of integrin types. Nat Mater 13: 631–637, 2014. [PubMed: 24793358]
109. Eng D, Caplan M, Preul M, Panitch A. Hyaluronan scaffolds: A balance between backbone functionalization and bioactivity. Acta Biomater 6: 2407–2414, 2010. [PubMed: 20051273]

110. Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D. Substrate compliance versus ligand density in cell on gel responses. Biophys J 86: 617–628, 2004. [PubMed: 14695306]
111. Engler AJ, Rehfeldt F, Sen S, Discher DE. Microtissue elasticity: Measurements by atomic force microscopy and its influence on cell differentiation. Methods Cell Biol 83: 521–545, 2007. [PubMed: 17613323]
112. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 126: 677–689, 2006. [PubMed: 16923388]
113. Enomoto T, Usuki J, Azuma A, Nakagawa T, Kudoh S. Diabetes mellitus may increase risk for idiopathic pulmonary fibrosis. Chest 123: 2007–2011, 2003. [PubMed: 12796182]
114. Evans ND, Minelli C, Gentleman E, LaPointe V, Patankar SN, Kallivretaki M, Chen X, Roberts CJ, Stevens MM. Substrate stiffness affects early differentiation events in embryonic stem cells. Eur Cell Mater 18: 1–13; discussion 13-14, 2009. [PubMed: 19768669]
115. Fairbanks BD, Schwartz MP, Halevi AE, Nuttelman CR, Bowman CN, Anseth KS. A versatile synthetic extracellular matrix mimic via thiol-norbornene photopolymerization. Adv Mater 21: 5005–5010, 2009. [PubMed: 25377720]
116. Feng J, Tang Y, Xu Y, Sun Q, Liao F, Han D. Substrate stiffness influences the outcome of antitumor drug screening in vitro. Clin Hemorheol Microcirc 55: 121–131, 2013. [PubMed: 23445634]
117. Feng X, Degese MS, Iglesias-Bartolome R, Vaque JP, Molinolo AA, Rodrigues M, Zaidi MR, Ksander BR, Merlino G, Sodhi A, Chen Q, Gutkind JS. Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry. Cancer Cell 25: 831–845, 2014. [PubMed: 24882515]
118. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, Loyd JE, Cosgrove GP, Lynch D, Groshong S, Collard HR, Wolters PJ, Bradford WZ, Kossen K, Seiwert SD, du Bois RM, Garcia CK, Devine MS, Gudmundsson G, Isaksson HJ, Kaminski N, Zhang Y, Gibson KF, Lancaster LH, Cogan JD, Mason WR, Maher TM, Molyneaux PL, Wells AU, Moffatt MF, Selman M, Pardo A, Kim DS, Crapo JD, Make BJ, Regan EA, Walek DS, Daniel JJ, Kamatani Y, Zelenika D, Smith K, McKean D, Pedersen BS, Talbert J, Kidd RN, Markin CR, Beckman KB, Lathrop M, Schwarz MI, Schwartz DA. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet 45: 613–620, 2013. [PubMed: 23583980]
119. Fiore VF, Strane PW, Bryksin AV, White ES, Hagood JS, Barker TH. Conformational coupling of integrin and Thy-1 regulates Fyn priming and fibroblast mechanotransduction. J Cell Biol 211: 173–190, 2015. [PubMed: 26459603]
120. Fiore VF, Wong SS, Tran C, Tan C, Xu W, Sulchek T, White ES, Hagood JS, Barker TH. αvβ3 Integrin drives fibroblast contraction and strain stiffening of soft provisional matrix during progressive fibrosis. JCI Insight 3 (20): 3, e97597, 2018.
121. Fischer RS, Gardel M, Ma X, Adelstein RS, Waterman CM. Local cortical tension by myosin II guides 3D endothelial cell branching. Curr Biol 19: 260–265, 2009. [PubMed: 19185493]
122. Forget A, Christensen J, Lüdeke S, Kohler E, Tobias S, Matloubi M, Thomann R, Shastri VP. Polysaccharide hydrogels with tunable stiffness and provasculogenic properties via α-helix to β-sheet switch in secondary structure. Proc Natl Acad Sci U S A 110: 12887–12892, 2013. [PubMed: 23886665]
123. Fortunati D, Chau DY, Wang Z, Collighan RJ, Griffin M. Cross-linking of collagen I by tissue transglutaminase provides a promising biomaterial for promoting bone healing. Amino Acids 46: 1751–1761, 2014. [PubMed: 24710705]
124. Frey MT, Engler A, Discher DE, Lee J, Wang YL. Microscopic methods for measuring the elasticity of gel substrates for cell culture: Microspheres, microindenters, and atomic force microscopy. Methods Cell Biol 83: 47–65, 2007. [PubMed: 17613304]
125. Frey MT, Wang YL. A photo-modulatable material for probing cellular responses to substrate rigidity. Soft Matter 5: 1918–1924, 2009. [PubMed: 19672325]
126. Friedman SL, Sheppard D, Duffield JS, Violette S. Therapy for fibrotic diseases: Nearing the starting line. Sci Transl Med 5: 167sr161, 2013.

127. Fu J, Wang YK, Yang MT, Desai RA, Yu X, Liu Z, Chen CS. Mechanical regulation of cell function with geometrically modulated elastomeric substrates. Nat Methods 7: 733–736, 2010. [PubMed: 20676108]
128. Fukui L, Henry JJ. FGF signaling is required for lens regeneration in *Xenopus laevis*. Biol Bull 221: 137–145, 2011. [PubMed: 21876116]
129. Gan CT, Lankhaar JW, Westerhof N, Marcus JT, Becker A, Twisk JW, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Noninvasively assessed pulmonary artery stiffness predicts mortality in pulmonary arterial hypertension. Chest 132: 1906–1912, 2007. [PubMed: 17989161]
130. García-Sancho Figueroa MC, Carrillo G, Pérez-Padilla R, Fernández-Plata MR, Buendía-Roldán I, Vargas MH, Selman M. Risk factors for idiopathic pulmonary fibrosis in a Mexican population. A case-control study. Respir Med 104: 305–309, 2010. [PubMed: 19782552]
131. Geerligs M, Peters GW, Ackermans PA, Oomens CW, Baaijens FP. Linear viscoelastic behavior of subcutaneous adipose tissue. Biorheology 45: 677–688, 2008. [PubMed: 19065014]
132. Ghajar CM, Chen X, Harris JW, Suresh V, Hughes CC, Jeon NL, Putnam AJ, George SC. The effect of matrix density on the regulation of 3-D capillary morphogenesis. Biophys J 94: 1930–1941, 2008. [PubMed: 17993494]
133. Giacomini MM, Travis MA, Kudo M, Sheppard D. Epithelial cells utilize cortical actin/myosin to activate latent TGF-β through integrin α(v)β(6)-dependent physical force. Exp Cell Res 318: 716–722, 2012. [PubMed: 22309779]
134. Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329: 1078–1081, 2010. [PubMed: 20647425]
135. Glaser KJ, Manduca A, Ehman RL. Review of MR elastography applications and recent developments. J Magn Reson Imaging 36: 757–774, 2012. [PubMed: 22987755]
136. Gould ST, Darling NJ, Anseth KS. Small peptide functionalized thiolene hydrogels as culture substrates for understanding valvular interstitial cell activation and de novo tissue deposition. Acta Biomater 8: 3201–3209, 2012. [PubMed: 22609448]
137. Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, Schwartz MA. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature 466: 263–266, 2010. [PubMed: 20613844]
138. Grevesse T, Versaevel M, Circelli G, Desprez S, Gabriele S. A simple route to functionalize polyacrylamide hydrogels for the independent tuning of mechanotransduction cues. Lab Chip 13: 777–780, 2013. [PubMed: 23334710]
139. Grevesse T, Versaevel M, Gabriele S. Preparation of hydroxy-PAAm hydrogels for decoupling the effects of mechanotransduction cues. J Vis Exp, 2014.
140. Gribbin J, Hubbard R, Smith C. Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. Respir Med 103: 927–931, 2009. [PubMed: 19058956]
141. Griffin M, Casadio R, Bergamini CM. Transglutaminases: Nature's biological glues. Biochem J 368: 377–396, 2002. [PubMed: 12366374]
142. Grosskopf AK, Truby RL, Kim H, Perazzo A, Lewis JA, Stone HA. Viscoplastic matrix materials for embedded 3D printing. ACS Appl Mater Interfaces 10: 23353–23361, 2018. [PubMed: 29493215]
143. Gu Z, Liu F, Tonkova EA, Lee SY, Tschumperlin DJ, Brenner MB. Soft matrix is a natural stimulator for cellular invasiveness. Mol Biol Cell, 2013.
144. Gundy S, Manning G, O'Connell E, Ellä V, Harwoko MS, Rochev Y, Smith T, Barron V. Human coronary artery smooth muscle cell response to a novel PLA textile/fibrin gel composite scaffold. Acta Biomater 4: 1734–1744, 2008. [PubMed: 18599374]
145. Guo L, Cui C, Zhang K, Wang J, Wang Y, Lu Y, Chen K, Yuan J, Xiao G, Tang B, Sun Y, Wu C. Kindlin-2 links mechano-environment to proline synthesis and tumor growth. Nat Commun 10: 845, 2019. [PubMed: 30783087]
146. Gupta M, Doss B, Lim CT, Voituriez R, Ladoux B. Single cell rigidity sensing: A complex relationship between focal adhesion dynamics and large-scale actin cytoskeleton remodeling. Cell Adh Migr 10: 554–567, 2016. [PubMed: 27050660]

147. Gutierrez E, Tkachenko E, Besser A, Sundd P, Ley K, Danuser G, Ginsberg MH, Groisman A. High refractive index silicone gels for simultaneous total internal reflection fluorescence and traction force microscopy of adherent cells. PLoS One 6: e23807, 2011. [PubMed: 21961031]

148. Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat Commun 3: 792, 2012. [PubMed: 22531177]

149. Guvendiren M, Perepelyuk M, Wells RG, Burdick JA. Hydrogels with differential and patterned mechanics to study stiffness-mediated myofibroblastic differentiation of hepatic stellate cells. J Mech Behav Biomed Mater 38: 198–208, 2014. [PubMed: 24361340]

150. Haak AJ, Kostallari E, Sicard D, Ligresti G, Choi KM, Caporarello N, Jones DL, Tan Q, Meridew J, Diaz Espinosa AM, Aravamudhan A, Maiers JL, Britt RD, Roden AC, Pabelick CM, Prakash YS, Nouraie SM, Li X, Zhang Y, Kass DJ, Lagares D, Tager AM, Varelas X, Shah VH, Tschumperlin DJ. Selective YAP/TAZ inhibition in fibroblasts via dopamine receptor D1 agonism reverses fibrosis. Sci Transl Med 11, 2019.

151. Hadden WJ, Young JL, Holle AW, McFetridge ML, Kim DY, Wijesinghe P, Taylor-Weiner H, Wen JH, Lee AR, Bieback K, Vo BN, Sampson DD, Kennedy BF, Spatz JP, Engler AJ, Choi YS. Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels. Proc Natl Acad Sci U S A 114: 5647–5652, 2017. [PubMed: 28507138]

152. Han YL, Ronceray P, Xu G, Malandrino A, Kamm RD, Lenz M, Broedersz CP, Guo M. Cell contraction induces long-ranged stress stiffening in the extracellular matrix. Proc Natl Acad Sci U S A 115: 4075–4080, 2018. [PubMed: 29618614]

153. Hansen ML, Rasmussen LM. Associations between plasma fibulin-1, pulse wave velocity and diabetes in patients with coronary heart disease. J Diabetes Complications 29: 362–366, 2015. [PubMed: 25633574]

154. Harada T, Swift J, Irianto J, Shin JW, Spinler KR, Athirasala A, Diegmiller R, Dingal PC, Ivanovska IL, Discher DE. Nuclear lamin stiffness is a barrier to 3D migration, but softness can limit survival. J Cell Biol 204: 669–682, 2014. [PubMed: 24567359]

155. Hayashi S, Dong B. Shape and geometry control of the *Drosophila tracheal* tubule. Dev Growth Differ 59: 4–11, 2017. [PubMed: 28093725]

156. Hecker L, Logsdon NJ, Kurundkar D, Kurundkar A, Bernard K, Hock T, Meldrum E, Sanders YY, Thannickal VJ. Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. Sci Transl Med 6: 231ra247, 2014.

157. Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH, Pellicoro A, Raschperger E, Betsholtz C, Ruminski PG, Griggs DW, Prinsen MJ, Maher JJ, Iredale JP, Lacy-Hulbert A, Adams RH, Sheppard D. Targeting of αv integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med 19: 1617–1624, 2013. [PubMed: 24216753]

158. Herrera J, Henke CA, Bitterman PB. Extracellular matrix as a driver of progressive fibrosis. J Clin Invest 128: 45–53, 2018. [PubMed: 29293088]

159. Hickey JW, Dong Y, Chung JW, Salathe SF, Pruitt HC, Li X, Chang C, Fraser AK, Bessell CA, Ewald AJ, Gerecht S, Mao HQ, Schneck JP. Engineering an artificial T-cell stimulating matrix for immunotherapy. Adv Mater 31: e1807359, 2019. [PubMed: 30968468]

160. Higuchi S, Watanabe TM, Kawauchi K, Ichimura T, Fujita H. Culturing of mouse and human cells on soft substrates promote the expression of stem cell markers. J Biosci Bioeng 117: 749–755, 2014. [PubMed: 24360205]

161. Hind LE, Dembo M, Hammer DA. Macrophage motility is driven by frontal-towing with a force magnitude dependent on substrate stiffness. Integr Biol (Camb) 7: 447–453, 2015. [PubMed: 25768202]

162. Hinz B, Gabbiani G, Chaponnier C. The NH2-terminal peptide of alpha-smooth muscle actin inhibits force generation by the myofibroblast in vitro and in vivo. J Cell Biol 157: 657–663, 2002. [PubMed: 11994316]

163. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J, De Wever O, Mareel M, Gabbiani G. Recent developments in myofibroblast biology: Paradigms for connective tissue remodeling. Am J Pathol, 2012.

164. Hobbs BD, de Jong K, Lamontagne M, Bossé Y, Shrine N, Artigas MS, Wain LV, Hall IP, Jackson VE, Wyss AB, London SJ, North KE, Franceschini N, Strachan DP, Beaty TH, Hokanson JE, Crapo JD, Castaldi PJ, Chase RP, Bartz TM, Heckbert SR, Psaty BM, Gharib SA, Zanen P, Lammers JW, Oudkerk M, Groen HJ, Locantore N, Tal-Singer R, Rennard SI, Vestbo J, Timens W, Paré PD, Latourelle JC, Dupuis J, O'Connor GT, Wilk JB, Kim WJ, Lee MK, Oh YM, Vonk JM, de Koning HJ, Leng S, Belinsky SA, Tesfaigzi Y, Manichaikul A, Wang XQ, Rich SS, Barr RG, Sparrow D, Litonjua AA, Bakke P, Gulsvik A, Lahousse L, Brusselle GG, Stricker BH, Uitterlinden AG, Ampleford EJ, Bleecker ER, Woodruff PG, Meyers DA, Qiao D, Lomas DA, Yim JJ, Kim DK, Hawrylkiewicz I, Sliwinski P, Hardin M, Fingerlin TE, Schwartz DA, Postma DS, MacNee W, Tobin MD, Silverman EK, Boezen HM, Cho MH, Investigators C, Investigators E, Investigators L, Group SR, Investigators ICGN, Investigators UB, and Consortium ICG. Genetic loci associated with chronic obstructive pulmonary disease overlap with loci for lung function and pulmonary fibrosis. Nat Genet 49: 426–432, 2017. [PubMed: 28166215]

165. Hoffman BD, Grashoff C, Schwartz MA. Dynamic molecular processes mediate cellular mechanotransduction. Nature 475: 316–323, 2011. [PubMed: 21776077]

166. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol 4: 435–459, 2009. [PubMed: 18954287]

167. Horowitz JC, Tschumperlin DJ, Kim KK, Osterholzer JJ, Subbotina N, Ajayi IO, Teitz-Tennenbaum S, Virk A, Dotson M, Liu F, Sicard D, Jia S, Sisson TH. Urokinase plasminogen activator overexpression reverses established lung fibrosis. Thromb Haemost 119: 1968–1980, 2019. [PubMed: 31705517]

168. Hsu SJ, Bouchard RR, Dumont DM, Wolf PD, Trahey GE. In vivo assessment of myocardial stiffness with acoustic radiation force impulse imaging. Ultrasound Med Biol 33: 1706–1719, 2007. [PubMed: 17698282]

169. Huang K, Mitzner W, Rabold R, Schofield B, Lee H, Biswal S, Tankersley CG. Variation in senescent-dependent lung changes in inbred mouse strains. J Appl Physiol 102 (1632-1639): 2007, 1985.

170. Huang X, Yang N, Fiore VF, Barker TH, Sun Y, Morris SW, Ding Q, Thannickal VJ, Zhou Y. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. Am J Respir Cell Mol Biol 47: 340–348, 2012. [PubMed: 22461426]

171. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater 9: 518–526, 2010. [PubMed: 20418863]

172. Hunter KS, Lammers SR, Shandas R. Pulmonary vascular stiffness: Measurement, modeling, and implications in normal and hypertensive pulmonary circulations. Compr Physiol 1: 1413–1435, 2011. [PubMed: 23733649]

173. Huse M. Mechanical forces in the immune system. Nat Rev Immunol 17: 679–690, 2017. [PubMed: 28757604]

174. Hynes RO. Stretching the boundaries of extracellular matrix research. Nat Rev Mol Cell Biol 15: 761–763, 2014. [PubMed: 25574535]

175. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 104: 2752–2760, 2004. [PubMed: 15226175]

176. Isenberg BC, Dimilla PA, Walker M, Kim S, Wong JY. Vascular smooth muscle cell durotaxis depends on substrate stiffness gradient strength. Biophys J 97: 1313–1322, 2009. [PubMed: 19720019]

177. Ishihara S, Yasuda M, Harada I, Mizutani T, Kawabata K, Haga H. Substrate stiffness regulates temporary NF-κB activation via actomyosin contractions. Exp Cell Res 319: 2916–2927, 2013. [PubMed: 24113574]

178. Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, Sands E, Suliman I, Trim N, Knorr A, Arthur MJ, Benyon RC, Iredale JP. Spontaneous recovery from micronodular cirrhosis: Evidence for incomplete resolution associated with matrix cross-linking. Gastroenterology 126: 1795–1808, 2004. [PubMed: 15188175]

179. Ito S, Ingenito EP, Brewer KK, Black LD, Parameswaran H, Lutchen KR, Suki B. Mechanics, nonlinearity, and failure strength of lung tissue in a mouse model of emphysema: Possible role of collagen remodeling. J Appl Physiol 98 (503-511): 2005, 1985.

180. Iwasaki A, Sakai K, Moriya K, Sasaki T, Keene DR, Akhtar R, Miya-zono T, Yasumura S, Watanabe M, Morishita S, Sakai T. Molecular mechanism responsible for fibronectin-controlled alterations in matrix stiffness in advanced chronic liver fibrogenesis. J Biol Chem 291: 72–88, 2016. [PubMed: 26553870]

181. Jacks T, Weinberg RA. Taking the study of cancer cell survival to a new dimension. Cell 111: 923–925, 2002. [PubMed: 12507419]

182. Jamieson RR, Stasiak SE, Polio SR, Augspurg RD, McCormick CA, Ruberti JW, Parameswaran H. Stiffening of the extracellular matrix is a sufficient condition for airway hyperreactivity. J Appl Physiol 130 (1635-1645): 2021, 1985.

183. Jang J, Kim TG, Kim BS, Kim SW, Kwon SM, Cho DW. Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Acta Biomater 33: 88–95, 2016. [PubMed: 26774760]

184. Jaspers M, Dennison M, Mabesoone MF, MacKintosh FC, Rowan AE, Kouwer PH. Ultra-responsive soft matter from strain-stiffening hydrogels. Nat Commun 5: 5808, 2014. [PubMed: 25510333]

185. Jaspers M, Pape AC, Voets IK, Rowan AE, Portale G, Kouwer PH. Bundle formation in biomimetic hydrogels. Biomacromolecules 17: 2642–2649, 2016. [PubMed: 27409975]

186. Jaspers M, Vaessen SL, van Schayik P, Voerman D, Rowan AE, Kouwer PHJ. Nonlinear mechanics of hybrid polymer networks that mimic the complex mechanical environment of cells. Nat Commun 8: 15478, 2017. [PubMed: 28541273]

187. Jeong J, Keum S, Kim D, You E, Ko P, Lee J, Kim J, Kim J-W, Rhee S. Spindle pole body component 25 homolog expressed by ECM stiffening is required for lung cancer cell proliferation. Biochem Biophys Res Commun 500: 937–943, 2018. [PubMed: 29709477]

188. Jiang S, Lyu C, Zhao P, Li W, Kong W, Huang C, Genin GM, Du Y. Cryoprotectant enables structural control of porous scaffolds for exploration of cellular mechano-responsiveness in 3D. Nat Commun 10: 3491, 2019. [PubMed: 31375674]

189. Jones DL, Meridew JA, Link PA, Ducharme MT, Lydon KL, Choi KM, Caporarello N, Tan Q, Diaz Espinosa AM, Xiong Y, Lee JH, Ye Z, Yan H, Ordog T, Ligresti G, Varelas X, Tschumperlin DJ. ZNF416 is a pivotal transcriptional regulator of fibroblast mechanoactivation. J Cell Biol 220, 2021.

190. Jones MG, Andriotis OG, Roberts JJ, Lunn K, Tear VJ, Cao L, Ask K, Smart DE, Bonfanti A, Johnson P, Alzetani A, Conforti F, Doherty R, Lai CY, Johnson B, Bourdakos KN, Fletcher SV, Marshall BG, Jogai S, Brereton CJ, Chee SJ, Ottensmeier CH, Sime P, Gauldie J, Kolb M, Mahajan S, Fabre A, Bhaskar A, Jarolimek W, Richeldi L, O'Reilly KM, Monk PD, Thurner PJ, Davies DE. Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. Elife 7, 2018.

191. Jorba I, Uriarte JJ, Campillo N, Farré R, Navajas D. Probing micromechanical properties of the extracellular matrix of soft tissues by atomic force microscopy. J Cell Physiol 232: 19–26, 2017. [PubMed: 27163411]

192. Judokusumo E, Tabdanov E, Kumari S, Dustin ML, Kam LC. Mechanosensing in T lymphocyte activation. Biophys J 102: L5–L7, 2012. [PubMed: 22339876]

193. Kadler KE, Baldock C, Bella J, Boot-Handford RP. Collagens at a glance. J Cell Sci 120: 1955–1958, 2007. [PubMed: 17550969]

194. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Investigat 119: 1420–1428, 2009.

195. Kandow CE, Georges PC, Janmey PA, Beningo KA. Polyacrylamide hydrogels for cell mechanics: Steps toward optimization and alternative uses. Methods Cell Biol 83: 29–46, 2007. [PubMed: 17613303]

196. Kapetanaki MG, Mora AL, Rojas M. Influence of age on wound healing and fibrosis. J Pathol 229: 310–322, 2013. [PubMed: 23124998]

197. Karki P, Birukova AA. Substrate stiffness-dependent exacerbation of endothelial permeability and inflammation: Mechanisms and potential implications in ALI and PH (2017 Grover Conference Series). Pulm Circ 8: 2045894018773044, 2018. [PubMed: 29714090]

198. Keating M, Kurup A, Alvarez-Elizondo M, Levine AJ, Botvinick E. Spatial distributions of pericellular stiffness in natural extracellular matrices are dependent on cell-mediated proteolysis and contractility. Acta Biomater 57: 304–312, 2017. [PubMed: 28483696]

199. Khatiwala CB, Peyton SR, Putnam AJ. Intrinsic mechanical properties of the extracellular matrix affect the behavior of pre-osteoblastic MC3T3-E1 cells. Am J Physiol Cell Physiol 290: C1640–C1650, 2006. [PubMed: 16407416]

200. Khunmanee S, Jeong Y, Park H. Crosslinking method of hyaluronic-based hydrogel for biomedical applications. J Tissue Eng 8: 2041731417726464, 2017. [PubMed: 28912946]

201. Kim D, You E, Jeong J, Ko P, Kim JW, Rhee S. DDR2 controls the epithelial-mesenchymal-transition-related gene expression via c-Myb acetylation upon matrix stiffening. Sci Rep 7: 6847, 2017. [PubMed: 28754957]

202. Kim YJ, Park JW, Kyung SY, Lee SP, Chung MP, Kim YH, Lee JH, Kim YC, Ryu JS, Lee HL, Park CS, Uh ST, Lee YC, Kim KH, Chun YJ, Park YB, Kim DS, Jegal Y, Park MS, Jeong SH. Clinical characteristics of idiopathic pulmonary fibrosis patients with diabetes mellitus: The national survey in Korea from 2003 to 2007. J Korean Med Sci 27: 756–760, 2012. [PubMed: 22787370]

203. Klingberg F, Chow ML, Koehler A, Boo S, Buscemi L, Quinn TM, Costell M, Alman BA, Genot E, Hinz B. Prestress in the extracellular matrix sensitizes latent TGF-β1 for activation. J Cell Biol 207: 283–297, 2014. [PubMed: 25332161]

204. Kloxin AM, Kasko AM, Salinas CN, Anseth KS. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science 324: 59–63, 2009. [PubMed: 19342581]

205. Kocgozlu L, Lavalle P, Koenig G, Senger B, Haikel Y, Schaaf P, Voegel JC, Tenenbaum H, Vautier D. Selective and uncoupled role of substrate elasticity in the regulation of replication and transcription in epithelial cells. J Cell Sci 123: 29–39, 2010. [PubMed: 20016064]

206. Kouwer PH, Koepf M, Le Sage VA, Jaspers M, van Buul AM, Eksteen-Akeroyd ZH, Woltinge T, Schwartz E, Kitto HJ, Hoogenboom R, Picken SJ, Nolte RJ, Mendes E, Rowan AE. Responsive biomimetic networks from polyisocyanopeptide hydrogels. Nature 493: 651–655, 2013. [PubMed: 23354048]

207. Kumar A, Placone JK, Engler AJ. Understanding the extracellular forces that determine cell fate and maintenance. Development 144: 4261–4270, 2017. [PubMed: 29183939]

208. Kyung SY, Byun KH, Yoon JY, Kim YJ, Lee SP, Park JW, Lee BH, Park JS, Jang AS, Park CS, Jeong SH. Advanced glycation endproducts and receptor for advanced glycation end-products expression in patients with idiopathic pulmonary fibrosis and NSIP. Int J Clin Exp Pathol 7: 221–228, 2014. [PubMed: 24427342]

209. Lammers SR, Kao PH, Qi HJ, Hunter K, Lanning C, Albietz J, Hofmeister S, Mecham R, Stenmark KR, Shandas R. Changes in the structure-function relationship of elastin and its impact on the proximal pulmonary arterial mechanics of hypertensive calves. Am J Physiol Heart Circ Physiol 295: H1451–H1459, 2008. [PubMed: 18660454]

210. Lang MR, Fiaux GW, Gillooly M, Stewart JA, Hulmes DJ, Lamb D. Collagen content of alveolar wall tissue in emphysematous and non-emphysematous lungs. Thorax 49: 319–326, 1994. [PubMed: 8202900]

211. Lee D, Rahman MM, Zhou Y, Ryu S. Three-dimensional confocal microscopy indentation method for hydrogel elasticity measurement. Langmuir 31: 9684–9693, 2015. [PubMed: 26270154]

212. Lee JY, Oh JG, Kim JS, Lee KW. Effects of chebulic acid on advanced glycation endproducts-induced collagen cross-links. Biol Pharm Bull 37: 1162–1167, 2014. [PubMed: 24759763]

213. Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. Prog Polym Sci 37: 106–126, 2012. [PubMed: 22125349]

214. Leiphart RJ, Chen D, Peredo AP, Loneker AE, Janmey PA. Mechanosensing at cellular interfaces. Langmuir 35: 7509–7519, 2019. [PubMed: 30346180]

215. Lepetit H, Eddahibi S, Fadel E, Frisdal E, Munaut C, Noel A, Humbert M, Adnot S, D'Ortho MP, Lafuma C. Smooth muscle cell matrix metalloproteinases in idiopathic pulmonary arterial hypertension. Eur Respir J 25: 834–842, 2005. [PubMed: 15863640]

216. Li L, Sharma N, Chippada U, Jiang X, Schloss R, Yarmush ML, Langrana NA. Functional modulation of ES-derived hepatocyte lineage cells via substrate compliance alteration. Ann Biomed Eng 36: 865–876, 2008. [PubMed: 18266108]

217. Liang Y, Walczak P, Bulte JW. The survival of engrafted neural stem cells within hyaluronic acid hydrogels. Biomaterials 34: 5521–5529, 2013. [PubMed: 23623429]

218. Ligresti G, Caporarello N, Meridew JA, Jones DL, Tan Q, Choi KM, Haak AJ, Aravamudhan A, Roden AC, Prakash YS, Lomberk G, Urrutia RA, Tschumperlin DJ. CBX5/G9a/H3K9me-mediated gene repression is essential to fibroblast activation during lung fibrosis. JCI Insight 5: e127111, 2019. [PubMed: 31095524]

219. Lima MJ, Correlo VM, Reis RL. Micro/nano replication and 3D assembling techniques for scaffold fabrication. Mater Sci Eng C Mater Biol Appl 42: 615–621, 2014. [PubMed: 25063161]

220. Lin DC, Yurke B, Langrana NA. Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel. J Biomech Eng 126: 104–110, 2004. [PubMed: 15171136]

221. Lin YC, Sung YK, Jiang X, Peters-Golden M, Nicolls MR. Simultaneously targeting myofibroblast contractility and extracellular matrix cross-linking as a therapeutic concept in airway fibrosis. Am J Transplant 17: 1229–1241, 2017. [PubMed: 27804215]

222. Link PA, Pouliot RA, Mikhaiel NS, Young BM, Heise RL. Tunable hydrogels from pulmonary extracellular matrix for 3D cell culture. J Vis Exp, 2017.

223. Liu CY, Chan SW, Guo F, Toloczko A, Cui L, Hong W. MRTF/SRF dependent transcriptional regulation of TAZ in breast cancer cells. Oncotarget 7: 13706–13716, 2016. [PubMed: 26885614]

224. Liu F, Haeger CM, Dieffenbach PB, Sicard D, Chrobak I, Coronata AM, Suárez Velandia MM, Vitali S, Colas RA, Norris PC, Marinković A, Liu X, Ma J, Rose CD, Lee SJ, Comhair SA, Erzurum SC, JD MD, Serhan CN, Walsh SR, Tschumperlin DJ, Fredenburgh LE. Distal vessel stiffening is an early and pivotal mechanobiological regulator of vascular remodeling and pulmonary hypertension. JCI Insight 1: e86987, 2016. [PubMed: 27347562]

225. Liu F, Lagares D, Choi KM, Stopfer L, Marinković A, Vrbanac V, Probst CK, Hiemer SE, Sisson TH, Horowitz JC, Rosas IO, Fredenburgh LE, Feghali-Bostwick C, Varelas X, Tager AM, Tschumperlin DJ. Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. Am J Physiol Lung Cell Mol Physiol 308: L344–L357, 2015. [PubMed: 25502501]

226. Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. J Cell Biol 190: 693–706, 2010. [PubMed: 20733059]

227. Liu F, Tschumperlin DJ. Micro-mechanical characterization of lung tissue using atomic force microscopy. J Vis Exp, 2011.

228. Liu HY, Greene T, Lin TY, Dawes CS, Korc M, Lin CC. Enzyme-mediated stiffening hydrogels for probing activation of pancreatic stellate cells. Acta Biomater 48: 258–269, 2017. [PubMed: 27769941]

229. Liu HY, Korc M, Lin CC. Biomimetic and enzyme-responsive dynamic hydrogels for studying cell-matrix interactions in pancreatic ductal adenocarcinoma. Biomaterials 160: 24–36, 2018. [PubMed: 29353105]

230. Liu L, Zhang SX, Liao W, Farhoodi HP, Wong CW, Chen CC, Ségaliny AI, Chacko JV, Nguyen LP, Lu M, Polovin G, Pone EJ, Downing TL, Lawson DA, Digman MA, Zhao W. Mechanoresponsive stem cells to target cancer metastases through biophysical cues. Sci Transl Med 9: eaan2966, 2017. [PubMed: 28747514]

231. Liu S, Young SM, Varisco BM. Dynamic expression of chymotrypsin-like elastase 1 over the course of murine lung development. Am J Physiol Lung Cell Mol Physiol 306: L1104–L1116, 2014. [PubMed: 24793170]

232. Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, Liu JO, Pan D. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev 26: 1300–1305, 2012. [PubMed: 22677547]

233. Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophys J 79: 144–152, 2000. [PubMed: 10866943]

234. Lockhart-Cairns MP, Newandee H, Thomson J, Weiss AS, Baldock C, Tarakanova A. Transglutaminase-mediated cross-linking of tropoelastin to fibrillin stabilises the elastin precursor prior to elastic fibre assembly. J Mol Biol 432: 5736–5751, 2020. [PubMed: 32898582]

235. Long R, Hall MS, Wu M, Hui CY. Effects of gel thickness on microscopic indentation measurements of gel modulus. Biophys J 101: 643–650, 2011. [PubMed: 21806932]

236. Lubinski MA, Emelianov SY, O'Donnell M. Speckle tracking methods for ultrasonic elasticity imaging using short-time correlation. IEEE Trans Ultrason Ferroelectr Freq Control 46: 82–96, 1999. [PubMed: 18238401]

237. Luft FC. Molecular mechanisms of arterial stiffness: New insights. J Am Soc Hypertens 6: 436–438, 2012. [PubMed: 23199674]

238. Luque T, Melo E, Garreta E, Cortiella J, Nichols J, Farré R, Navajas D, Local micromechanical properties of decellularized lung scaffolds measured with atomic force microscopy. Acta Biomater 9: 6852–6859, 2013. [PubMed: 23470549]

239. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. Proc Natl Acad Sci U S A 100: 5413–5418, 2003. [PubMed: 12686696]

240. Lyck Hansen M, Beck HC, Irmukhamedov A, Jensen PS, Olsen MH, Rasmussen LM. Proteome analysis of human arterial tissue discloses associations between the vascular content of small leucine-rich repeat proteoglycans and pulse wave velocity. Arterioscler Thromb Vasc Biol 35: 1896–1903, 2015. [PubMed: 26069235]

241. Macaya D, Spector M. Injectable hydrogel materials for spinal cord regeneration: A review. Biomed Mater 7: 012001, 2012. [PubMed: 22241481]

242. Machahua C, Montes-Worboys A, Llatjos R, Escobar I, Dorca J, Molina-Molina M, Vicens-Zygmunt V. Increased AGE-RAGE ratio in idiopathic pulmonary fibrosis. Respir Res 17: 144, 2016. [PubMed: 27816054]

243. Mahapatra S, Nishimura RA, Sorajja P, Cha S, McGoon MD. Relationship of pulmonary arterial capacitance and mortality in idiopathic pulmonary arterial hypertension. J Am Coll Cardiol 47: 799–803, 2006. [PubMed: 16487848]

244. Majedi FS, Hasani-Sadrabadi MM, Thauland TJ, Li S, Bouchard LS, Butte MJ. T-cell activation is modulated by the 3D mechanical microenvironment. Biomaterials 252: 120058, 2020. [PubMed: 32413594]

245. Mammoto A, Mammoto T, Kanapathipillai M, Wing Yung C, Jiang E, Jiang A, Lofgren K, Gee EP, Ingber DE. Control of lung vascular permeability and endotoxin-induced pulmonary oedema by changes in extracellular matrix mechanics. Nat Commun 4: 1759, 2013. [PubMed: 23612300]

246. Mammoto T, Jiang E, Jiang A, Mammoto A. Extracellular matrix structure and tissue stiffness control postnatal lung development through the lipoprotein receptor-related protein 5/Tie2 signaling system. Am J Respir Cell Mol Biol 49: 1009–1018, 2013. [PubMed: 23841513]

247. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: Synthetic ECM analogs for tissue engineering. Biomaterials 22: 3045–3051, 2001. [PubMed: 11575479]

248. Marangon I, Silva AA, Guilbert T, Kolosnjaj-Tabi J, Marchiol C, Natkhunarajah S, Chamming's F, Ménard-Moyon C, Bianco A, Gennisson JL, Renault G, Gazeau F. Tumor stiffening, a key determinant of tumor progression, is reversed by nanomaterial-induced photothermal therapy. Theranostics 7: 329–343, 2017. [PubMed: 28042338]

249. Mariappan YK, Glaser KJ, Ehman RL. Magnetic resonance elastography: A review. Clin Anat 23: 497–511, 2010. [PubMed: 20544947]

250. Marinković A, Liu F, Tschumperlin DJ. Matrices of physiologic stiffness potently inactivate idiopathic pulmonary fibrosis fibroblasts. Am J Respir Cell Mol Biol 48: 422–430, 2013. [PubMed: 23258227]

251. Marinković A, Mih JD, Park JA, Liu F, Tschumperlin DJ. Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-β responsiveness. Am J Physiol Lung Cell Mol Physiol 303: L169–L180, 2012. [PubMed: 22659883]
252. Mason BN, Starchenko A, Williams RM, Bonassar LJ, Reinhart-King CA. Tuning three-dimensional collagen matrix stiffness independently of collagen concentration modulates endothelial cell behavior. Acta Biomater 9: 4635–4644, 2013. [PubMed: 22902816]
253. Matalon O, Ben-Shmuel A, Kivelevitz J, Sabag B, Fried S, Joseph N, Noy E, Biber G, Barda-Saad M. Actin retrograde flow controls natural killer cell response by regulating the conformation state of SHP-1. EMBO J 37, 2018.
254. Mathai SK, Pedersen BS, Smith K, Russell P, Schwarz MI, Brown KK, Steele MP, Loyd JE, Crapo JD, Silverman EK, Nickerson D, Fingerlin TE, Yang IV, Schwartz DA. Desmoplakin variants are associated with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 193: 1151–1160, 2016. [PubMed: 26669357]
255. Meli VS, Atcha H, Veerasubramanian PK, Nagalla RR, Luu TU, Chen EY, Guerrero-Juarez CF, Yamaga K, Pandori W, Hsieh JY, Downing TL, Fruman DA, Lodoen MB, Plikus MV, Wang W, Liu WF. YAP-mediated mechanotransduction tunes the macrophage inflammatory response. Sci Adv 6, 6 (49): eabb8471, 2020.
256. Melo E, Cárdenes N, Garreta E, Luque T, Rojas M, Navajas D, Farré R. Inhomogeneity of local stiffness in the extracellular matrix scaffold of fibrotic mouse lungs. J Mech Behav Biomed Mater 37: 186–195, 2014. [PubMed: 24946269]
257. Melo E, Garreta E, Luque T, Cortiella J, Nichols J, Navajas D, Farré R. Effects of the decellularization method on the local stiffness of acellular lungs. Tissue Eng Part C Methods 20: 412–422, 2014. [PubMed: 24083889]
258. Meng KP, Majedi FS, Thauland TJ, Butte MJ. Mechanosensing through YAP controls T cell activation and metabolism. J Exp Med 217, 2020.
259. Meng Z, Qiu Y, Lin KC, Kumar A, Placone JK, Fang C, Wang KC, Lu S, Pan M, Hong AW, Moroishi T, Luo M, Plouffe SW, Diao Y, Ye Z, Park HW, Wang X, Yu FX, Chien S, Wang CY, Ren B, Engler AJ, Guan KL. RAP2 mediates mechanoresponses of the Hippo pathway. Nature 560: 655–660, 2018. [PubMed: 30135582]
260. Mennens SFB, Bolomini-Vittori M, Weiden J, Joosten B, Cambi A, van den Dries K. Substrate stiffness influences phenotype and function of human antigen-presenting dendritic cells. Sci Rep 7: 17511, 2017. [PubMed: 29235514]
261. Mierke CT, Sauer F, Grosser S, Puder S, Fischer T, Käs JA. The two faces of enhanced stroma: Stroma acts as a tumor promoter and a steric obstacle. NMR Biomed 31: e3831, 2018. [PubMed: 29215759]
262. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell 113: 329–342, 2003. [PubMed: 12732141]
263. Miron-Mendoza M, Seemann J, Grinnell F. The differential regulation of cell motile activity through matrix stiffness and porosity in three dimensional collagen matrices. Biomaterials 31: 6425–6435, 2010. [PubMed: 20537378]
264. Miyazawa A, Ito S, Asano S, Tanaka I, Sato M, Kondo M, Hasegawa Y. Regulation of PD-L1 expression by matrix stiffness in lung cancer cells. Biochem Biophys Res Commun 495: 2344–2349, 2018. [PubMed: 29274784]
265. Mižíková I, Pfeffer T, Nardiello C, Surate Solaligue DE, Steenbock H, Tatsukawa H, Silva DM, Vadász I, Herold S, Pease RJ, Iismaa SE, Hitomi K, Seeger W, Brinckmann J, Morty RE. Targeting transglutaminase 2 partially restores extracellular matrix structure but not alveolar architecture in experimental bronchopulmonary dysplasia. FEBS J 285: 3056–3076, 2018. [PubMed: 29935061]
266. Moon JJ, Saik JE, Poché RA, Leslie-Barbick JE, Lee SH, Smith AA, Dickinson ME, West JL. Biomimetic hydrogels with pro-angiogenic properties. Biomaterials 31: 3840–3847, 2010. [PubMed: 20185173]

267. Mordechay L, Le Saux G, Edri A, Hadad U, Porgador A, Schvartzman M. Mechanical Regulation of the Cytotoxic Activity of Natural Killer Cells. ACS Biomater Sci Eng 7: 122–132, 2021. [PubMed: 33455204]

268. Muthupillai R, Lomas DJ, Rossman PJ, Greenleaf JF, Manduca A, Ehman RL. Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. Science 269: 1854–1857, 1995. [PubMed: 7569924]

269. Nagy N, de la Zerda A, Kaber G, Johnson PY, Hu KH, Kratochvil MJ, Yadava K, Zhao W, Cui Y, Navarro G, Annes JP, Wight TN, Heilshorn SC, Bollyky PL, Butte MJ. Hyaluronan content governs tissue stiffness in pancreatic islet inflammation. J Biol Chem 293: 567–578, 2018. [PubMed: 29183997]

270. Navab R, Strumpf D, To C, Pasko E, Kim KS, Park CJ, Hai J, Liu J, Jonkman J, Barczyk M, Bandarchi B, Wang YH, Venkat K, Ibrahimov E, Pham NA, Ng C, Radulovich N, Zhu CQ, Pintilie M, Wang D, Lu A, Jurisica I, Walker GC, Gullberg D, Tsao MS. Integrin α11β1 regulates cancer stromal stiffness and promotes tumorigenicity and metastasis in non-small cell lung cancer. Oncogene 35: 1899–1908, 2016. [PubMed: 26148229]

271. Nave AH, Mižíková I, Niess G, Steenbock H, Reichenberger F, Talav-era ML, Veit F, Herold S, Mayer K, Vadász I, Weissmann N, Seeger W, Brinckmann J, Morty RE. Lysyl oxidases play a causal role in vascular remodeling in clinical and experimental pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 34: 1446–1458, 2014. [PubMed: 24833797]

272. Nerem RM, Seliktar D. Vascular tissue engineering. Annu Rev Biomed Eng 3: 225–243, 2001. [PubMed: 11447063]

273. Nichols JE, Niles J, Riddle M, Vargas G, Schilagard T, Ma L, Edward K, La Francesca S, Sakamoto J, Vega S, Ogadegbe M, Mlcak R, Deyo D, Woodson L, McQuitty C, Lick S, Beckles D, Melo E, Cortiella J. Production and assessment of decellularized pig and human lung scaffolds. Tissue Eng Part A 19: 2045–2062, 2013. [PubMed: 23638920]

274. Nightingale K, Soo MS, Nightingale R, Trahey G. Acoustic radiation force impulse imaging: In vivo demonstration of clinical feasibility. Ultrasound Med Biol 28: 227–235, 2002. [PubMed: 11937286]

275. Nonaka PN, Uriarte JJ, Campillo N, Melo E, Navajas D, Farré R, Oliveira LV. Mechanical properties of mouse lungs along organ decellularization by sodium dodecyl sulfate. Respir Physiol Neurobiol 200: 1–5, 2014. [PubMed: 24837837]

276. Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ. Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. Nature 417: 424–428, 2002. [PubMed: 12024209]

277. Oakes PW, Patel DC, Morin NA, Zitterbart DP, Fabry B, Reichner JS, Tang JX. Neutrophil morphology and migration are affected by substrate elasticity. Blood 114: 1387–1395, 2009. [PubMed: 19491394]

278. O'Brien XM, Loosley AJ, Oakley KE, Tang JX, Reichner JS. Technical advance: Introducing a novel metric, directionality time, to quantify human neutrophil chemotaxis as a function of matrix composition and stiffness. J Leukoc Biol 95: 993–1004, 2014. [PubMed: 24511103]

279. O'Connor RS, Hao X, Shen K, Bashour K, Akimova T, Hancock WW, Kam LC, Milone MC. Substrate rigidity regulates human T cell activation and proliferation. J Immunol 189: 1330–1339, 2012. [PubMed: 22732590]

280. Oh K, Park HB, Byoun OJ, Shin DM, Jeong EM, Kim YW, Kim YS, Melino G, Kim IG, Lee DS. Epithelial transglutaminase 2 is needed for T cell interleukin-17 production and subsequent pulmonary inflammation and fibrosis in bleomycin-treated mice. J Exp Med 208: 1707–1719, 2011. [PubMed: 21746810]

281. Oh RS, Haak AJ, Smith KMJ, Ligresti G, Choi KM, Xie T, Wang S, Walters PR, Thompson MA, Freeman MR, Manlove LJ, Chu VM, Feghali-Bostwick C, Roden AC, Schymeinsky J, Pabelick CM, Prakash YS, Vassallo R, Tschumperlin DJ. RNAi screening identifies a mechanosensitive ROCK-JAK2-STAT3 network central to myofibroblast activation. J Cell Sci 131, 2018.

282. Oh SH, An DB, Kim TH, Lee JH. Wide-range stiffness gradient PVA/HA hydrogel to investigate stem cell differentiation behavior. Acta Biomater 35: 23–31, 2016. [PubMed: 26883774]

283. Ohashi K, Fujiwara S, Mizuno K. Roles of the cytoskeleton, cell adhesion and rho signalling in mechanosensing and mechanotransduction. J Biochem 161: 245–254, 2017. [PubMed: 28082721]

284. Ohlmeier S, Nieminen P, Gao J, Kanerva T, Rönty M, Toljamo T, Bergmann U, Mazur W, Pulkkinen V. Lung tissue proteomics identifies elevated transglutaminase 2 levels in stable chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 310: L1155–L1165, 2016. [PubMed: 27084846]

285. Okamoto T, Takagi Y, Kawamoto E, Park EJ, Usuda H, Wada K, Shi-maoka M. Reduced substrate stiffness promotes M2-like macrophage activation and enhances peroxisome proliferator-activated receptor gamma expression. Exp Cell Res 367: 264–273, 2018. [PubMed: 29627321]

286. O'Leary LE, Fallas JA, Bakota EL, Kang MK, Hartgerink JD. Multihierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel. Nat Chem 3: 821–828, 2011. [PubMed: 21941256]

287. Olsen KC, Sapinoro RE, Kottmann RM, Kulkarni AA, Iismaa SE, Johnson GV, Thatcher TH, Phipps RP, Sime PJ. Transglutaminase 2 and its role in pulmonary fibrosis. Am J Respir Crit Care Med 184: 699–707, 2011. [PubMed: 21700912]

288. Onochie OE, Zollinger A, Rich CB, Smith M, Trinkaus-Randall V. Epithelial cells exert differential traction stress in response to substrate stiffness. Exp Eye Res 181: 25–37, 2019. [PubMed: 30653966]

289. Ophir J, Céspedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: A quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging 13: 111–134, 1991. [PubMed: 1858217]

290. Osman M, Cantor JO, Roffman S, Keller S, Turino GM, Mandl I. Cigarette smoke impairs elastin resynthesis in lungs of hamsters with elastase-induced emphysema. Am Rev Respir Dis 132: 640–643, 1985. [PubMed: 3929658]

291. Ott HC, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, Kotton D, Vacanti JP. Regeneration and orthotopic transplantation of a bioartificial lung. Nat Med 16: 927–933, 2010. [PubMed: 20628374]

292. Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8: 839–845, 2007. [PubMed: 17684528]

293. Pankova D, Jiang Y, Chatzifrangkeskou M, Vendrell I, Buzzelli J, Ryan A, Brown C, O'Neill E. RASSF1A controls tissue stiffness and cancer stem-like cells in lung adenocarcinoma. EMBO J 38: e100532, 2019. [PubMed: 31268606]

294. Pankova D, Jiang Y, Chatzifrangkeskou M, Vendrell I, Buzzelli J, Ryan A, Brown C, O'Neill E. RASSF 1A controls tissue stiffness and cancer stem-like cells in lung adenocarcinoma. The EMBO J 38: e100532, 2019. [PubMed: 31268606]

295. Parameswaran K, Willems-Widyastuti A, Alagappan VK, Radford K, Kranenburg AR, Sharma HS. Role of extracellular matrix and its regulators in human airway smooth muscle biology. Cell Biochem Biophys 44: 139–146, 2006. [PubMed: 16456242]

296. Park JS, Burckhardt CJ, Lazcano R, Solis LM, Isogai T, Li L, Chen CS, Gao B, Minna JD, Bachoo R, DeBerardinis RJ, Danuser G. Mechanical regulation of glycolysis via cytoskeleton architecture. Nature 578: 621–626, 2020. [PubMed: 32051585]

297. Park JS, Chu JS, Tsou AD, Diop R, Tang Z, Wang A, Li S. The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF-β. Biomaterials 32: 3921–3930, 2011. [PubMed: 21397942]

298. Patel NR, Bole M, Chen C, Hardin CC, Kho AT, Mih J, Deng L, Butler J, Tschumperlin D, Fredberg JJ, Krishnan R, Koziel H. Cell elasticity determines macrophage function. PLoS One 7: e41024, 2012. [PubMed: 23028423]

299. Pedersen JA, Swartz MA. Mechanobiology in the third dimension. Ann Biomed Eng 33: 1469–1490, 2005. [PubMed: 16341917]

300. Pelham RJ Jr, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A 94: 13661–13665, 1997. [PubMed: 9391082]

301. Pelham RJ, Wang YL. Cell locomotion and focal adhesions are regulated by the mechanical properties of the substrate. Biol Bull 194: 348–349; discussion 349-350, 1998. [PubMed: 11536880]
302. Pereira RF, Barrias CC, Bártolo PJ, Granja PL. Cell-instructive pectin hydrogels crosslinked via thiol-norbornene photo-click chemistry for skin tissue engineering. Acta Biomater 66: 282–293, 2018. [PubMed: 29128530]
303. Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E, Niklason LE. Tissue-engineered lungs for in vivo implantation. Science 329: 538–541, 2010. [PubMed: 20576850]
304. Peyton SR, Putnam AJ. Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. J Cell Physiol 204: 198–209, 2005. [PubMed: 15669099]
305. Phelps EA, Enemchukwu NO, Fiore VF, Sy JC, Murthy N, Sulchek TA, Barker TH, García AJ. Maleimide cross-linked bioactive PEG hydrogel exhibits improved reaction kinetics and cross-linking for cell encapsulation and in situ delivery. Adv Mater 24: 64–70, 62, 2012. [PubMed: 22174081]
306. Philp CJ, Siebeke I, Clements D, Miller S, Habgood A, John AE, Navaratnam V, Hubbard RB, Jenkins G, Johnson SR. Extracellular matrix cross-linking enhances fibroblast growth and protects against matrix proteolysis in lung fibrosis. Am J Respir Cell Mol Biol 58: 594–603, 2018. [PubMed: 29053339]
307. Pogoda K, Bucki R, Byfield FJ, Cruz K, Lee T, Marcinkiewicz C, Janmey PA. Soft substrates containing hyaluronan mimic the effects of increased stiffness on morphology, motility, and proliferation of glioma cells. Biomacromolecules 18: 3040–3051, 2017. [PubMed: 28858529]
308. Polio SR, Stasiak SE, Jamieson RR, Balestrini JL, Krishnan R, Parameswaran H. Extracellular matrix stiffness regulates human airway smooth muscle contraction by altering the cell-cell coupling. Sci Rep 9:9564, 2019. [PubMed: 31267003]
309. Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically based pharmacokinetic and pharmacodynamic analysis enabled by microfluidically linked organs-on-chips. Annu Rev Pharmacol Toxicol 58: 37–64, 2018. [PubMed: 29309256]
310. Price AP, England KA, Matson AM, Blazar BR, Panoskaltsis-Mortari A. Development of a decellularized lung bioreactor system for bioengineering the lung: The matrix reloaded. Tissue Eng Part A 16: 2581–2591, 2010. [PubMed: 20297903]
311. Puig M, Lugo R, Gabasa M, Giménez A, Velásquez A, Galgoczy R, Ramírez J, Gómez-Caro A, Busnadiego Ó, Rodríguez-Pascual F. Matrix stiffening and β1 integrin drive subtype-specific fibroblast accumulation in lung cancer. Molecul Cancer Res 13: 161–173, 2015.
312. Qu J, Yang SZ, Zhu Y, Guo T, Thannickal VJ, Zhou Y. Targeting mechanosensitive MDM4 promotes lung fibrosis resolution in aged mice. J Exp Med 218, 2021.
313. Qu J, Zhu L, Zhou Z, Chen P, Liu S, Locy ML, Thannickal VJ, Zhou Y. Reversing mechanoinductive DSP expression by CRISPR/dCas9-mediated epigenome editing. Am J Respir Crit Care Med 198: 599–609, 2018. [PubMed: 29924937]
314. Raghu G, Brown KK, Collard HR, Cottin V, Gibson KF, Kaner RJ, Lederer DJ, Martinez FJ, Noble PW, Song JW, Wells AU, Whelan TP, Wuyts W, Moreau E, Patterson SD, Smith V, Bayly S, Chien JW, Gong Q, Zhang JJ, O'Riordan TG. Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: A randomised, double-blind, controlled, phase 2 trial. Lancet Respir Med 5: 22–32, 2017. [PubMed: 27939076]
315. Rahaman SO, Grove LM, Paruchuri S, Southern BD, Abraham S, Niese KA, Scheraga RG, Ghosh S, Thodeti CK, Zhang DX, Moran MM, Schilling WP, Tschumperlin DJ, Olman MA. TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. J Clin Invest 124: 5225–5238, 2014. [PubMed: 25365224]
316. Raqeeb A, Jiao Y, Syyong HT, Paré PD, Seow CY. Regulatable stiffness in relaxed airway smooth muscle: A target for asthma treatment? J Appl Physiol 112 (337-346): 2012, 1985.
317. Reagan MR, Kaplan DL. Concise review: Mesenchymal stem cell tumor-homing: Detection methods in disease model systems. Stem Cells 29: 920–927, 2011. [PubMed: 21557390]

318. Rehfeldt F, Engler AJ, Eckhardt A, Ahmed F, Discher DE. Cell responses to the mechanochemical microenvironment–implications for regenerative medicine and drug delivery. Adv Drug Deliv Rev 59: 1329–1339, 2007. [PubMed: 17900747]
319. Riento K, Ridley AJ. Rocks: Multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol 4: 446–456, 2003. [PubMed: 12778124]
320. Robinson KA, Sun M, Barnum CE, Weiss SN, Huegel J, Shetye SS, Lin L, Saez D, Adams SM, Iozzo RV, Soslowsky LJ, Birk DE. Decorin and biglycan are necessary for maintaining collagen fibril structure, fiber realignment, and mechanical properties of mature tendons. Matrix Biol 64: 81–93, 2017. [PubMed: 28882761]
321. Roeder BA, Kokini K, Sturgis JE, Robinson JP, Voytik-Harbin SL. Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure. J Biomech Eng 124: 214–222, 2002. [PubMed: 12002131]
322. Rogers CI, Qaderi K, Woolley AT, Nordin GP. 3D printed microfluidic devices with integrated valves. Biomicrofluidics 9, 2015. DOI: 016501.
323. Rosales AM, Anseth KS. The design of reversible hydrogels to capture extracellular matrix dynamics. Nat Rev Mater 1, 2016. DOI: 15012.
324. Rosales AM, Vega SL, DelRio FW, Burdick JA, Anseth KS. Hydrogels with reversible mechanics to probe dynamic cell microenvironments. Angew Chem Int Ed Engl 56: 12132–12136, 2017. [PubMed: 28799225]
325. Rozario T, Dzamba B, Weber GF, Davidson LA, DeSimone DW. The physical state of fibronectin matrix differentially regulates morphogenetic movements in vivo. Dev Biol 327: 386–398, 2009. [PubMed: 19138684]
326. Ruffenach G, Chabot S, Tanguay VF, Courboulin A, Boucherat O, Potus F, Meloche J, Pflieger A, Breuils-Bonnet S, Nadeau V, Paradis R, Tremblay E, Girerd B, Hautefort A, Montani D, Fadel E, Dorfmuller P, Humbert M, Perros F, Paulin R, Provencher S, Bonnet S. Role for runt-related transcription factor 2 in proliferative and calcified vascular lesions in pulmonary arterial hypertension. Am J Respir Crit Care Med 194: 1273–1285, 2016. [PubMed: 27149112]
327. Sakai T, Larsen M, Yamada KM. Fibronectin requirement in branching morphogenesis. Nature 423: 876–881, 2003. [PubMed: 12815434]
328. Salameh N, Larrat B, Abarca-Quinones J, Pallu S, Dorvillius M, Leclercq I, Fink M, Sinkus R, Van Beers BE. Early detection of steatohepatitis in fatty rat liver by using MR elastography. Radiology 253: 90–97, 2009. [PubMed: 19587308]
329. Samuel MS, Lopez JI, McGhee EJ, Croft DR, Strachan D, Timpson P, Munro J, Schröder E, Zhou J, Brunton VG, Barker N, Clevers H, Sansom OJ, Anderson KI, Weaver VM, Olson MF. Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer Cell 19: 776–791, 2011. [PubMed: 21665151]
330. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: A new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 29: 1705–1713, 2003. [PubMed: 14698338]
331. Sarvazyan AP, Rudenko OV, Swanson SD, Fowlkes JB, Emelianov SY. Shear wave elasticity imaging: A new ultrasonic technology of medical diagnostics. Ultrasound Med Biol 24: 1419–1435, 1998. [PubMed: 10385964]
332. Sato S, Chong SG, Upagupta C, Yanagihara T, Saito T, Shimbori C, Bellaye PS, Nishioka Y, Kolb MR. Fibrotic extracellular matrix induces release of extracellular vesicles with pro-fibrotic miRNA from fibrocytes. Thorax, 2021.
333. Saums MK, Wang W, Han B, Madhavan L, Han L, Lee D, Wells RG. Mechanically and chemically tunable cell culture system for studying the myofibroblast phenotype. Langmuir 30: 5481–5487, 2014. [PubMed: 24787894]
334. Saxena N, Mogha P, Dash S, Majumder A, Jadhav S, Sen S. Matrix elasticity regulates mesenchymal stem cell chemotaxis. J Cell Sci 131, 2018.
335. Schäfer M, Myers C, Brown RD, Frid MG, Tan W, Hunter K, Stenmark KR. Pulmonary arterial stiffness: Toward a new paradigm in pulmonary arterial hypertension pathophysiology and assessment. Curr Hypertens Rep 18: 4, 2016. [PubMed: 26733189]

336. Schiller HB, Hermann MR, Polleux J, Vignaud T, Zanivan S, Friedel CC, Sun Z, Raducanu A, Gottschalk KE, Théry M, Mann M, Fässler R. β1- and αv-class integrins cooperate to regulate myosin II during rigidity sensing of fibronectin-based microenvironments. Nat Cell Biol 15: 625–636, 2013. [PubMed: 23708002]

337. Schittny JC, Paulsson M, Vallan C, Burri PH, Kedei N, Aeschlimann D. Protein cross-linking mediated by tissue transglutaminase correlates with the maturation of extracellular matrices during lung development. Am J Respir Cell Mol Biol 17: 334–343, 1997. [PubMed: 9308920]

338. Scholze A, Bladbjerg EM, Sidelmann JJ, Diederichsen AC, Mickley H, Nybo M, Argraves WS, Marckmann P, Rasmussen LM. Plasma concentrations of extracellular matrix protein fibulin-1 are related to cardiovascular risk markers in chronic kidney disease and diabetes. Cardiovasc Diabetol 12: 6, 2013. [PubMed: 23294625]

339. Schuliga M, Grainge C, Westall G, Knight D. The fibrogenic actions of the coagulant and plasminogen activation systems in pulmonary fibrosis. Int J Biochem Cell Biol 97: 108–117, 2018. [PubMed: 29474926]

340. Scott JE. Proteoglycan: Collagen interactions and corneal ultrastructure. Biochem Soc Trans 19: 877–881, 1991. [PubMed: 1794577]

341. Scott JE. Supramolecular organization of extracellular matrix glycosaminoglycans, in vitro and in the tissues. FASEB J 6: 2639–2645, 1992. [PubMed: 1612287]

342. Seidlits SK, Khaing ZZ, Petersen RR, Nickels JD, Vanscoy JE, Shear JB, Schmidt CE. The effects of hyaluronic acid hydrogels with tunable mechanical properties on neural progenitor cell differentiation. Biomaterials 31: 3930–3940, 2010. [PubMed: 20171731]

343. Selman M, Montaño M, Ramos C, Vanda B, Becerril C, Delgado J, Sansores R, Barrios R, Pardo A. Tobacco smoke-induced lung emphysema in guinea pigs is associated with increased interstitial collagenase. Am J Physiol 271:L734–L743, 1996. [PubMed: 8944716]

344. Seow CY. Passive stiffness of airway smooth muscle: The next target for improving airway distensibility and treatment for asthma? Pulm Pharmacol Ther 26: 37–41, 2013. [PubMed: 22776694]

345. Shah MA, Scaman CH, Palcic MM, Kagan HM. Kinetics and stereospecificity of the lysyl oxidase reaction. J Biol Chem 268: 11573–11579, 1993. [PubMed: 8099354]

346. Shaheen S, Wan Z, Li Z, Chau A, Li X, Zhang S, Liu Y, Yi J, Zeng Y, Wang J, Chen X, Xu L, Chen W, Wang F, Lu Y, Zheng W, Shi Y, Sun X, Li Z, Xiong C, Liu W. Substrate stiffness governs the initiation of B cell activation by the concerted signaling of PKCbeta and focal adhesion kinase. Elife 6: e23063, 2017. [PubMed: 28195531]

347. Shkumatov A, Thompson M, Choi KM, Sicard D, Baek K, Kim DH, Tschumperlin DJ, Prakash YS, Kong H. Matrix stiffness-modulated proliferation and secretory function of the airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 308: L1125–L1135, 2015. [PubMed: 25724668]

348. Sicard D, Haak AJ, Choi KM, Craig AR, Fredenburgh LE, Tschumperlin DJ. Aging and anatomical variations in lung tissue stiffness. Am J Physiol Lung Cell Mol Physiol 314: L946–L955, 2018. [PubMed: 29469613]

349. Sieminski AL, Hebbel RP, Gooch KJ. The relative magnitudes of endothelial force generation and matrix stiffness modulate capillary morphogenesis in vitro. Exp Cell Res 297: 574–584, 2004. [PubMed: 15212957]

350. Sinkus R, Siegmann K, Xydeas T, Tanter M, Claussen C, Fink M. MR elastography of breast lesions: Understanding the solid/liquid duality can improve the specificity of contrast-enhanced MR mammography. Magn Reson Med 58: 1135–1144, 2007. [PubMed: 17969009]

351. Sinkus R, Tanter M, Xydeas T, Catheline S, Bercoff J, Fink M. Viscoelastic shear properties of in vivo breast lesions measured by MR elastography. Magn Reson Imaging 23: 159–165, 2005. [PubMed: 15833607]

352. Sisson TH, Ajayi IO, Subbotina N, Dodi AE, Rodansky ES, Chibucos LN, Kim KK, Keshamouni VG, White ES, Zhou Y, Higgins PD, Larsen SD, Neubig RR, Horowitz JC. Inhibition of myocardin-related transcription factor/serum response factor signaling decreases lung fibrosis and promotes mesenchymal cell apoptosis. Am J Pathol 185: 969–986, 2015. [PubMed: 25681733]

353. Sisson TH, Simon RH. The plasminogen activation system in lung disease. Curr Drug Targets 8: 1016–1029, 2007. [PubMed: 17896953]
354. Sokocevic D, Bonenfant NR, Wagner DE, Borg ZD, Lathrop MJ, Lam YW, Deng B, Desarno MJ, Ashikaga T, Loi R, Hoffman Am, Weiss DJ. The effect of age and emphysematous and fibrotic injury on the re-cellularization of de-cellularized lungs. Biomaterials 34: 3256–3269, 2013. [PubMed: 23384794]
355. Soucy PA, Werbin J, Heinz W, Hoh JH, Romer LH. Microelastic properties of lung cell-derived extracellular matrix. Acta Biomater 7: 96–105. [PubMed: 20656080]
356. Southern BD, Grove LM, Rahaman SO, Abraham S, Scheraga RG, Niese KA, Sun H, Herzog EL, Liu F, Tschumperlin DJ, Egelhoff TT, Rosenfeld SS, Olman MA. Matrix-driven myosin II mediates the profibrotic fibroblast phenotype. J Biol Chem 291: 6083–6095, 2016. [PubMed: 26763235]
357. Speight P, Kofler M, Szászi K, Kapus A. Context-dependent switch in chemo/mechanotransduction via multilevel crosstalk among cytoskeleton-regulated MRTF and TAZ and TGFβ-regulated Smad3. Nat Commun 7: 11642, 2016. [PubMed: 27189435]
358. Sridharan R, Cavanagh B, Cameron AR, Kelly DJ, O'Brien FJ. Material stiffness influences the polarization state, function and migration mode of macrophages. Acta Biomater 89: 47–59, 2019. [PubMed: 30826478]
359. STEINBERG MS. Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. Science 141: 401–408, 1963. [PubMed: 13983728]
360. Stephens P, Grenard P, Aeschlimann P, Langley M, Blain E, Errington R, Kipling D, Thomas D, Aeschlimann D. Crosslinking and G-protein functions of transglutaminase 2 contribute differentially to fibroblast wound healing responses. J Cell Sci 117: 3389–3403, 2004. [PubMed: 15199098]
361. Stone PJ, Gottlieb DJ, O'Connor GT, Ciccolella DE, Breuer R, Bryan-Rhadfi J, Shaw HA, Franzblau C, Snider GL. Elastin and collagen degradation products in urine of smokers with and without chronic obstructive pulmonary disease. Am J Respir Crit Care Med 151: 952–959, 1995. [PubMed: 7697272]
362. Stowers RS, Allen SC, Suggs LJ. Dynamic phototuning of 3D hydrogel stiffness. Proc Natl Acad Sci U S A 112: 1953–1958, 2015. [PubMed: 25646417]
363. Stowers RS, Shcherbina A, Israeli J, Gruber JJ, Chang J, Nam S, Rabiee A, Teruel MN, Snyder MP, Kundaje A, Chaudhuri O. Matrix stiffness induces a tumorigenic phenotype in mammary epithelium through changes in chromatin accessibility. Nat Biomed Eng 3: 1009–1019, 2019. [PubMed: 31285581]
364. Streitberger KJ, Sack I, Krefting D, Pfüller C, Braun J, Paul F, Wuerfel J. Brain viscoelasticity alteration in chronic-progressive multiple sclerosis. PLoS One 7: e29888, 2012. [PubMed: 22276134]
365. Stroka KM, Aranda-Espinoza H. Neutrophils display biphasic relationship between migration and substrate stiffness. Cell Motil Cytoskeleton 66: 328–341, 2009. [PubMed: 19373775]
366. Stroka KM, Aranda-Espinoza H. Endothelial cell substrate stiffness influences neutrophil transmigration via myosin light chain kinase-dependent cell contraction. Blood 118: 1632–1640, 2011. [PubMed: 21652678]
367. Stroka KM, Levitan I, Aranda-Espinoza H. OxLDL and substrate stiffness promote neutrophil transmigration by enhanced endothelial cell contractility and ICAM-1. J Biomech 45: 1828–1834, 2012. [PubMed: 22560286]
368. Suh JK, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. Biomaterials 21: 2589–2598, 2000. [PubMed: 11071608]
369. Suki B. Assessing the functional mechanical properties of bioengineered organs with emphasis on the lung. J Cell Physiol 229: 1134–1140, 2014. [PubMed: 24604423]
370. Sun W, Chan SY. Pulmonary arterial stiffness: An early and pervasive driver of pulmonary arterial hypertension. Front Med (Lausanne) 5: 204, 2018. [PubMed: 30073166]
371. Sun Z, Guo SS, Fässler R. Integrin-mediated mechanotransduction. J Cell Biol 215: 445–456, 2016. [PubMed: 27872252]

372. Sunyer R, Jin AJ, Nossal R, Sackett DL. Fabrication of hydrogels with steep stiffness gradients for studying cell mechanical response. PLoS One 7: e46107, 2012. [PubMed: 23056241]

373. Sweers KK, Bennink ML, Subramaniam V. Nanomechanical properties of single amyloid fibrils. J Phys Condens Matter 24: 243101, 2012. [PubMed: 22585542]

374. Swift J, Ivanovska IL, Buxboim A, Harada T, Dingal PC, Pinter J, Pajerowski JD, Spinler KR, Shin JW, Tewari M, Rehfeldt F, Speicher DW, Discher DE. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 341: 1240104, 2013. [PubMed: 23990565]

375. Szeto SG, Narimatsu M, Lu M, He X, Sidiqi AM, Tolosa MF, Chan L, De Freitas K, Bialik JF, Majumder S, Boo S, Hinz B, Dan Q, Advani A, John R, Wrana JL, Kapus A, Yuen DA. YAP/TAZ are mechanoregulators of TGF. J Am Soc Nephrol 27: 3117–3128, 2016. [PubMed: 26961347]

376. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: An approach to isolate mechanical force. Proc Natl Acad Sci U S A 100: 1484–1489, 2003. [PubMed: 12552122]

377. Tapial Martínez P, López Navajas P, Lietha D. FAK structure and regulation by membrane interactions and force in focal adhesions. Biomolecules 10, 2020.

378. Tatler AL, John AE, Jolly L, Habgood A, Porte J, Brightling C, Knox AJ, Pang L, Sheppard D, Huang X, Jenkins G. Integrin αvβ5-mediated TGF-β activation by airway smooth muscle cells in asthma. J Immunol 187: 6094–6107, 2011. [PubMed: 22025551]

379. Tazdait M, Mezquita L, Lahmar J, Ferrara R, Bidault F, Ammari S, Balleyguier C, Planchard D, Gazzah A, Soria J. Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. Eu J Cancer 88: 38–47, 2018.

380. Thiele J, Ma Y, Bruekers SM, Ma S, Huck WT. 25th anniversary article: Designer hydrogels for cell cultures: A materials selection guide. Adv Mater 26: 125–147, 2014. [PubMed: 24227691]

381. Tian B, Manley JL. Alternative polyadenylation of mRNA precursors. Nat Rev Mol Cell Biol 18: 18–30, 2017. [PubMed: 27677860]

382. Tian W, Hao C, Fan Z, Weng X, Qin H, Wu X, Fang M, Chen Q, Shen A, Xu Y. Myocardin related transcription factor A programs epigenetic activation of hepatic stellate cells. J Hepatol 62: 165–174, 2015. [PubMed: 25109772]

383. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng 103: 655–663, 2009. [PubMed: 19472329]

384. Tjin G, White ES, Faiz A, Sicard D, Tschumperlin DJ, Mahar A, Kable EPW, Burgess JK. Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. Dis Model Mech 10: 1301–1312, 2017. [PubMed: 29125826]

385. Todorovic V, Jurukovski V, Chen Y, Fontana L, Dabovic B, Rifkin DB. Latent TGF-beta binding proteins. Int J Biochem Cell Biol 37: 38–41, 2005. [PubMed: 15381147]

386. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349–363, 2002. [PubMed: 11988769]

387. Torrino S, Roustan FR, Kaminski L, Bertero T, Pisano S, Ambrosetti D, Dufies M, Uhler JP, Lemichez E, Mettouchi A. UBTD1 is a mechanoregulator controlling cancer aggressiveness. EMBO Rep 20: e46570, 2019. [PubMed: 30804013]

388. Totaro A, Castellan M, Battilana G, Zanconato F, Azzolin L, Giulitti S, Cordenonsi M, Piccolo S. YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. Nat Commun 8: 15206, 2017. [PubMed: 28513598]

389. Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: A material's perspective. Curr Opin Biotechnol 24: 948–953, 2013. [PubMed: 23611564]

390. Trappmann B, Gautrot JE, Connelly JT, Strange DG, Li Y, Oyen ML, Cohen Stuart MA, Boehm H, Li B, Vogel V, Spatz JP, Watt FM, Huck WT. Extracellular-matrix tethering regulates stem-cell fate. Nat Mater 11: 642–649, 2012. [PubMed: 22635042]

391. Truby RL, Lewis JA. Printing soft matter in three dimensions. Nature 540: 371–378, 2016. [PubMed: 27974748]

392. Tschumperlin DJ. Matrix, mesenchyme, and mechanotransduction. Ann Am Thorac Soc 12 (Suppl 1): S24–S29, 2015. [PubMed: 25830830]

393. Tschumperlin DJ, Lagares D. Mechano-therapeutics: Targeting mechanical signaling in fibrosis and tumor stroma. Pharmacol Ther 107575, 2020.

394. Tse JR, Engler AJ. Preparation of hydrogel substrates with tunable mechanical properties. Curr Protoc Cell Biol. Chapter 10: Unit 10: 16, 2010.

395. Tse JR, Engler AJ. Stiffness gradients mimicking in vivo tissue variation regulate mesenchymal stem cell fate. PLoS One 6: e15978, 2011. [PubMed: 21246050]

396. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: A meta-analysis of diagnostic accuracy. J Hepatol 54: 650–659, 2011. [PubMed: 21146892]

397. Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res 56: 1–21, 2001. [PubMed: 11237208]

398. Urciuoli E, Peruzzi B. Involvement of the FAK Network in pathologies related to altered mechanotransduction. Int J Mol Sci 21, 2020. [PubMed: 32188047]

399. Venkatasubramanian RT, Wolkers WF, Shenoi MM, Barocas VH, Lafontaine D, Soule CL, Iaizzo PA, Bischof JC. Freeze-thaw induced biomechanical changes in arteries: Role of collagen matrix and smooth muscle cells. Ann Biomed Eng 38: 694–706, 2010. [PubMed: 20108044]

400. Vining KH, Mooney DJ. Mechanical forces direct stem cell behaviour in development and regeneration. Nat Rev Mol Cell Biol 18: 728–742, 2017. [PubMed: 29115301]

401. Wagenseil JE, Mecham RP. Elastin in large artery stiffness and hypertension. J Cardiovasc Transl Res 5: 264–273, 2012. [PubMed: 22290157]

402. Wagh AA, Roan E, Chapman KE, Desai LP, Rendon DA, Eckstein EC, Waters CM. Localized elasticity measured in epithelial cells migrating at a wound edge using atomic force microscopy. Am J Physiol Lung Cell Mol Physiol 295: L54–L60, 2008. [PubMed: 18487359]

403. Walker CJ, Crocini C, Ramirez D, Killaars AR, Grim JC, Aguado BA, Clark K, Allen MA, Dowell RD, Leinwand LA, Anseth KS. Nuclear mechanosensing drives chromatin remodelling in persistently activated fibroblasts. Nat. Biomed Eng, 2021.

404. Wallis JM, Borg ZD, Daly AB, Deng B, Ballif BA, Allen GB, Jaworski DM, Weiss DJ. Comparative assessment of detergent-based protocols for mouse lung de-cellularization and re-cellularization. Tissue Eng Part C Methods 18: 420–432, 2012. [PubMed: 22165818]

405. Walraven M, Hinz B. Therapeutic approaches to control tissue repair and fibrosis: Extracellular matrix as a game changer. Matrix Biol 71–72: 205–224, 2018.

406. Wang JY, Mesquida P, Lee T. Young's modulus measurement on pig trachea and bronchial airways. Annu Int Conf IEEE Eng Med Biol Soc 2089-2092: 2011, 2011.

407. Wang T, Hamilla S, Cam M, Aranda-Espinoza H, Mili S. Extracellular matrix stiffness and cell contractility control RNA localization to promote cell migration. Nat Commun 8: 896, 2017. [PubMed: 29026081]

408. Wang W, Lollis EM, Bordeleau F, Reinhart-King CA. Matrix stiffness regulates vascular integrity through focal adhesion kinase activity. FASEB J 33: 1199–1208, 2019. [PubMed: 30102569]

409. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J Cell Biol 137: 231–245, 1997. [PubMed: 9105051]

410. Webber MJ, Tongers J, Newcomb CJ, Marquardt KT, Bauersachs J, Losordo DW, Stupp SI. Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. Proc Natl Acad Sci U S A 108: 13438–13443, 2011. [PubMed: 21808036]

411. Wei Y, Dong W, Jackson J, Ho TC, Le Saux CJ, Brumwell A, Li X, Klesney-Tait J, Cohen ML, Wolters PJ, Chapman HA. Blocking LOXL2 and TGFβ1 signalling induces collagen I turnover in precision-cut lung slices derived from patients with idiopathic pulmonary fibrosis. Thorax, 2021.

412. Wen JH, Vincent LG, Fuhrmann A, Choi YS, Hribar KC, Taylor-Weiner H, Chen S, Engler AJ. Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 13: 979–987, 2014. [PubMed: 25108614]

413. Weng T, Ko J, Masamha CP, Xia Z, Xiang Y, Chen NY, Molina JG, Collum S, Mertens TC, Luo F, Philip K, Davies J, Huang J, Wilson C, Thandavarayan RA, Bruckner BA, Jyothula SS, Volcik

KA, Li L, Han L, Li W, Assassi S, Karmouty-Quintana H, Wagner EJ, Blackburn MR. Cleavage factor 25 deregulation contributes to pulmonary fibrosis through alternative polyadenylation. J Clin Invest 130, 2019.

414. Wetzl V, Tiede SL, Faerber L, Weissmann N, Schermuly RT, Ghofrani HA, Gall H. Plasma MMP2/TIMP4 ratio at follow-up assessment predicts disease progression of idiopathic pulmonary arterial hypertension. Lung 195: 489–496, 2017. [PubMed: 28516393]
415. Wilgus ML, Borczuk AC, Stoopler M, Ginsburg M, Gorenstein L, Sonett JR, Powell CA. Lysyl oxidase: A lung adenocarcinoma biomarker of invasion and survival. Cancer 117: 2186–2191, 2011. [PubMed: 21523732]
416. Wilson JW, Li X, Pain MC. The lack of distensibility of asthmatic airways. Am Rev Respir Dis 148: 806–809, 1993. [PubMed: 8368652]
417. Wilusz RE, Defrate LE, Guilak F. A biomechanical role for perlecan in the pericellular matrix of articular cartilage. Matrix Biol 31: 320–327, 2012. [PubMed: 22659389]
418. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol 179: 1311–1323, 2007. [PubMed: 18086923]
419. Witsch TJ, Niess G, Sakkas E, Likhoshvay T, Becker S, Herold S, Mayer K, Vadász I, Roberts JD, Seeger W, Morty RE. Transglutaminase 2: A new player in bronchopulmonary dysplasia? Eur Respir J 44: 109–121, 2014. [PubMed: 24603819]
420. Wood JA, Shah NM, McKee CT, Hughbanks ML, Liliensiek SJ, Russell P, Murphy CJ. The role of substratum compliance of hydrogels on vascular endothelial cell behavior. Biomaterials 32: 5056–5064, 2011. [PubMed: 21501863]
421. Woodcock CC, Hafeez N, Handen A, Tang Y, Harvey LD, Estephan LE, Speyer G, Kim S, Bertero T, Chan SY. Matrix stiffening induces a pathogenic QKI-miR-7-SRSF1 signaling axis in pulmonary arterial endothelial cells. Am J Physiol Lung Cell Mol Physiol, 2021.
422. Wouters OY, Ploeger DT, van Putten SM, Bank RA. 3,4-Dihydroxy-L-phenylalanine as a novel covalent linker of extracellular matrix proteins to polyacrylamide hydrogels with a tunable stiffness. Tissue Eng Part C Methods, 2016.
423. Xia T, Liu W, Yang L. A review of gradient stiffness hydrogels used in tissue engineering and regenerative medicine. J Biomed Mater Res A 105: 1799–1812, 2017. [PubMed: 28187512]
424. Xia XD, Lee J, Khan S, Ye L, Li Y, Dong L. Suppression of phosphatidylinositol 3-kinase/Akt signaling attenuates hypoxia-induced pulmonary hypertension through the downregulation of Lysyl oxidase. DNA Cell Biol 35: 599–606, 2016. [PubMed: 27383273]
425. Xie SA, Zhang T, Wang J, Zhao F, Zhang YP, Yao WJ, Hur SS, Yeh YT, Pang W, Zheng LS, Fan YB, Kong W, Wang X, Chiu JJ, Zhou J. Matrix stiffness determines the phenotype of vascular smooth muscle cell in vitro and in vivo: Role of DNA methyltransferase 1. Biomaterials 155: 203–216, 2018. [PubMed: 29182961]
426. Xu MY, Porte J, Knox AJ, Weinreb PH, Maher TM, Violette SM, McAnulty RJ, Sheppard D, Jenkins G. Lysophosphatidic acid induces alphavbeta6 integrin-mediated TGF-beta activation via the LPA2 receptor and the small G protein G alpha(q). Am J Pathol 174: 1264–1279, 2009. [PubMed: 19147812]
427. Xu X, Li Z, Cai L, Calve S, Neu CP. Mapping the nonreciprocal micromechanics of individual cells and the surrounding matrix within living tissues. Sci Rep 6: 24272, 2016. [PubMed: 27067516]
428. Xu X, Li Z, Leng Y, Neu CP, Calve S. Knockdown of the pericellular matrix molecule perlecan lowers in situ cell and matrix stiffness in developing cartilage. Dev Biol 418: 242–247, 2016. [PubMed: 27578148]
429. Yamashita H, Ichikawa T, Matsuyama D, Kimura Y, Ueda K, Craig SW, Harada I, Kioka N. The role of the interaction of the vinculin proline-rich linker region with vinexin α in sensing the stiffness of the extracellular matrix. J Cell Sci 127: 1875–1886, 2014. [PubMed: 24554436]
430. Yang C, Tibbitt MW, Basta L, Anseth KS. Mechanical memory and dosing influence stem cell fate. Nat Mater 13: 645–652, 2014. [PubMed: 24633344]
431. Yaqoob U, Cao S, Shergill U, Jagavelu K, Geng Z, Yin M, De Assuncao TM, Cao Y, Szabolcs A, Thorgeirsson S. Neuropilin-1 stimulates tumor growth by increasing fibronectin fibril assembly in the tumor microenvironment. Cancer Res 72: 4047–4059, 2012. [PubMed: 22738912]

432. Yasmin MRA, McEniery CM, Cleary SE, Li Y, Siew K, Figg NL, Khir AW, Cockcroft jR, Wilkinson IB, O'Shaughnessy KM. The matrix proteins aggrecan and fibulin-1 play a key role in determining aortic stiffness. Sci Rep 8: 8550, 2018. [PubMed: 29867203]

433. Yeh YC, Corbin EA, Caliari SR, Ouyang L, Vega SL, Truitt R, Han L, Margulies KB, Burdick JA. Mechanically dynamic PDMS substrates to investigate changing cell environments. Biomaterials 145: 23–32, 2017. [PubMed: 28843064]

434. Yeh YT, Hur SS, Chang J, Wang KC, Chiu JJ, Li YS, Chien S. Matrix stiffness regulates endothelial cell proliferation through septin 9. PLoS One 7: e46889, 2012. [PubMed: 23118862]

435. Yin H, Ding Y, Zhai Y, Tan W, Yin X. Orthogonal programming of heterogeneous micro-mechano-environments and geometries in three-dimensional bio-stereolithography. Nat Commun 9: 4096, 2018. [PubMed: 30291242]

436. Yin M, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. Clin Gastroenterol Hepatol 5: 1207–1213.e1202, 2007. [PubMed: 17916548]

437. Young DA, Choi YS, Engler AJ, Christman KL. Stimulation of adipogenesis of adult adipose-derived stem cells using substrates that mimic the stiffness of adipose tissue. Biomaterials 34: 8581–8588, 2013. [PubMed: 23953825]

438. Yu OM, Miyamoto S, Brown JH. Myocardin-related transcription factor A and Yes-associated protein exert dual control in G protein-coupled receptor- and RhoA-mediated transcriptional regulation and cell proliferation. Mol Cell Biol 36: 39–49, 2016. [PubMed: 26459764]

439. Yuan Y, Zhong W, Ma G, Zhang B, Tian H. Yes-associated protein regulates the growth of human non-small cell lung cancer in response to matrix stiffness. Molecul Med Rep 11: 4267–4272, 2015.

440. Yurke B, Turberfield AJ, Mills AP, Simmel FC, Neumann JL. A DNA-fuelled molecular machine made of DNA. Nature 406: 605–608, 2000. [PubMed: 10949296]

441. Zemel A, Rehfeldt F, Brown AE, Discher DE, Safran SA. Optimal matrix rigidity for stress fiber polarization in stem cells. Nat Phys 6: 468–473, 2010. [PubMed: 20563235]

442. Zeng Y, Yi J, Wan Z, Liu K, Song P, Chau A, Wang F, Chang Z, Han W, Zheng W, Chen YH, Xiong C, Liu W. Substrate stiffness regulates B-cell activation, proliferation, class switch, and T-cell-independent antibody responses in vivo. Eur J Immunol 45: 1621–1634, 2015. [PubMed: 25756957]

443. Zhang H, Yousem SA, Franklin WA, Elder E, Landreneau R, Ferson P, Keenan R, Whiteside T, Levitt ML. Differentiation and programmed cell death-related intermediate biomarkers for the development of non-small cell lung cancer: A pilot study. Hum Pathol 29: 965–971, 1998. [PubMed: 9744313]

444. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A. Self-complementary oligopeptide matrices support mammalian cell attachment. Biomaterials 16: 1385–1393, 1995. [PubMed: 8590765]

445. Zhang X, Chen X, Yang T, Zhang N, Dong L, Ma S, Liu X, Zhou M, Li B. The effects of different crossing-linking conditions of genipin on type I collagen scaffolds: An in vitro evaluation. Cell Tissue Bank 15: 531–541, 2014. [PubMed: 24442821]

446. Zhang X, Kim TH, Thauland TJ, Li H, Majedi FS, Ly C, Gu Z, Butte MJ, Rowat AC, Li S. Unraveling the mechanobiology of immune cells. Curr Opin Biotechnol 66: 236–245, 2020. [PubMed: 33007634]

447. Zhao B, Li L, Wang L, Wang CY, Yu J, Guan KL. Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. Genes Dev 26: 54–68, 2012. [PubMed: 22215811]

448. Zhou J, Kim HY, Davidson LA. Actomyosin stiffens the vertebrate embryo during crucial stages of elongation and neural tube closure. Development 136: 677–688, 2009. [PubMed: 19168681]

449. Zhou Y, Hagood JS, Lu B, Merryman WD, Murphy-Ullrich JE. Thy-1-integrin alphav beta5 interactions inhibit lung fibroblast contraction-induced latent transforming growth factor-beta1 activation and myofibroblast differentiation. J Biol Chem 285: 22382–22393, 2010. [PubMed: 20463011]

450. Zhou Y, Horowitz JC, Naba A, Ambalavanan N, Atabai K, Balestrini J, Bitterman PB, Corley RA, Ding BS, Engler AJ, Hansen KC, Hagood JS, Kheradmand F, Lin QS, Neptune E, Niklason L, Ortiz LA, Parks WC, Tschumperlin DJ, White ES, Chapman HA, Thannickal VJ. Extracellular matrix in lung development, homeostasis and disease. Matrix Biol 73: 77–104, 2018. [PubMed: 29524630]

451. Zhou Y, Huang X, Hecker L, Kurundkar D, Kurundkar A, Liu H, Jin TH, Desai L, Bernard K, Thannickal VJ. Inhibition of mechanosensitive signaling in myofibroblasts ameliorates experimental pulmonary fibrosis. J Clin Invest 123: 1096–1108, 2013. [PubMed: 23434591]

452. Zhou Z, Qu J, He L, Zhu Y, Yang SZ, Zhang F, Guo T, Peng H, Chen P, Zhou Y. Stiff matrix instigates type I collagen biogenesis by mammalian cleavage factor I complex-mediated alternative polyadenylation. JCI Insight 5: e133972, 2020. [PubMed: 31935199]

453. Zhu CQ, Popova SN, Brown ER, Barsyte-Lovejoy D, Navab R, Shih W, Li M, Lu M, Jurisica I, Penn LZ, Gullberg D, Tsao MS. Integrin alpha 11 regulates IGF2 expression in fibroblasts to enhance tumorigenicity of human non-small-cell lung cancer cells. Proc Natl Acad Sci U S A 104: 11754–11759, 2007. [PubMed: 17600088]

454. Zhu J, Marchant RE. Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devices 8: 607–626, 2011. [PubMed: 22026626]

455. Zimmermann H, Wählisch F, Baier C, Westhoff M, Reuss R, Zimmermann D, Behringer M, Ehrhart F, Katsen-Globa A, Giese C, Marx U, Sukhorukov VL, Vásquez JA, Jakob P, Shirley SG, Zimmermann U. Physical and biological properties of barium cross-linked alginate membranes. Biomaterials 28: 1327–1345, 2007. [PubMed: 17166581]

456. Ziv K, Nuhn H, Ben-Haim Y, Sasportas LS, Kempen PJ, Niedringhaus TP, Hrynyk M, Sinclair R, Barron AE, Gambhir SS. A tunable silk-alginate hydrogel scaffold for stem cell culture and transplantation. Biomaterials 35: 3736–3743, 2014. [PubMed: 24484675]

457. Zustiak SP. The role of matrix compliance on cell responses to drugs and toxins: Towards predictive drug screening platforms. Macromol Biosci 15: 589–599, 2015. [PubMed: 25654999]

Didactic Synopsis

Major teaching points

- The mechanical properties of the ECM regulate the function and behavior of various types of cells in the lung.
- The mechanisms underlying the mechanical interplays between cells and the ECM involve mechanosensing and mechanotransduction.
- Mechanointeractions of cells and the ECM are essential for the maintenance of lung homeostasis. Aberrant ECM mechanical signals are associated with the development of many common lung diseases.
- Matrix stiffness is regulated by the degree of ECM crosslinks, specific ECM components, cellular contractility, and other factors.
- An increasing availability of stiffness-tunable matrix substrates enables the study of cellular responses to matrix stiffness.
- The advent of high-resolution atomic force microscopy (AFM) allows mechanical testing of lung ECM at the microscale level. Ongoing development of noninvasive imaging modalities will benefit earlier diagnoses and potentially lead to better patient outcomes.
- Therapeutically targeting ECM stiffness is an emerging field of mechanomedicine.

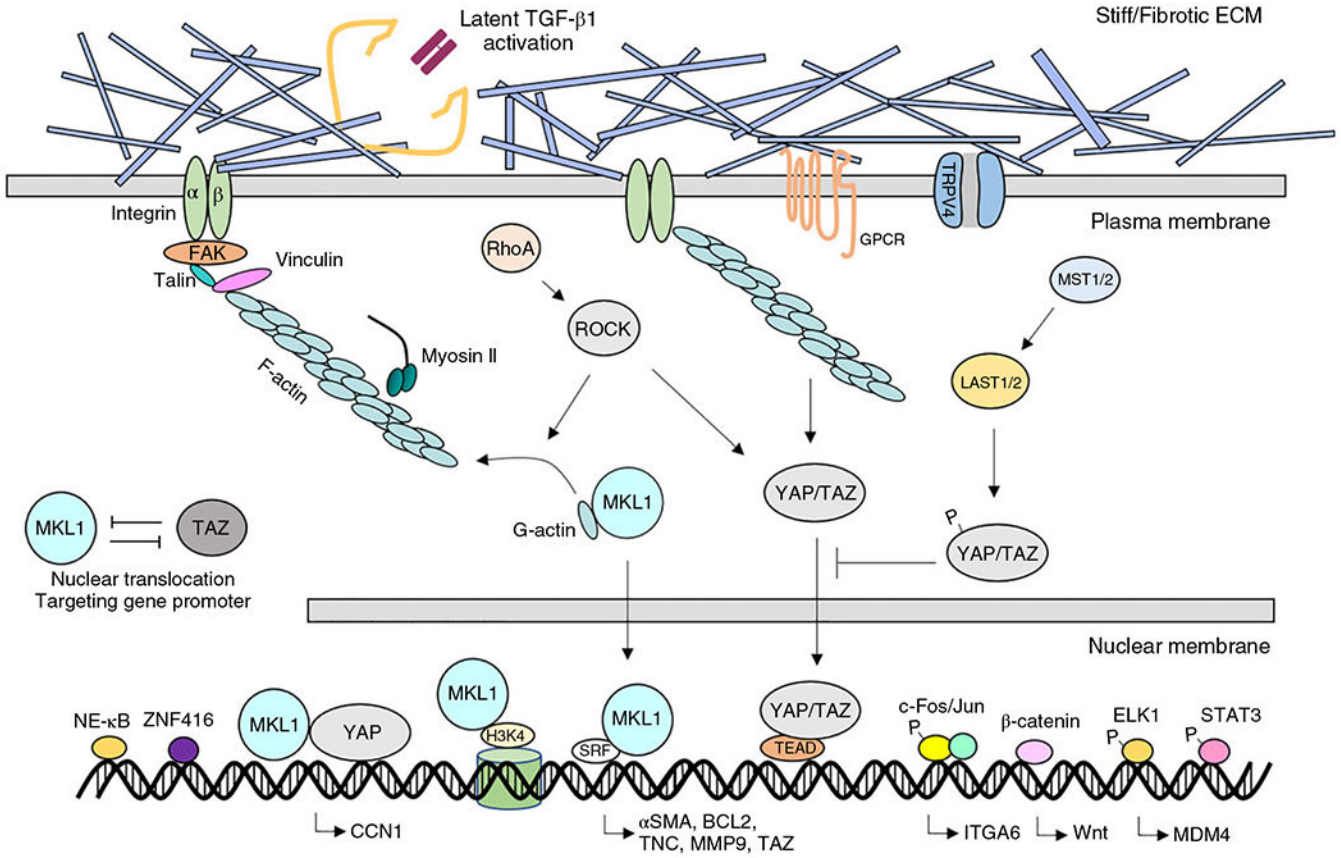

Figure 1. A scheme of matrix stiffness-mediated mechanosensing and mechanotransduction pathways.

ECM-derived mechanical cues are sensed by cell surface receptors, including integrin-containing FAs, GPCRs, and TRPV4. Mechanoactivation of RhoA and ROCK promotes G-actin polymerization into F-actin, resulting in the release of MKL1 and its nuclear translocation. RhoA/ROCK activation and the remodeling of actin cytoskeleton can also activate YAP/TAZ and promote their nuclear translocation, whereas the canonical Hippo signaling phosphorylates YAP/TAZ and inhibits their nuclear translocation. In the nucleus, MKL1 interacts with SRF to activate the transcription of target genes, including αSMA and BCL2. MKL1 binds to YAP, which regulates CCN1 gene expression. In contrast, MKL1 and TAZ appear to antagonize each other for the cytoplasmic-nuclear shuttling and the binding of gene promoter. MKL1 also regulates histone modifications through binding to H3K4 methyltransferase. Other mechanosensitive transcription factors include NF-κB, β-catenin, c-Fos/c-Jun, STAT3, ZNF416, and ELK1. Furthermore, the contractile cytoskeleton pulling on a stiffened ECM may change the conformation of latent TGF-β complex, leading to the release or exposure of active TGF-β.

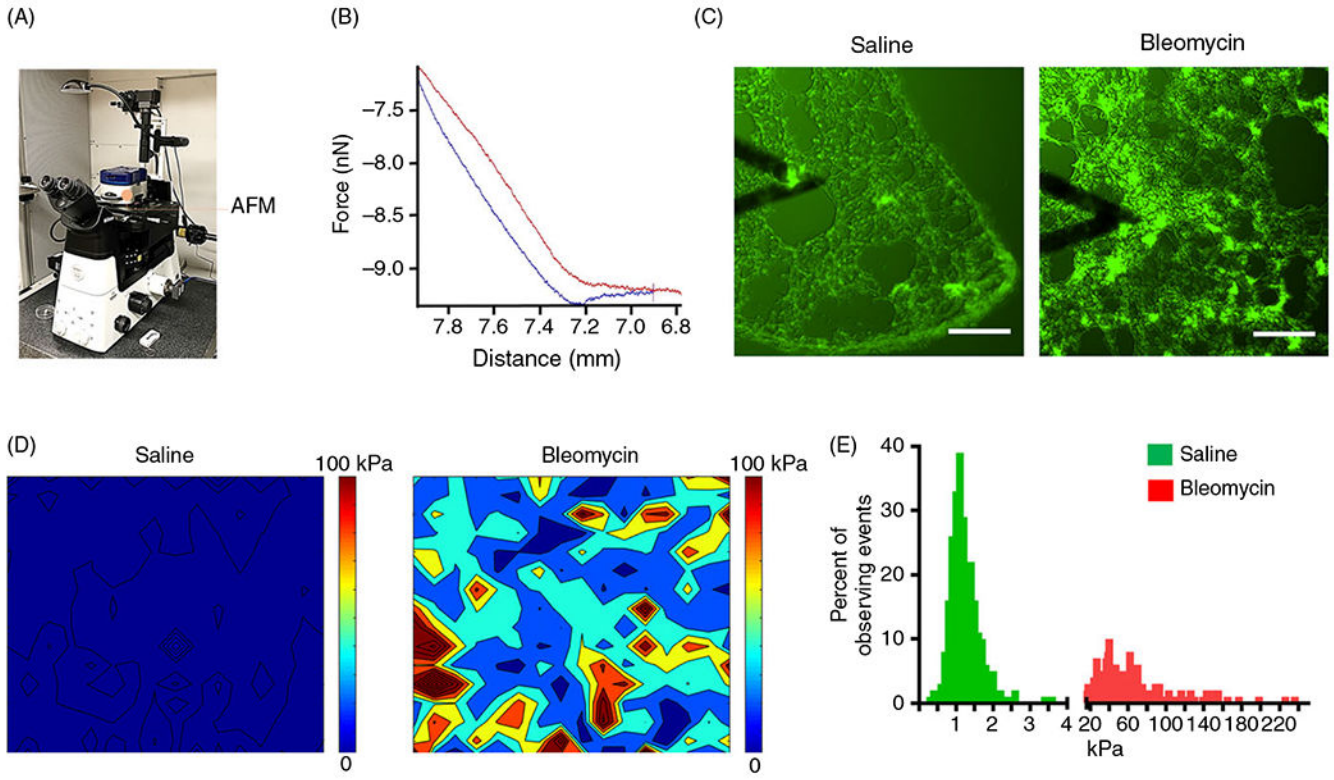

Figure 2. Mechanical testing of normal and fibrotic mouse lung tissues by AFM microindentation.

(A) A MFP-3D-BIO atomic force microscope (Asylum Research) mounted on a Nikon eclipse Ti2 microscope. (B) A typical force-distance curve generated by AFM on mouse lung tissues. (C to E) C57BL/6 mice were administered 1.5 U/kg body weight bleomycin intratracheally to induce lung fibrosis. Control mice were given an equal volume (50 μL) of saline. Lung tissues were harvested at 28 d. Mechanical testing of unfixed native mouse lung tissues was performed by AFM microindentation. Tissue sections were stained with a collagen-Col-F collagen-binding reagent to facilitate localization of specific lung areas (e.g., the alveolar wall and collagen-enriched fibrotic area) and positioning of the cantilever tip (C). Heat maps show the elastic moduli (Young's moduli) of the alveolar wall in the saline-treated lungs and the fibrotic area in bleomycin-treated lungs (D). Bar graphs show the distribution of stiffness in saline- and bleomycin-treated mouse lungs (E). Scale bars = 100 μm. (Panels C-E: Reused, with permission, from Sato S, et al., 2021, 332)

Table 1

Advantages and Disadvantages of Various Techniques for Tissue Stiffness Measurements

Method	Advantages	Disadvantages
Atomic force microscopy (AFM)	• High scan speed • Mapping of tissue stiffness heterogeneity • Minimal damage to sample • Mechanical testing in a liquid environment • Accurate targeting of the region of interest at the microscale level	• High scan speed • Limited to scan of tissue surface • Neglect of viscoelastic behavior
Ultrasound elastography	• Noninvasive technique • Ease of use • Low cost • Identification of spatial stiffness variations in a tissue volume	• Low reproducibility of measurements • Relatively high measurement failure rate • Lack of accurate targeting of the region of interest
Magnetic resonance elastography	• Noninvasive technique • Measurements of 3D tissue stiffness • Measurements of tissue viscosity • Low variability	• Limited accessibility to human body parts • Inability to distinguish tissue stiffening caused by fibrosis, inflammation, or altered perfusion • Longer imaging time • Higher cost
Fluorescent microscopy	• Cost-effective measurements • No special equipment needed	• Relatively longer operating time • User-dependent measurement

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Table 2

Lung Tissue Stiffness by Region and Health Status

Species	Region	Status	Young's modulus by AFM (mean ± SE)	References
Mouse	Parenchyma	Control (saline treated)	Median = ~0.5 kPa[a]	(226)
		Fibrosis (bleomycin treated)	Median = ~3kPa[a]	
Mouse	Parenchyma	Control (saline treated)	0.73 kPa	(227)
		Fibrosis (bleomycin treated)	13.39 kPa	
Mouse	Unspecified	Control (saline treated)	1.96 ± 1.21 kPa[b]	(48)
		Fibrosis (bleomycin treated)	17.25 ± 11.06 kPa[b]	
Mouse	Airway	Normal lung	23.1 ± 14.0 kPa[b]	(347)
Mouse	Alveolar septa	Control (saline treated)	27.2 ± 1.6 kPa	(256)
	Visceral pleura		56.6 ± 4.6 kPa	
	Tunica adventitia		41.1 ± 8.0 kPa	
	Tunica intima		79.3 ± 7.2 kPa	
	Alveolar septa	Fibrosis (bleomycin treated)	64.8 ± 7.1 kPa	
	Visceral pleura		99.9 ± 11.7 kPa	
	Tunica adventitia		105.2 ± 13.6 kPa	
	Tunica intima		146.6 ± 28.8 kPa	
Rat	Alveolar septa	Decellularized normal lung	16.8 ± 2.4 kPa	(257)
	Alveolar junction		15.6 ± 2.5 kPa	
	Pleura		34.6 ± 2.4 kPa	
	Tunica adventitia		30.9 ± 2.5 kPa	
	Tunica intima		59.6 ± 2.5 kPa	
Rat	Alveolar septa	Decellularized normal lung	5.6 ± 3.4 kPa[b]	(238)
	Alveolar junction		6.8 ± 3.9 kPa[b]	
	Pleura		15.8 ± 13.7 kPa[b]	
Human	Unspecified	Native lung, control subjects	1.96 ± 0.13 kPa	(41)
		Native lung, IPF subjects	16.52 ± 2.25 kPa	
		Decellularized lung, control subjects	1.61 ± 0.08 kPa	

Species	Region	Status	Young's modulus by AFM (mean ± SE)	References
Human	Unspecified	Decellularized lung, IPF subjects	7.34 ± 0.60 kPa	(85)
		Decellularized normal lung	3.7 ± 1.3 kPa[c]	
		Decellularized IPF lung	18.9 ± 11.1 kPa[c]	
		Decellularized COPD lung	2.9 ± 0.8 kPa[c]	

[a] Shear modulus.

[b] Mean ± SD.

[c] Stiffness measured by a low-load compression tester.

Table 3

Advantages and Disadvantages of Various Hydrogels

Type	Advantages	Disadvantages
Natural hydrogels		
Collagen	• Biocompatibility • High availability • Simple gelation • 3D cell culture	• Weak mechanical properties
Hyaluronic acid	• Biocompatibility • User-defined crosslinking chemistry	• Poor mechanical properties • Rapid degradation
Alginate	• Low toxicity • Low cost • Mild gelation conditions	• Poor cell adhesion • Limited long-term stability
Agarose	• Simple gelation • Tunable mechanical properties	• Variable pore size
Pectin	• High availability • Low cost • Easy functionalization	• Low stability • Poor mechanical properties
Decellularized ECM	• Structure and composition mimicking the native ECM • Inherent bioactivity	• Harsh chemical or mechanical treatments • Incomplete decellularization • Damage to the ECM's ultrastructure
Synthetic hydrogels		
Polyacrylamide	• Cost-effectiveness • Easy preparation • Constant surface chemistry • Reproductivity • Tunable mechanical properties	• Limited to 2D cell culture
Polyethylene glycol	• Cytocompatibility • User-defined crosslinking chemistry	• Poor cell adhesion
Polydimethylsiloxane	• Easy preparation • Long-term storage	• Absorption of small molecules
Polyisocyanopeptide-based hydrogels	• Nonlinear strain-stiffening properties • Easy cell recovery	• Fragileness
Self-assembling peptide hydrogels	• Biocompatibility • Structure mimicking the native ECM • Tunable mechanical properties	• Poor stability and consistency
Hybrid hydrogels	• Combined strengths of both natural and synthetic hydrogels	• High cost • Limited mechanical properties
Gradient stiffness matrices	• Mimicking the mechanical heterogeneity observed *in vivo* • Simulation of pathological conditions such as fibrosis and cancer	• Time consuming • Complex methodologies

Type	Advantages	Disadvantages
Mechanically dynamic matrices	• Mimicking the dynamic nature of the cellular microenvironment	• Limited long-term stability • Slow reaction kinetics