



Extracellular matrix mechanics in lung parenchymal diseases

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ABSTRACT

In this review, we examine how the extracellular matrix (ECM) of the lung contributes to the overall mechanical properties of the parenchyma, and how these properties change in disease. The connective tissues of the lung are composed of cells and ECM, which includes a variety of biological macromolecules and water. The macromolecules that are most important in determining the mechanical properties of the ECM are collagen, elastin, and proteoglycans. We first discuss the various components of the ECM and how their architectural organization gives rise to the mechanical properties of the parenchyma. Next, we examine how mechanical forces can affect the physiological functioning of the lung parenchyma. Collagen plays an especially important role in determining the homeostasis and cellular responses to injury because it is the most important load-bearing component of the parenchyma. We then demonstrate how the concept of percolation can be used to link microscopic pathologic alterations in the parenchyma to clinically measurable lung function during the progression of emphysema and fibrosis. Finally, we speculate about the possibility of using targeted tissue engineering to optimize treatment of these two major lung diseases.

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1. Introduction

Biomechanics can be defined as the application of the principles of mechanics to biology and physiology. According to Fung (1993), “Biomechanics aims to explain the mechanics of life and living. From molecules to organisms, everything must obey the laws of mechanics.” Beyond its power to explain the mechanical behavior of the living, biomechanics is now recognized to be part of mechanobiology in that the mechanical properties of biological tissues, also called biomechanical properties, play fundamental roles in the normal functioning of virtually all connective tissue, organ and organism. Indeed, these biomechanical properties are critical determinants of how mechanical interactions of the body with the environment produce physical forces at the cellular level.

In the lung, mechanical forces can directly influence physiological function via cellular signaling (Wirtz and Dobbs, 2000) such as during lung development (Torday et al., 1998), surfactant release by alveolar epithelial cells (Wirtz and Dobbs, 1990), contraction of airway smooth muscle (Fredberg et al., 1997), and tissue remodeling (Lambert et al., 1992). It is now well recognized that mechanical interactions between cells and the extracellular matrix (ECM) have major regulatory effects on cellular physiology and cell-cycle kinetics which can lead to the reorganization and remodeling of the ECM

(Carver et al., 1991; Breen, 2000). This in turn influences the macroscopic biomechanical properties and hence the functioning of the lung.

Traditional biomechanics has focused on characterizing the macroscopic structural and mechanical properties of living tissues and organs by establishing mathematical relations, called the constitutive equations, that describe how mechanical stresses (force per unit area) change in response to a change in the size and/or shape of a body usually given in terms of strain (relative change in dimension). The constitutive equations are often nonlinear and can describe the static relationship between stress and strain. When the constitutive equations also include time-dependent or dynamic relations between stresses and strains, the tissue is referred to as viscoelastic. All living tissues display viscoelastic behavior (Fung, 1993), reflecting the finite time required for the constituent fibers, cells and fluids to adjust their local configurations in response to a change in macroscopic tissue strain. The precise link between the complex rheology of biological soft tissue and its microstructure is still poorly understood, although various theories have been proposed such as the notion that stress relaxation may occur through cascades of micro-ruptures within the tissue (Bates, 2007) or slow undulation of fibers (Suki et al., 1994). In any case, the constitutive equations are commonly determined from measured static or dynamic stress–strain relationships. These relations generally reflect behavior emergent from the mechanical properties of the individual constituents as well as their structural arrangement in the tissue (Fung, 1993).

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All connective tissues are composed of cells and ECM that includes water and a variety of biological macromolecules. The macromolecules that are most important in determining the mechanical properties of these tissues are collagen, elastin and proteoglycans. Among the macromolecules, the most abundant and perhaps most critical for structural integrity is collagen. One might expect therefore that the amount of collagen in a tissue is the primary determinant of its mechanical properties. However, different connective tissues with similar collagen content can exhibit different mechanical behavior matching the specific needs of the organ (Fung, 1993). During the last decade, the advent of novel imaging techniques (Cox et al., 2003) and quantitative computational modeling (Redaelli et al., 2003) have allowed the study of micromechanics of specific components of tissues and hence improved our understanding of the relationships between tissue composition, microstructure, and macrophysiology. In particular, it has become evident that macrophysiology reflects both the mechanical properties of the individual components of the tissues, as well as the complexity of its structure (Suki et al., 2005).

This review article focuses on the biomechanical and structural properties of the lung and how these properties change in disease. The first several sections will closely follow a previous review article (Suki et al., 2005). First, we will summarize the constituents of the connective tissue of the lung and their complex structural organization. Next, we will describe how the mechanical properties of the parenchyma arise from the constituents and structural organization of the alveolar walls. In places where there is a lack of experimental data on specific ECM properties of the lung, we will use results from other tissue or organs. We will then argue that collagen, being the most important load-bearing element of the parenchyma, is critical to determining the homeostasis and cellular responses to injury. The deformation of the alveolar wall network is heterogeneous and hence the distribution of mechanical forces on the collagen is also heterogeneous in the lung. This heterogeneity in the ECM properties likely plays a role in the regional distribution of cell signaling and hence on the early remodeling of the connective tissue in diseases. The question of how this heterogeneity grows during the progression of two key parenchymal diseases, emphysema and fibrosis, will be discussed in the context of the emergent properties of lung function (Bates et al., 2007). Finally, we will briefly speculate about how future tissue engineering approaches might take advantage of this emergent behavior of lung function to optimally treat these diseases (Suki et al., 2007).

2. Main constituents of the lung connective tissue

2.1. Properties of collagens

There are over 20 different types of collagen molecule. Most interstitial collagens (I, II, III, V, XI) are helical in structure, consisting of three polypeptide chains each of which is a left-handed coil of approximately 1000 amino acids, with the three chains forming a right-handed super helix (Brown and Timpl, 1995). These helical molecules are rod-like rigid structures with length and diameter of about 300 and 1.5 nm, respectively, and capable of spontaneous fibril forming (Silver et al., 2003). The helical subunits are first assembled in the endoplasmic reticulum of interstitial cells in precursor forms called procollagens which have amino and carboxyl terminal globular regions known as propeptides. These serve to solubilize the procollagen, and correctly align the individual peptide chains to facilitate helix formation (Silver et al., 2003). Following secretion, the propeptides are enzymatically cleaved, which allows the collagen molecules to associate both axially and laterally to start forming fibrils. Apparently, type I collagen is thermally unstable

at body temperature and folding of the least stable microdomains can trigger self-assembly of fibrils where the helices are protected from complete unfolding (Leikina et al., 2002). The lateral and axial growth of the fibrils appears to be, in part, determined through interactions with other matrix components such as proteoglycans (Hukins, 1984; Hulmes et al., 1995; Raspanti et al., 1997). The fibril structure itself also shows tremendous hierarchical complexity. For example, the lateral packing of molecules can exhibit significant fluid-like disorder (Hukins, 1984; Holmes et al., 2001). The collagen fibrils can further organize into thicker fibers through cross-linking of lysine and hydroxylysine residues present within the overlapping terminal helical and teleopeptide regions of the molecules (Silver et al., 2003). These fibrils and fibers may be arranged either randomly (e.g. in lung tissue or cartilage) or as quasi-deterministic networks (e.g. in tendon) within an organ.

The interstitium of the lung parenchyma contains mostly types I and III collagens which provide the structural framework for the alveolar wall. Fiber thickness ranges from several hundred nm to well over a micron (Sobin et al., 1988). The distribution of fiber thickness is skewed, and has a long “tail” (Sobin et al., 1988) similar to a power law (Suki, 2002), indicating broad variability of fiber structure. This blend of deterministic order (exact amino sequence and axial packing) and random disorder (from fluid-like lateral packing to random networks) may partly be responsible for the existence of a broad range of time constants that characterizes the viscoelastic properties of the connective tissue of the lung (Bates et al., 1994; Suki et al., 1994). These collagen fibers in the parenchyma are further organized to form an axial fiber network extending down from the central airways to the alveolar ducts, a peripheral fiber network extending centrally from the visceral pleura, and a parenchymal interstitium which connects the two (Weibel and Gil, 1977). Variations in the collagen content of the parenchyma during development (Tanaka and Ludwig, 1999; Tanaka et al., 2001), in fibrosis (Dolhnikoff et al., 1999) or following *in vitro* digestion (Yuan et al., 2000) have suggested an important role for these protein fibers in the biomechanical properties of the parenchyma.

2.2. Properties of elastic fibers

Elastic fibers are composed of elastin and microfibrils which are mostly fibrillin and fibullin (Reinhardt et al., 1996). The elasticity of microfibrils is controversial and their role in lung elasticity has not been studied. Values of the Young's modulus (increase in stress due to a small uniaxial stretching normalized by strain) of microfibrils have been reported to be as low as 0.2 MPa (Thurmond and Trotter, 1996), which is about 3–5 times lower than the stiffness of elastin (Sherebrin et al., 1983; Fung, 1993), and as high as 96 MPa (Sherratt et al., 2003), which is closer to that of collagen (Fung, 1993). The microfibrils often form a fibrous outer mantle surrounding the more amorphous elastin. The elastin is composed of insoluble flexible cross-linked polypeptides. While the 3D molecular structure of elastin fibers is not as well understood (Hukins, 1984; Kozel et al., 2003), elastin organizes itself into easily extensible fibers and has a linear stress–strain relation up to 200% strain (Fung, 1993). The distributions of the diameters and lengths of elastin fibers in the lung are skewed with long “tails,” and appear similar to the distribution of collagen fiber properties. Thus, the elastic fibers exhibit significant structural heterogeneity and are also known to be mechanically connected to the collagen (Brown et al., 1994) via microfibrils and/or proteoglycans (Hukins, 1984; Raspanti et al., 1997; Kielty et al., 2002). Traditionally, elastin is thought to dominate lung elasticity at normal breathing lung volumes (Setnikar, 1955), while collagen becomes progressively more important as volume approaches total lung capacity. However, comparing the effects of elastin and collagen digestion on the constitutive equa-

tion of parenchymal strips suggests that collagen and elastin may be equally important even at lower lung volumes (Yuan et al., 2000).

2.3. Properties of proteoglycans

Within the lung, collagen and elastin fibers of the connective tissues are embedded in a hydrated gel, also called “ground substance”. The composition of the matrix and the ratio of fiber to gel vary among tissues (Hukins, 1984) and change during maturation and with disease states (Juul et al., 1993). Critical constituents of this matrix are the glycosaminoglycans (GAG), a family of highly charged polysaccharides (Hukins, 1984). There are several different types of GAGs (e.g. hyaluronic acid, chondroitin sulphate, dermatan sulphate, keratan sulphate) whose molecular weights vary over 3 orders of magnitude implying that the polymer chains can contain as many as 10^4 units with a huge variability in size and structure (Buckwalter and Rosenberg, 1982; Scott, 1992). Within the lung parenchyma, the most abundant GAGs are heparin sulphate and chondroitin sulphate. Most GAGs are usually covalently linked to a core protein to form proteoglycans. Similar to collagen, GAGs can also have secondary and tertiary structures by forming helical and randomly organized regions depending on the ionic environment and pH of the matrix (Scott, 1992). Proteoglycans can also associate ionically with one another to form large aggregates that exhibit a higher level of hierarchical organization. Images of the proteoglycans obtained by electron microscopy reveal an extraordinarily complex structure (Buckwalter and Rosenberg, 1982; Fratzl and Daxer, 1993). The majority of the above studies on proteoglycan mechanics have been done in cartilage and it is likely that the role of proteoglycans in lung function has been underestimated. Indeed, only a few studies have examined their role in lung mechanics (Al Jamal et al., 2001; Cavalcante et al., 2005).

2.4. Interstitial cells

The lung parenchyma contains a variety cell types. From the point of view of mechanics, the most important are the contractile cells that include smooth muscle cells in the alveolar duct and vessel walls and myofibroblasts and fibroblasts in the alveolar walls (Weibel, 1986). Stimulation of these cells with different agonists induces local internal stresses in the fiber network which can lead to changes in the viscoelastic properties of the lung tissue (Fredberg et al., 1993; Navajas et al., 1995; Salerno et al., 1995; Yuan et al., 1997; Dolhnikoff et al., 1998). However, during contractile challenge the mechanical properties of tissue strips have also been found to vary with the number of medium size airways in the sample (Salerno et al., 1995). It is therefore possible that the previously observed changes in mechanics during agonist challenge were in fact partly related to smooth muscle contraction and airway–parenchymal interaction. Nevertheless, the viscoelastic properties of the lung parenchyma are only moderately affected by the active tone of the interstitial cells (Yuan et al., 1997; Dolhnikoff et al., 1998). A more important function of the interstitial cells is to actively remodel and repair the connective tissue during growth or following injury. As a result of such cellular processes, the nonlinear viscoelastic properties of the lung tissue can change significantly both at the organ and the alveolar wall level (Brewer et al., 2003). Thus, while cells contribute little to the biomechanical properties of the tissue in response to physical (e.g. deformation) or chemical (e.g. histamine challenge) stimuli over a short time period, they are responsible for the longer term maintenance and remodeling of the composition and structure of the matrix.

2.5. Interaction among the components

Conventional histological or immunohistochemical techniques do not truly reflect the spatial orientations of different tissue components *in vivo* due to artifacts resulting from fixation. Recent atomic force microscopic studies have demonstrated the existence of ridges and filaments along the surface of unfixed, untreated collagen fibrils from rat tail tendon (Sakai et al., 2001) which likely represent the core protein and the GAG side chains of proteoglycans, respectively. These data suggest that the organized network of proteoglycan aggregates have multiple interactions, including chemical and topological, with collagen fibril surfaces as well as with each other. Collagen may reinforce the proteoglycan–water gel and provide a safety limit against excessive stretching of the matrix. This results in a composite structure with significant load-bearing capacity over a wide range of external forces to which the lung is exposed. An additional level of structural complexity comes from the fact that the fibers of the lung parenchyma within the alveolar walls are arranged in a hexagonal-like lattice (Stamenovic, 1990; Kononov et al., 2001). By combining fluorescent imaging of the alveolar walls with computational modeling following external deformation, it appears that interconnectedness of the alveolar wall network also contributes to parenchymal viscoelasticity (Brewer et al., 2003).

3. Mechanical properties of the normal lung

3.1. Molecular, fibril and fiber elasticity

The structure of the lung is largely determined by the connective tissue network. The complex organization and the nonlinear mechanical properties of these tissue components lead to complex mechanical behavior. The Young's modulus of the type I collagen molecule has been estimated to be between 3 and 9 GPa (Sasaki and Odajima, 1996b; Silver et al., 2003). The elasticity of a single collagen molecule has been attributed to the existence of amino acid sequences along the triple helix that lack proline and hydroxyproline (Silver et al., 2002). These regions are more flexible than other regions of the helix. Such variation of rigid and flexible regions likely has a significant effect on the fibril-forming ability and hence the elasticity of the fibrils. Additionally, the unfolding of thermally activated molecular kinks or “crimps” along the molecule may also contribute to molecular elasticity (Misof et al., 1997). The stress–strain curve of fibrils appears reasonably linear up to 3–5% strain, with a modulus in the order of 0.5–5 MPa (Silver et al., 2003). It is notable that the stiffness of elastin is at least 2 orders of magnitude smaller than that of collagen (Fung, 1993). This is likely a result of the amorphous nature of elastin compared to the near crystalline organization of collagen fibers. Crimps also exist at the fibril and fiber levels (Silver et al., 2003).

When thicker fibers in the tissue are stretched, it is the crimps along the fibers that first unfold followed by an unfolding of the crimps in the fibrils (Misof et al., 1997). Further stretching of the fibers results in stretching of the triple helices and the cross-links which also raises the possibility of slipping of molecules and fibrils within a fiber (Fratzl et al., 1998) (Fig. 1).

In addition to the elasticity of a single molecule, collagen fiber stiffness may depend on the number of fibrils through a given cross section, i.e. the diameter as well as the type of cross-linking between molecules and fibrils. Increasing both diameter and cross-linking tend to increase fiber stiffness in normal collagen (Silver et al., 2003; Angele et al., 2004). Furthermore, fibril length as well as small proteoglycan bridges between fibrils can contribute to the stiffness of collagen fibers (Redaelli et al., 2003). The stress–strain curve of tendon composed of many fibrils arranged in paral-

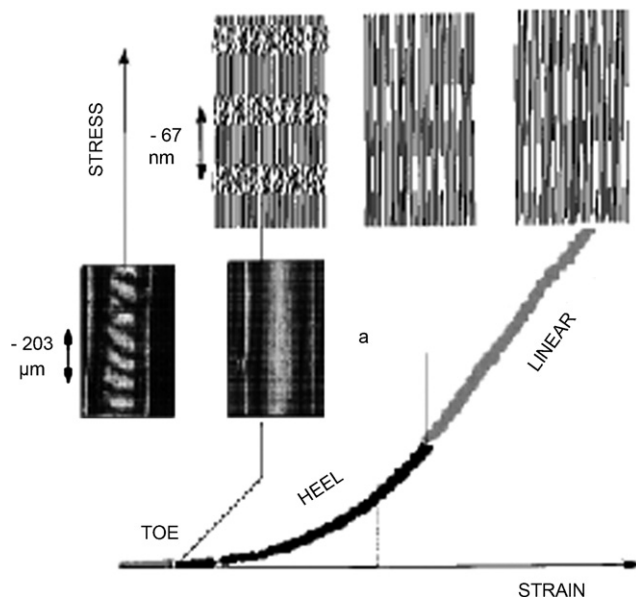


Fig. 1. Stress–strain curve of collagen in tendon. Large crimps and unfolding result in little stress in the “toe” region. Nonlinearity characterized by the “heel” region originates from the crimps (a) unfolding with stretching (with permission from [Fratzl et al. \(1998\)](#)).

lel is nonlinear with a toe and a steep region ([Stromberg and Wiederhielm, 1969](#); [Sasaki and Odajima, 1996a](#)). The toe region is usually attributed to the crimps along the fibrils which, upon stretching, straighten out ([Giraud-Guille and Besseau, 1998](#)) (Fig. 1). The composition of the fibrils and fibers is also important because fibers can contain a mixture of different collagen types. It has been argued that type I collagen is stiffer than type III ([Silver et al., 2002](#)) implying that fiber stiffness can depend on the relative amounts of type I and type III within the fiber. Furthermore, there are notable species related differences. A small amount (5–10%) of variation in amino acid composition between bovine and equine collagen can lead to a 2–3 fold difference in elastic modulus of cross-linked collagen gels *in vitro* ([Angele et al., 2004](#)). All these factors can give rise to significant inter- and intra-species variability in the mechanical properties of the alveolar walls. However, as both mechanical and biochemical factors contribute to collagen production and assembly, it is likely that there are significant regional variations in collagen fiber properties within a single lung. This variation is in addition to the different fiber contents and mechanical properties of alveolar ducts and alveolar walls ([Mercer and Crapo, 1990](#); [Denny and Schroter, 1997](#)). The former has been argued to be stiffer and hence contribute more to lung elasticity at higher lung volumes ([Mercer et al., 1987](#)).

3.2. Elasticity of lung collagen, alveolar wall, tissue strip and whole lung

As shown in Fig. 1, the stress–strain curve of individual fibers may become linear once the crimps are unfolded. However, for macroscopic samples of lung tissue and many other tissues, the stress–strain curve often exhibits exponential stiffening ([Fukaya et al., 1968](#); [Mijailovich et al., 1994](#); [Navajas et al., 1995](#); [Mishima et al., 1999](#); [Yuan et al., 2000](#)). For example, during uniaxial stretching, stress (σ) is related to strain (ϵ) in the material by the equation

$$\sigma = A\epsilon \exp(b\epsilon) \quad (1)$$

where A is the amplitude of the stress–strain curve and can be related to the incremental elastic modulus near zero strain (as can be seen from a Taylor expansion of Eq. (1)) and b is a nonlinearity

parameter which is thought to reflect the progressive recruitment of collagen fibers. That is, at low strain most of the stress in the tissue is borne by fibers of elastin that are relatively compliant, but as strain increases the initially flaccid collagen fibers start to straighten and take up the load-bearing role. As progressively more of the stiff collagen fibers get recruited in this way, the bulk stiffness of the tissue increases commensurately. Macroscopic tissue stiffness thus arises largely because of the way in which the various constituent fibers are organized with respect to each other, rather than being a reflection of the constitutive properties of any particular fiber type. [Maksym and Bates \(1997\)](#) showed this can be modeled analytically in terms of a linear chain of pairs of parallel springs and strings. If the springs (representing extensible elastin fibers) are all identical then the overall stress–strain behavior of the model is determined by the length distribution of the strings (representing inextensible collagen fibers). Alternatively, the model can be constructed using strings of equal length and springs with distributed stiffnesses. [Maksym et al. \(1998\)](#) extended this model into two dimensions using a triangular network of line elements each containing a parallel combination of a crimped collagen fiber and an elastin fiber. They found that the exponential nonlinearity in Eq. (1) could be explained by a power law distribution of collagen fiber properties, which is compatible with morphometric assessment of collagen in the lung ([Sobin et al., 1988](#); [Mercer and Crapo, 1990](#)).

Including realistic geometry in a model of parenchymal tissue further improves our understanding of the mechanism of alveolar wall deformation. Recently, simulations using a hexagonal network to mimic the observed deformation of the alveoli in normal, hypotonic and hypertonic solutions suggest that the folding of the alveolar wall and collagen during uniaxial stretching is elastically limited by the proteoglycan matrix ([Cavalcante et al., 2005](#)). The reason is that the proteoglycans are highly sensitive to the local osmolality whereas collagen and elastin are much less sensitive. Under hypertonic conditions the negatively charged proteoglycans collapse (low stiffness), whereas under hypotonic conditions they become inflated (high stiffness) and thus hinder the folding of collagen fibers into the direction of the macroscopic strain, contributing to the elastic behavior of the tissue.

A simple hexagonal model, however, is unable to mimic the exponentially increasing stress–strain curve of lung tissue strip (e.g. Eq. (1)) because the angles of the hexagons collapse upon stretching, causing the model to become unstable and exhibit a much lower elasticity than is observed experimentally. Thus, a structurally reasonable model needs to include both line element elasticity and a mechanism that hinders the collapse of angles. This can be achieved by using angular springs between neighboring line elements to represent resistance to bond-bending. Such a model is shown in Fig. 2 for two configurations corresponding to two values of the angular spring constant, while the line element stiffness was kept constant. Notice that the minimum energy configuration of the structure is very different when the angular spring constant is low (left panel) compared to when it is high (right panel). This means that (1) the macroscopic Poisson ratio (the negative of the lateral strain normalized by the axial strain) is significantly influenced by mechanical interactions between collagen and proteoglycans, and (2) the deformation pattern of the alveolar wall network does not follow the macroscopic deformation, i.e. it is not affine. However, by incorporating this mechanical interaction between collagen and proteoglycans into the hexagonal network model and comparing its stress–strain curve to measured data, the average Young's modulus of a single alveolar wall is estimated to be about 5 kPa. Furthermore, by taking into account the volume fraction of collagen fibers in the alveolar walls, a lower limit of collagen fiber stiffness in the alveolar wall is estimated to be about 300 kPa for 30% uniaxial macroscopic strain ([Cavalcante et al., 2005](#)).

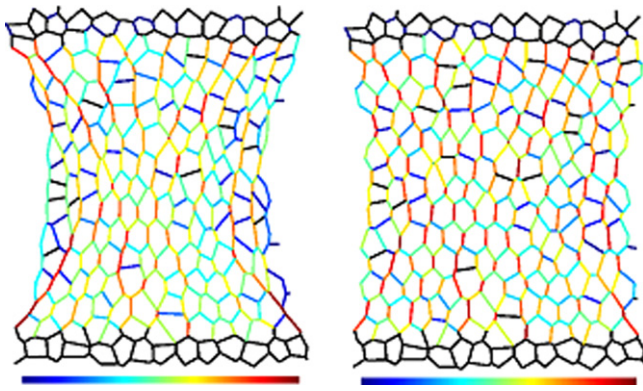


Fig. 2. Two-dimensional elastic network model of the lung parenchyma. Line elements are nonlinear springs and their folding is resisted by angular springs. When the angular spring (bond-bending) constant is low (left), the uniaxial stretching in the vertical direction results in large changes in angle. When the angular spring constant is high (right), macroscopic stretching lengthens the line elements rather than folding them, which results in higher forces and hence more red color. The microscopic deformation is not affine, it does not follow the macroscopic deformation leading to significant heterogeneity both in structure and force distribution (color) which also depend on the bond-bending to line element stiffness ratio. The black bonds at the top and bottom boundaries are rigid (based on Cavalcante et al. (2005)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The elasticity of the whole lung is encapsulated in its quasi-static pressure–volume curve, the shape and position of which has been attributed variously to surface tension, alveolar duct properties, and constitutive equations for the lung fibers in an average alveolar duct unit (Wilson and Bachofen, 1982; Stamenovic, 1990; Denny and Schroter, 1995). At the same time, it is clear that distributed fiber properties are also crucial in determining overall lung mechanical behavior (Maksym and Bates, 1997; Maksym et al., 1998), as the exponential relation between transpulmonary pressure and lung volume in Eq. (1) is related to the gradual recruitment of collagen fibers. Thus, it remains difficult to directly relate the bulk modulus of the lungs to the physico-chemical properties of collagen unless the spatial distributions of collagen and elastin (Mercer and Crapo, 1990) as well as alveolar duct and septal geometries (Weibel, 1986) together with their distributed natures (Cavalcante et al., 2005) are appropriately taken into account.

In summary, while the phenomenological quasi-static stress–strain curve of lung tissue can be accurately accounted for by various models, the relationship between the molecular organization of collagen and elastin fibers and the *in vivo* pressure–volume curve is not fully understood. Such a mechanistic link would obviously be valuable to better understand the pathophysiology observed in emphysema and fibrosis, two diseases in which the pressure–volume curve and hence the mechanical properties of the lung change in a manner that implies alteration in collagen and elastin elasticity and organization.

4. Biomechanics of the lung parenchyma in diseases

4.1. Structure and properties of elastin and collagen in lung tissue disease

Using scanning electron microscopy, the three-dimensional microstructure of normal lung parenchyma has recently been visualized in extraordinary detail and shows complex intermingled networks of elastin and collagen (Toshima et al., 2004). In diseases of the parenchyma these structures become remodeled (Lucey et al., 1998; Vlahovic et al., 1999). While the detailed cellular machinery behind this remodeling is not well understood, it has significant

consequences for the structural and mechanical properties of the parenchyma. In mouse models of emphysema, for example, these elastin and collagen fiber networks show tortuous and substantially damaged structures (O'Donnell et al., 1999). Cultures of cells exposed to elastase exhibit damaged elastic fibers with a frayed, porous appearance and a granular texture (Morris et al., 1998). Following 7 days of repair, the granular texture of damaged fibers disappears and the porous areas become filled in. By 22 days following elastase exposure, the elastic fibers are indistinguishable from fibers in control cultures. Nevertheless, this repair is likely to be aberrant because elastase treatment has been shown to lead to progressive airspace enlargement in rat lungs despite normal levels of elastin (Mercer and Crapo, 1992).

A recent study characterized the force–extension curves of collagen fibrils and whole tendons from rats that had been pretreated with β -APN, which inhibits the formation of cross-links among fibrils (Puxkandl et al., 2002). In the absence of cross-links, the stress required to stretch the tendon decreased dramatically from 120 MPa in the normal to 15 MPa in the tendon from the treated rats. Furthermore, the stress quickly reached a plateau and in certain samples actually started to decrease beyond a critical stretch, signifying the breakdown of the tendon. It has also been shown that the collagen in the alveolar walls, and indeed the walls themselves, mechanically fail at a lower stress in emphysematous lungs than in normal lungs (Kononov et al., 2001; Suki et al., 2003; Ito et al., 2005, 2006). Since the failure stress of the alveolar wall is determined by the strength of collagen, this could indicate abnormal cross-linking of the collagen in emphysematous lungs as a consequence of inflammation and remodeling.

During fibrosis, there is significant collagen deposition in the lung that is accompanied by stiffening of the lung tissue (Dolhnikoff et al., 1999; Ebihara et al., 2000; Faffe et al., 2001). Interestingly, while both the elastic and collagen fiber contents increase, changes in the overall viscoelastic properties of the lung tissue appear to correlate with the contents of collagen (Dolhnikoff et al., 1999) or proteoglycans (Ebihara et al., 2000), suggesting that it is reorganization of the fibers that is most responsible for the altered mechanical properties of the tissue. However, little is known about the organization and stiffness of individual collagen fibrils and fibers in fibrosis, making this an important area for future research. In cardiovascular tissues, the fibrotic increase in stiffness is attributed to the deposition of extra collagen and also possibly to increased collagen cross-linking associated with the formation of advanced glycosylation end-products. If the maturation of cross-linking is faster than the turnover of collagen itself then fibrotic lesions develop and lead to an increased local stiffness, a process similar to aging (Avery and Bailey, 2005). It is unclear, however, how the locally formed fibrotic lesions influence organ level function. Indeed, the few published studies in the lung are somewhat contradictory in their accounts of the correlation between mechanical properties and collagen content (Goldstein et al., 1979; Ebihara et al., 2000). Below, we show that, in fact, collagen content alone cannot account for the organ level functional properties of the lung, regardless of how chronic mechanical forces may have influenced cellular metabolism and tissue remodeling. Of key importance is how the remodeled tissue is organized spatially.

5. Effects of mechanical forces on the lung parenchyma

5.1. Mechanical forces, cell signaling and biomechanical properties of the ECM

The lung tissue is constantly under a pre-existing tensile stress, or prestress, which is a result of the distension of the lung by

the transpulmonary pressure. The regional distribution of prestress is determined by the hydrostatic pressure in the pleural space and the shape of the lung (West, 1971; Hubmayr, 2002). Prestress also changes cyclically and irregularly with breathing. The prestress in the alveolar walls is transferred through the ECM to the adhering cells with important consequences for cellular biophysics, biochemistry and phenotype (Giancotti and Ruoslahti, 1999). Such mechanical interactions between cells and the matrix are known to modulate cell contractility and myosin light chain phosphorylation (Polte et al., 2004), cell rheology (Rosenblatt et al., 2004), and focal adhesion assembly (Chen et al., 2003), all of which are critical for control of cell adhesion, migration, growth, contractility and viability. Additionally, the mechanical properties of the ECM may influence angiogenesis (Ingber, 2002), as well as connective tissue homeostasis itself (Chiquet et al., 2003). The direct interaction between the ECM and cellular biochemistry also has important implications for the biomechanical properties of the connective tissues.

It has been suggested by Ingber (1991) that cells in the interstitium sense mechanical forces via the integrin adhesion receptors that connect the cytoskeleton to the ECM. In addition to collagen, integrin receptors can also anchor to other ECM molecules such as fibronectin or laminin. However, since collagen is the main load-bearing component in connective tissue, any prestress in the lung would likely be transferred from the collagen to the other ECM molecules. Although the exact molecular mechanisms by which this mechanotransduction occurs are not entirely clear (Shyy and Chien, 2002), it has been found that modulation of stresses on the cell surface leads to a dynamic remodeling of focal adhesions (Davies et al., 2001). Thus, integrins serve as a mechanotransduction device and can activate various cellular processes (Giancotti and Ruoslahti, 1999) when the forces along the collagen change. For example, integrins have been shown to down-regulate collagen α -1(I) and up-regulate interstitial collagenase when fibroblasts were grown on a collagen matrix without prestress (Lambert et al., 1992). However, mechanical forces can also induce direct secretion of various growth factors that accelerate the remodeling of the matrix. For example, tensile force mediated up-regulation of the α -1(I) procollagen gene was found to depend on the release of transforming growth factor beta (TGF- β) (Gutierrez and Perr, 1999). Tensile force also appears to regulate the connective tissue growth factor that is able to stimulate extracellular protein release through a TGF- β independent pathway (Schild and Trueb, 2002). Additionally, fibroblasts appear to respond differentially to various types of mechanical stimuli (e.g. uniaxial versus biaxial deformation) (Berry et al., 2003).

It is clear that the presence of specific types of mechanical forces and various signaling cues in the ECM jointly regulate how the cells create their microenvironment to maintain an optimal structure and biomechanical properties of an organ (Huskes et al., 2000). Thus, cells and the prestressed ECM live in a dynamic balance in which about 10% of total lung collagen is newly synthesized each day, 40% of which is immediately degraded, resulting in continuous remodeling of the matrix (McAnulty and Laurent, 1987). A schematic diagram of the hierarchical transmission of mechanical stimuli from the level of the whole lung down to single cells, and the various possible feedback loops controlling ECM remodeling and ultimately organ level mechanics, are shown in Fig. 3. We note, however, that several other important mechanisms related to the effects of mechanical forces on lung biology, such as surfactant secretion (Wirtz and Dobbs, 1990) and smooth muscle contractility (Fredberg et al., 1997; Polte et al., 2004) are not considered here.

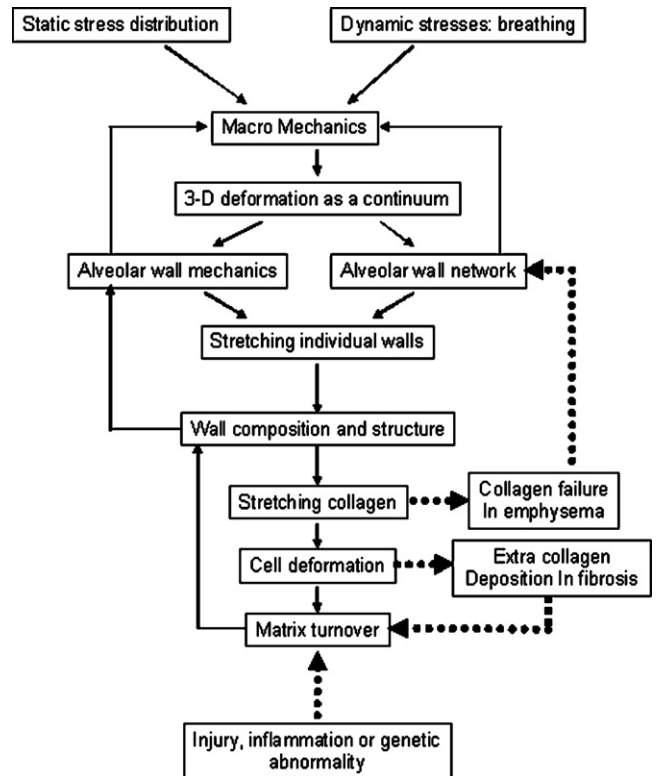


Fig. 3. Schematic diagram of force transmission from the level of the whole lung to single cell with various feedback mechanisms influencing ECM composition and lung mechanics. Dotted lines show external or internal influences as well as various possible feedback loops in disease states.

5.2. Mechanical forces in the diseased lung

In disease, the biochemical cues within the alveolar walls change either because of the expression of enzymes and/or cytokines, or secondary to injury caused by external agents such as cigarette smoke. This can drastically change the turnover half-time of matrix molecules. For example, increased collagen mRNA expression in the lung was detected within 6 h of elastase treatment of hamsters (Lucey et al., 1998). In this case, matrix remodeling was directly initiated by the acute injury caused by elastase. It is conventional to think that diseases such as emphysema or fibrosis develop as a result of the changes in the biochemical microenvironment. However, as soon as the composition of the ECM is altered, either because of direct injury or cellular remodeling, corresponding changes occur in the biomechanical properties of the matrix and consequently of the alveolar wall. Such changes affect the local deformation of the alveolar wall, causing the wall network to reorganize itself in order to re-establish mechanical equilibrium. Thus, it is conceivable that disease can change local prestress on the alveolar wall which in turn can have a positive feedback effect on cell signaling, leading to further assembly of matrix molecules. In this way, the initiation of a disease process has the potential to lead to chronic and progressive tissue remodeling.

While virtually nothing is known about the remodeling effects of mechanical forces in the fibrotic lung, the possibility that mechanical forces contribute to the progression of emphysema has been put forth as early as 1971 by West (West, 1971) who showed that the topological distribution of emphysema scores closely resemble the regional distribution of mechanical stresses in the lung due to its own weight. Consequently, he argued that mechanical forces should contribute to tissue pathophysiology. In a two-dimensional

finite element model of the alveolar structure, Gefen et al. (1999) demonstrated that alterations in the elastic properties of the alveolar wall in emphysema can lead to a stress concentration up to 6 times higher than in the normal lung tissue. Such high stresses might lead to rupture of the remodeled fibers. This idea has been explored recently using imaging of the alveolar walls coupled with simultaneous measurement of the mechanical properties of tissue strips during uniaxial stretching (Kononov et al., 2001). It was found that the alveolar walls from elastase-treated rats can break under the influence of mechanical forces akin to those likely occur *in vivo*. The notion that mechanical force might contribute to tissue destruction is also consistent with computed tomography images of the lung (Mishima et al., 1999).

The breakdown of the alveolar wall network driven by mechanical forces has been shown to lead to significant heterogeneities in structure at the parenchymal tissue level, with collagen expected to play a major role in this process due to its being the most important load-bearing constituent of the alveolar wall (Suki et al., 2003). At high lung volumes the collagen in the alveolar wall normally protects the parenchyma from rupture, so rupture of alveolar walls in the emphysematous lung at strains corresponding to normal breathing suggests that the yield stress of the collagen must be weaker following the remodeling process (Kononov et al., 2001). This notion is supported by a recent study reporting that tissue strips from elastase-treated mice failed at ~50% less stress than control even though the treated animals had a 50% increase in total collagen content of the lung (Ito et al., 2005). It is important to mention, however, that the mean linear intercept, a measure of alveolar airspace size, was also increased, indicating that the number of alveolar walls available to support the stress per unit cross sectional area perpendicular to the direction of stretching was reduced. One may thus argue that the reduction in failure stress was simply due to the larger stress per alveolar wall in the emphysematous tissue. On the other hand, it has also been argued that the increase in mean linear intercept cannot fully explain the decrease in failure stress, especially when the collagen content per alveolar wall is increased (Ito et al., 2005). Therefore, despite the increased collagen content of alveolar walls in the emphysematous lung, wall stiffness is still smaller than in healthy animals.

These findings suggest that the normal dynamic equilibrium between matrix turnover and mechanical forces is disturbed in emphysema, which would lead to the additional positive feedback loops in Fig. 3 shown by dotted lines. For example, direct injury to the alveoli might lead to an alteration in matrix turnover and reduction in collagen failure strength that ultimately results in the breakdown of alveolar walls, which then propagates the stress further to the remaining walls so that they themselves can break. Such positive feedback loops can cause a continuous deterioration of lung function and may explain the progressive nature of emphysema (Suki et al., 2003). One might also speculate that positive feedback loops would persist in fibrosis if local increases in stiffness stimulate fibroblasts to secrete even more collagen. In any case, the finding in emphysema that increased collagen content is accompanied by decreased stiffness is in stark contrast to the mechanics of the fibrotic lung in which total collagen content also increases but is accompanied by an increase in stiffness (Dolhnikoff et al., 1999). The conclusion is thus inescapable that, following the remodeling process, the internal structural organization of the collagen fibers in the alveolar wall is drastically different in emphysematous and fibrotic lungs. If, as is widely suspected, both emphysema and fibrosis are triggered by an aberrant cellular repair response to an initial insult, the grossly different mechanical and structural fates of the parenchyma in these two diseases could be due to a bifurcation at some point in the process of tissue repair. To further complicate matters, however, there is evidence to suggest that emphysema and

fibrosis can co-exist, so it may be that different repair processes can proceed concurrently within the same lung (Lundblad et al., 2005).

6. Linking microscopic disease progression to lung function: the concept of percolation

Following remodeling in emphysema, alveolar walls become weakened (Lucey et al., 1998; Vlahovic et al., 1999) and are eventually ruptured by the cyclic mechanical stresses imposed during breathing (Kononov et al., 2001). Successive mechanical failures result in a lung that becomes progressively more compliant with time (Suki et al., 2003). In contrast, the fibrotic lung becomes progressively stiffer due to the deposition of collagen in the tissues (Ebihara et al., 2000), which reduces lung volume and increases the work of breathing (Hart et al., 2002). Nevertheless, the derangements in lung mechanics at the organ level accompanying both emphysema (Foronjy et al., 2005) and fibrosis (Goldstein et al., 1979) are not simply determined by the amount of tissue loss or remodeling. As we discussed above, lung parenchymal stiffness is, to a significant extent, determined by the complex interconnections of the protein, fibrils and fibers of the tissue. In other words, alterations in lung elasticity are emergent phenomena that arise from the way in which the lesions of disease are arranged spatially.

6.1. Network models of the fibrotic and emphysematous lung

Computer models have proven essential for investigating the link between microscopic alterations and macroscopic function, both in emphysema (Suki et al., 2003; Ito et al., 2006; Bates et al., 2007) and fibrosis (Bates et al., 2007). To illustrate this point, we briefly present here a model published recently by Bates et al. (2007). The lung parenchyma is modeled as a two-dimensional hexagonal network of ideal linearly elastic springs with a rectangular boundary. The individual springs represent alveolar walls as in Fig. 2. The outer borders of the network are held fixed such that the springs are prestressed to correspond to a given lung volume. The equilibrium configuration of the network is calculated by minimizing the elastic energy of the network using simulated annealing (Kirkpatrick et al., 1983) optimized for elastic networks (Cavalcante et al., 2005). As a measure of the functional properties of the model, the two-dimensional bulk modulus (B) of the network is calculated by increasing its linear dimensions biaxially by 0.001%, recalculating the new equilibrium configurations of all the springs, and then normalizing the increase in stress to the increase in linear strain. Thus, B is a measure of the overall stiffness of the network, and is the model equivalent of the inverse of lung compliance. Further details can be found in Bates et al. (2007).

6.2. Linking microscopic alterations to lung function via percolation

To simulate the collagen deposition that occurs in fibrosis, springs are randomly stiffened by a factor of 100. The modulus B for our network model of fibrosis versus the fraction c of randomly stiffened springs changes relatively slowly when c is below about 0.6 and much more quickly when c is above 0.6 (Fig. 4). The knee in the relationship at about $c = 0.6$ indicates a threshold, called the percolation threshold, at which at least one contiguous pathway of stiff springs spans the entire network. The percolation threshold separates widely differing rates of change of mechanical function. Fig. 4 also shows a diagonal straight line corresponding to the constant rate of change in B occurring when the stiffness of each spring in the network increases progressively and simultaneously. Also shown in Fig. 4 are example network configurations at baseline (uniform spring stiffness) and when the model has two different fractions of stiff springs. When c is about 0.5, the stiff springs clump together

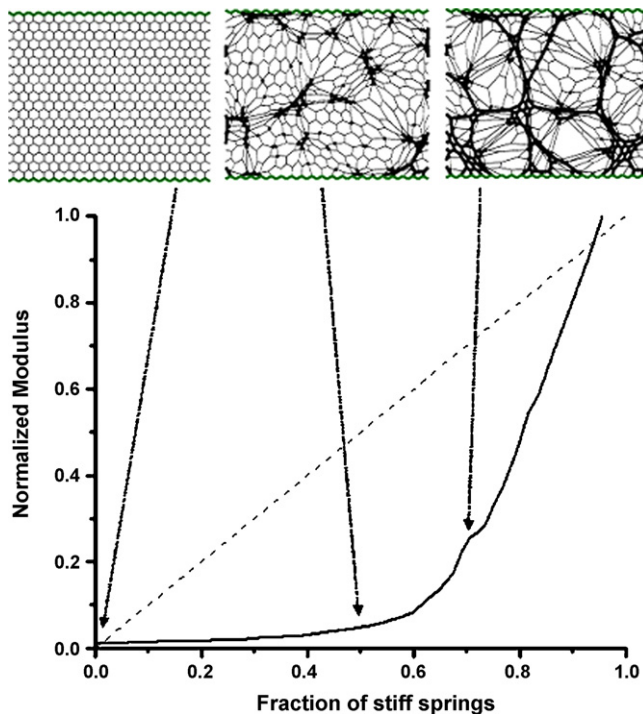


Fig. 4. Simulation of the progression of pulmonary fibrosis. The solid line shows the bulk modulus B of the elastic network as a function of the fraction of springs c randomly stiffened by a factor of 100. If all of the spring constants were uniformly stiffened in a gradual manner from the baseline value of 1 to 100, the modulus would follow the dashed diagonal line. Shown at the top are the network configurations obtained when $c=0$, $c=0.5$ and $c=0.67$ with thick line showing stiff springs (with permission from Bates et al. (2007)).

in groups that are still separated by soft springs. At $c=0.65$ the stiff springs percolate, i.e. they span the network both vertically and horizontally in unbroken chains. Note how the network begins in a homogeneous configuration at $c=0$, and becomes increasingly heterogeneous reaching maximum heterogeneity at about $c=0.65$.

To mimic emphysema, rather than cutting the springs randomly, the notion that alveolar walls experiencing high stress are more likely to break than walls under less stress is invoked (Kononov et al., 2001). The simulation starts by randomly breaking 1% of the springs in the network in order to seed a random destructive process in the beginning. The new equilibrium configuration of the network is determined and then the next 7% of the springs that are under the greatest stress are eliminated. This process is repeated until B falls to zero. This causes the holes in the network to be spatially correlated and makes B fall toward zero much more rapidly than if the cutting had been completely random. Fig. 5 shows the progression of B with the fraction c of cut springs and demonstrates an initially accelerating rate of decline that eventually decelerates as B approaches zero when about 15% of the spring have been cut, i.e. $c=0.15$. The point at which $B=0$ represents another percolation threshold, this time corresponding to the complete loss of network elasticity. The three examples of the network shown in Fig. 5 demonstrate how an initial set of randomly located small holes progresses to a series of large holes that span the network in an almost contiguous pathway. The heterogeneity of the structure also increases as the network undergoes progressive breakdown.

The network structures in Figs. 4 and 5 imply that the geographical nature of lung pathology should exert an important influence on its physiological manifestations. Nevertheless, merely ascribing mechanical dysfunction of the parenchyma to the vagaries of lesional organization is unhelpful without an understanding of how

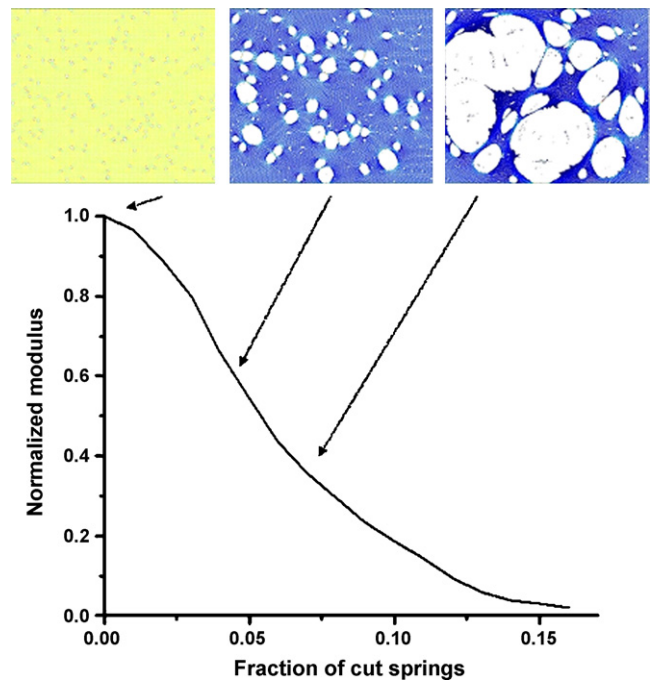


Fig. 5. Simulation of the progression of emphysema. The curve shows the bulk modulus B of the elastic network (normalized to the modulus of the network when fully intact) as a function of the fraction of springs c cut on the basis of the amount of force they carry. Shown at the top are the network configurations obtained at three points along this process. The forces in the individual springs are indicated by colors with yellow indicating high stress and decreasing stress corresponding to progressively darker shades of blue (with permission from Bates et al. (2007)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the two are linked. Our above results show that such a link can be forged through the concept of percolation (Bates et al., 2007), which has been invoked previously to account for numerous natural processes involving transmission of events across networks (Stauffer and Aharony, 1992). These simulations suggest that the tissue undergoes a sharp change in its macroscopic mechanical properties at the percolation threshold, which may correspond to the point in the disease course when patients experience a sudden downturn in clinical symptoms. Percolation may thus explain why there is often a disconnect between the microscopic progression of parenchymal pathology and the development of symptomatic decrements in lung function.

These simulations clearly suggest that due to the network-like arrangement of the alveolar walls and the inherent microscopic heterogeneity of mechanical properties and mechanical forces, organ level function in the lung should be an emergent property and hence strongly influenced by the nature of heterogeneity. We further speculate that the progression of emphysema, fibrosis and likely most other diseases should necessarily lead to an increasing spatial heterogeneity of tissue remodeling and biomechanical properties of the ECM because, as we argued above, local changes in composition alter biomechanical properties and the distribution of mechanical forces which in turn can act as a positive feedback mechanism to produce additional remodeling. Such a mechanical–biochemical feedback process might be at the core of the early pathogenesis of interstitial lung diseases.

6.3. Implications for tissue engineering

Currently, there is no way to reverse either emphysema (Barnes and Stockley, 2005) or pulmonary fibrosis (Raghu and Chang, 2004),

so lung transplantation remains the only intervention that could be considered curative. Partial relief from the symptoms of emphysema can be achieved in some patients by Lung Volume Reduction Surgery (LVRS) (Fessler and Permutt, 1998), where selected regions of the lung are surgically removed. This allows the remaining portions of the lung to inflate within the thoracic cavity, restoring lung elastic recoil to some degree and reducing dyspnea (Gelb et al., 1999). Unfortunately, the benefits of LVRS are temporary and seem to disappear at a rate inversely related to the degree of initial improvement (Brenner et al., 1998). No such stop-gap measure currently exists for fibrosis.

Progress in tissue engineering and stem cell biology gives hope that cures may eventually be developed for emphysema and fibrosis, perhaps similar to the technique now being used to replace blood vessels with tissue engineered vascular grafts (Shin'oka et al., 2005). However, the selection of location in the treatment of blood vessels is not objective, but rather is based on the judgment of the physician. In the case of the lung, the efficacy of treatments would clearly be maximized by targeted delivery of drugs, cells, or engineered tissues to those sites that result in the greatest reversal of symptoms. The question thus arises as to where treatment should be directed, and in what quantity, to achieve reasonable improvements in lung function. Unfortunately, methods do not currently exist that can identify regions in the lung where treatment should be directed even if such treatment technology was available. Recently, however, we have addressed this issues using computer modeling and have demonstrated that the reverse process of percolation, which we called depercolation, has the potential to allow us to identify and evaluate the efficacy of various treatment schemes (Suki et al., 2007).

Briefly, suppose that reversing the percolation process we described above corresponds to the repair or regeneration of damaged tissue. In the fibrotic case, this would be equivalent to replacing stiff springs with normal ones, while for emphysema it would mean the addition of missing springs. There are a large number of different sequences in which the individual springs could be repaired or replaced, not all of which will lead to the same time-course of symptomatic relief. Indeed, some sequences will lead to more rapid relief than others. For example, we might expect a much larger reversal of abnormal lung function in fibrosis if a treatment breaks up a percolating pathway of contiguous lesions into two or more isolated clusters, than if the treatment merely ablates an isolated lesion. For simplicity, we will assume that the repair is ideal in the sense that it returns a local region of tissue to normality without adverse biological side effects. In the case of fibrosis, this means that local parenchymal stiffness can be returned from pathologically high to normal, while in the case of emphysema it means that we can either regenerate alveolar walls in situ or insert engineered parenchymal tissue into the lung at desired locations. Using the inverse processes shown in Figs. 4 and 5, we compared the functional consequences of these in silico therapies in terms of their effects on B during both random and targeted repair (i.e. softening springs in fibrosis or adding springs in emphysema). Although the technology is not yet available to achieve this in practice, our modeling results (Suki et al., 2007) conclusively indicated that substantial recovery of function can be achieved even with therapies that produce minimal repair if the tissue therapies are optimally targeted.

7. Conclusions

The connective tissue of the lung is not a static structure even during normal functioning. The tissue is the end result of a molecular hierarchical organization living in a dynamic balance between

continuous breakdown and remodeling that is also modulated by mechanical forces. When this delicate dynamic balance is perturbed by external or internal chemical changes, such as those occurring in a disease process or environmental stimuli, the system dynamically remodels itself through an excess or lack of the processes that either produce or break up these large complex macromolecular structures. The result is a chemically and structurally different tissue with accordingly altered biomechanical properties. We have also argued that changes in matrix stiffness will alter the mechanical forces on cells which in turn may also influence the way cells remodel the interstitium. An important consequence of this is that a complete understanding of tissue biomechanics and lung function will not result solely from biochemical purification and biophysical study of the molecules in isolation. Connective tissues must be studied as an integrated system within their natural biochemical and mechanical environments. Much further research is needed to better understand the relation among local mechanical forces, enzyme activity and cellular remodeling. Additionally, we have shown that heterogeneity plays an important role in lung function, and have demonstrated that the concept of percolation can be used to link the progression of microscopic pathogenesis to clinically relevant functional indexes. Thus, in order to fully appreciate how diseases propagate spatially in the lung and how they progress with time, it will be essential to link the regional composition of the ECM and the local biomechanical properties of the tissue to the geometric organization of these properties.

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