# Lung Extracellular Matrix and Fibroblast Function

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## Abstract

Extracellular matrix (ECM) is a tissue-specific macromolecular structure that provides physical support to tissues and is essential for normal organ function. In the lung, ECM plays an active role in shaping cell behavior both in health and disease by virtue of the contextual clues it imparts to cells. Qualities including dimensionality, molecular composition, and intrinsic stiffness all promote normal function of the lung ECM. Alterations in composition and/or modulation of stiffness of the focally injured or diseased lung ECM microenvironment plays a part in reparative processes performed by fibroblasts. Under conditions of remodeling or in disease states, inhomogeneous stiffening (or softening) of the pathologic ECM may both precede modifications in cell behavior and be a result of disease progression. The ability of ECM to stimulate further ECM production by fibroblasts and drive disease progression has potentially significant implications for mesenchymal stromal cell–based therapies; in the setting of pathologic ECM stiffness or composition, the therapeutic intent of progenitor cells may be subverted. Taken together, current data suggest that lung ECM actively contributes to health and disease; thus, mediators of cell–ECM signaling or factors that influence ECM stiffness may represent viable therapeutic targets in many lung disorders.

**Keywords:** extracellular matrix; fibroblasts; disease progression; cell shape; cellular mechanotransduction

(Received in original form June 6, 2014; accepted in final form July 21, 2014)

Supported by National Institutes of Health grants U01 HL111016 and R01 HL109118 (E.S.W.).

*Author Contributions*: E.S.W. is solely responsible for the content of this manuscript.

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Ann Am Thorac Soc Vol 12, Supplement 1, pp S30–S33, Mar 2015

DOI: 10.1513/AnnalsATS.201406-240MG
Internet address: www.atsjournals.org

Extracellular matrix (ECM) is a highly dynamic complex of fibrous proteins, glycoproteins, and proteoglycans that composes the noncellular aspect of tissues and varies in composition according to its tissue localization and physiological circumstances. In addition to providing structural integrity, ECM delivers important spatial and contextual cues to drive cellular phenotypes. The ECM in the lung is typically restricted to two basic compartments: basement membranes and the interstitial spaces. Basement membranes are thin, specialized layers of ECM found under all epithelial and endothelial cell layers, whereas interstitial spaces form the parenchyma of the lung (1). Within the lung interstitium, resident fibroblasts are the most commonly identified cell and are mainly responsible for ECM production; they also serve as effector cells during injury repair.

The term "matrisome" has been introduced to describe the various fibrillar proteins, glycoproteins, proteoglycans, and their associated modifying molecules (e.g., metalloproteases, matricellular proteins) that compose the ECM of tissues (2). Recently, the matrisome of both rodent (3) and human lungs (4) has been characterized. Not surprisingly, qualitative differences between rodent and human lung ECM are observed, although the bulk of matrisome constituents are conserved between the two species. Importantly, both studies make clear that the extracellular lung parenchyma is not solely composed of collagens, elastin, glycosaminoglycans, and basement membrane laminin, as has been traditionally thought.

The approach used to identify the matrisome, including removal of all cellular and nuclear material followed by digestion of resultant matrix and application of unbiased mass spectrometry technology, allows for the identification of previously unrecognized ECM components in the lung; this paves the way for potentially new areas of study in cell–matrix interactions. It is worthwhile mentioning that not all methods of decellularizing the lung are necessarily equivalent; variable loss of proteins, growth factors, and matrix-associated molecules occur depending on the detergents used, the pressures applied, and the length of the process (5–7). Aside from rodent and human lungs and rodent colonic tissue (3), the matrisome of other organs in either human or experimental animals has not yet been defined,

representing a critical, untapped aspect of biomedical research.

Besides influencing cellular behavior based on specific molecular signaling, dimensionality and biomechanical cues in the lung clearly impact the effect of ECM on interstitial cell phenotype (8–15). Although traditional *in vitro* studies of cell–matrix interactions usually rely on matrix-coated culture dishes, these artificial environments do not truly recapitulate the *in vivo* conditions under which cells reside. Notwithstanding the uniformity of the culture substrate (cells *in vivo* typically encounter multiple ECM molecules simultaneously, as described above) and the nearly constant stiffness of the tissue culture plastic (to be described below), fibroblasts cultivated in planar dishes are maintained in a two-dimensional orientation. In stark contrast, cells within the normal three-dimensional microenvironment of the lung extend membrane protrusions in all dimensions; for example, alveolar type I cells reach through pores of Kohn, and fibroblasts extend to contact endothelial cells and type I cells in numerous alveolar spaces to "sample" multiple alveoli simultaneously (16). Studies have also shown that phenotypic behavior depends on the position of the cell in space, with quite disparate phenotypes observed between fibroblasts in two-dimensional, compared with three-dimensional, environments (8–10).

*In vivo*, cells are subject to stress and strain forces. Stress is defined as the magnitude and direction of forces acting on a cell, whereas strain is the magnitude and direction of deformation (17). In the alveolus, cells (endothelial, epithelial, and fibroblasts) are exposed to rhythmic stretch (stress) throughout the respiratory cycle, leading to cell strain. Indeed, these strain forces are necessary for cells to perform their characteristic functions. For instance, airway epithelium enhances production of surfactant and improves barrier function when cultured at an air–liquid interface in a microfluidic device with cyclic stretch that simulates breathing (18).

Elastic (Young's) modulus is defined as stress/strain (19) and describes the force necessary to deform a material. Based on a variety of factors (including constituent cell types, composition of the ECM, fat content, and degree of mineralization, among others) different tissues and organs possess different elastic moduli (intrinsic stiffness). These range from extremely soft (blood plasma, approximately 50 Pa) to exceedingly stiff (bone, approximately 100,000 kPa) (20). In stark contrast, tissue culture plastic and glass possess elastic moduli in the 2- to 4-GPa range (20). Normal human lung parenchyma elastic modulus has been measured at 0.44 to 7.5 kPa (4), and this inhomogeneity depends in part on the region measured (alveolar wall vs. airway wall vs. airway epithelium, for example). Therefore, human lung fibroblasts *in vivo* are likely to experience an elastic modulus in the ~1 kPa range and are maintained in a quiescent state as a result. Various disease states may result in perturbations in the lung elastic modulus, with emphysema being associated with decreased lung stiffness (21) and fibrotic disorders with increased lung stiffness (4, 22).

## Modulation of ECM Stiffness

Defining the intrinsic human lung stiffness is an important first step in determining the contribution of mechanical forces to cell behavior; however, it is truly only the beginning. Important advances in our understanding of cell biology in lung disease will come when we better delineate the mechanisms by which lung stiffness undergoes change. Because lung stiffness primarily reflects biomechanical properties of the ECM, it makes sense that appreciation of the ways in which lung ECM may change over time (i.e., with aging) and in disease states will enhance our comprehension of the influences of biomechanical forces on cell function. Globally, changes in ECM stiffness may occur due to: (*1*) changes in the matrix molecules that make up the ECM, (*2*) fluctuations in the degree of protein cross-linking that occurs due to matrix-modifying enzymes, (*3*) alterations in the remodeling process, and (*4*) extent of mineralization.

It is widely accepted that fibrillar proteins, such as collagens and fibronectins, account for the tensile strength of the lung, whereas elastin molecules account for the elastic recoil (23, 24). Modifications in the levels and ratios of these proteins (among others) thus alter relative contributions of each molecule to ECM stiffness. Homeostatic turnover of these proteins is markedly different, with collagen fibers estimated to undergo remodeling at a rate of 3 to 10% per day (25), whereas elastin fibers are long lived, with a mean stability of 74 years in humans (26). In disease, these rates likely vary, although data are sparse in humans. Pathologic fragmentation and degradation of ECM also contribute to tissue stiffness; recent data suggest damage to elastin fibers with increased fractional area of fibronectin and tenascin in chronic obstructive pulmonary disease (COPD) airways, which will alter stiffness of the ECM (27). Similarly, robust collagen deposition in fibrotic disorders such as idiopathic pulmonary fibrosis (IPF) is associated with marked increases in lung stiffness (4).

ECM cross-linking from either enzymatic or nonenzymatic pathways contributes to matrix stiffness by strengthening protein–protein interactions and altering the physical characteristics of the ECM resulting in resistance to proteolysis, emergence of neoepitopes, and enhanced cell–matrix interactions (for a recent review, *see* Reference 28). Enzymatic cross-linking is typically mediated by the lysyl oxidase family of enzymes and/or by tissue transglutaminase, whereas nonenzymatic reactions, such as isomerization, glycosylation, and sulfation, occur either spontaneously or in response to increased blood glucose levels (as in the case of advanced glycation end products) (28). These various post-translational modifications can result in significant stiffening of ECM and resultant pathology when normal remodeling functions are subverted.

## ECM Stiffness in Pulmonary Fibrosis: Implications for the Fibroblast

Chronic lung injury and subsequent repair invariably lead to scar tissue formation, which is usually self-limited. In certain settings, however, scar tissue formation becomes progressive, with ongoing lung parenchymal destruction and replacement by pathologic fibrosis; the prototypical example of this is IPF. Replacement of normal elastic lung tissue with densely collagenous tissue, fragmented elastin fibers, and decreased ECM turnover in a histopathologic pattern termed usual interstitial pneumonia is a hallmark feature of the disease. In usual interstitial

pneumonia and in animal models of lung fibrosis, the ECM becomes exceedingly stiff (4, 22); however, it has yet to be fully elucidated whether ECM stiffening occurs due to ECM deposition and cross-linking or whether it precedes the development of fibrosis. Interestingly, one study in experimental liver fibrosis demonstrated that liver stiffness increased before measurable increases in liver collagen (29), highlighting the possibility that locally increased stiffness contributes to, rather than solely results from, fibrogenesis. Of course, an alternate explanation is that expression of other noncollagenous ECM molecules results in augmented stiffness before the deposition of collagen. One possibility includes the matrix proteoglycan versican, which has been shown to be deposited before mature collagens in IPF, fibroproliferative ARDS, and bronchiolitis obliterans and may cause collagen deposition through localized matrix stiffening (30). These possibilities require further mechanistic exploration in model systems, including lung, and should be explored in human tissues.

For years it has been recognized that fibroblasts adhere more strongly to and proliferate more robustly on stiffer matrices compared with softer ones (22, 31). Newer data support the hypothesis that ECM stiffness in IPF promotes a profibrotic phenotype in fibroblasts, such as myofibroblast differentiation (4), matrix synthesis (22, 32), and down-regulation of antifibrotic molecules (22). Indeed, recent data suggest that ECM protein translation in IPF is positively influenced by the IPF ECM, even in fibroblasts derived from normal donors (32). In line with these findings, a study now demonstrates that ECM within a physiologic range of stiffness is capable of reversing the activated myofibroblast phenotype (33), lending further credence to the idea that targeting ECM stiffness may be an appropriate therapeutic approach in fibrotic disorders. However, no studies to date have uncoupled pathologic ECM stiffness from pathologic ECM composition, raising the prospect that ECM composition, or the combination of pathologic ECM composition and stiffness, drives a feed-forward loop in fibrotic lung disease. This possibility will need to be more fully explored to better understand the relationships between ECM composition, ECM stiffness, and fibroblast behavior in fibrotic lung disease.

## ECM Stiffness in Pulmonary Fibrosis: Implications for Repair Strategies

Recently, there has been a burgeoning interest in using progenitor cells to enhance tissue repair in numerous organs, including lung (34–36). Mesenchymal stromal cells (MSCs), resident within lung tissue, appear to represent a viable pool of progenitor cells (reviewed in Reference 37) and have been investigated in animal models of lung disease (38–40). These studies demonstrate the reparative potential of MSCs and have led to a wave of clinical trials of MSC therapy in patients with various lung disorders (e.g., ARDS, IPF, and COPD). Although results are not yet available for most of these trials, preclinical studies suggest that MSCs are capable of restoring alveolar fluid clearance in *ex vivo* perfused and ventilated human lung (41).

The mechanism of action of these cells in lung repair is not fully understood, with some authors proposing that engraftment of MSCs into fibrotic or diseased tissues mediates repair (42) and others suggesting that MSC modulation of the host immune system may be responsible for the reparative effect (43). Regardless, this will require further study. Notably, investigation of MSCs on ECM substrates of varying stiffness shows that MSCs adopt a phenotype reflecting the stiffness of ECM: when cultured on ECM the stiffness of brain (0.1–1 kPa), MSCs adopted a branched, neuron-like appearance; when the stiffness approximates muscle (8–17 kPa), the same cell assumes a spindle shape; when cultured on stiff, crosslinked collagen (25–40 kPa), the cell assumes a polygonal, fibroblastic shape (44). The effect of ECM on other progenitor cell types, such as bone marrow–derived stem cells or embryonic stem cells, is less clear. Coupled with evidence that fibrotic ECM drives further ECM protein translation (32), one must consider the possibility that MSCs used for lung repair may in fact be "hijacked" to develop a profibrotic phenotype. Early clinical studies suggest that bone marrow–derived MSC therapy is safe in patients with COPD (45), but, as mentioned previously, ECM stiffness may be markedly different between COPD and IPF lung.

## Conclusions

ECM is a dynamic, ordered aggregate of macromolecules that plays an active role in shaping fibroblast and other cell behavior. Qualities of the ECM, including composition, dimensionality, and stiffness, all impart critical cues to help orient cells to their position and the context in which they exist. In the lung, fibroblasts reside enmeshed in ECM within the interstitial space until they are required for wound repair. Changes to ECM quality likely promote fibroblast behavior in the early and late wound repair response. As mesenchymal progenitor cell therapy is a promising approach for patients with IPF and other chronic lung diseases, investigators should be aware that abnormal ECM may drive profibrotic, rather than reparative, cellular phenotypes. In disorders characterized by excessive ECM deposition, like IPF, evidence suggests that the surrounding matrix is more than just an endpoint; as a result, mediators of cell–ECM signaling or factors that influence ECM stiffness may represent viable therapeutic targets in many lung disorders. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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