Tissue mechanics and fibrosis

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1. Introduction

Mechanical forces are essential to the development, progression, and (potentially) regression of tissue fibrosis. Although often ignored in studies and models of fibrosis, particularly in the era of genomics and proteomics, mechanical signals are similar to chemical signals in their range of effects and are likely to be equally important. The mechanical forces that act in fibrosis are highly varied, and may mediate individual cell phenotypes as well as global architectural changes. Understanding the role of mechanics in fibrosis is key to understanding the basic pathophysiological mechanisms of fibrotic diseases as well as developing new therapies.

2. Forces

There are multiple forces at work in tissues. These include tension and compressive forces (forces which pull or push perpendicular to the surface of an object) and shear forces (which are parallel to the surface) (Fig. 1A). These forces exert stress on objects, defined as force (in Newtons (N)) normalized to the area over which it acts and expressed in units of pascals (1 Pa = 1 pN/μm²). Forces in tissues result from cell-generated tension, fluid flow, stretch, and hydrostatic/osmotic pressure, which are resisted to variable extents by tissue stiffness. These forces collectively regulate the phenotype and proliferation of myofibroblasts and other cells in damaged tissues, the activation of growth factors, and the structure and mechanics of the matrix — all of which are central to fibrosis.

Mechanical forces are essential to the development and progression of fibrosis, and are likely to be as important as soluble factors. These forces regulate the phenotype and proliferation of myofibroblasts and other cells in damaged tissues, the activation of growth factors, the structure and mechanics of the matrix, and, potentially, tissue patterning. Better understanding of the variety and magnitude of forces, the characteristics of those forces in biological tissues, and their impact on fibrosis in multiple tissues is needed and may lead to identification of important new therapeutic targets. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

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3. Tissue stiffness and stiffness sensing

The best-studied force in tissue fibrosis is tension generated in response to tissue stiffness. Tissue stiffness is measured as the elastic modulus, defined as the resistance to deformation, and is expressed as the magnitude of a stress (compression, elongation, or shear force, normalized to area) divided by the strain (deformation) induced by the stress (Fig. 1B). Young’s elastic modulus (E) describes the resistance to a compressive or elongating force, while the shear elastic modulus (G) describes the resistance to a shear force. E and G are both expressed in units of Pa; for a perfectly elastic material that conserves volume (one that returns to its original shape when the stress is removed), E is three times G. Tissues, however, are not perfectly elastic but are viscoelastic, meaning that, like liquids, they have a viscosity, and that the strain in response to a stress changes with time [3,4]. Although the role of the elastic modulus in regulating cell behavior is the subject of increasing study, the role of the viscous component of tissues is poorly understood [5]. Tissues are also structurally heterogeneous and resist deformation to different extents depending on the direction in which a force is applied. Additionally, neither the elastic nor the viscous stress of most biological tissues varies linearly with strain; although this can be important in maintaining the mechanical characteristics and integrity of a given tissue, it is difficult to model and study [6].

Tissue stiffness is sensed when cells adhere to matrix proteins and apply tension, meeting resistance that reflects the stiffness of the tissue. The cellular actin–myosin cytoskeleton exerts forces on extracellular matrix proteins via integrin attachments located within focal adhesions; stiffer tissues result in increased resistance to the pulling force exerted by cells, contributing to strengthening that force [7,8]. Whether the mechanical force originating at the cell boundary is transmitted directly to the nucleus or to nuclear proteins (Yap/Taz, for example) [9], and whether signaling cascades are activated (potentially via focal adhesion kinase (FAK) or other focal adhesion proteins) as a result of tension at the site of the focal adhesion, are issues that have stimulated extensive investigation [10].

Normal tissues vary in their stiffness when measured over the same strains and time scales. The brain is very soft, with an elastic modulus around 100 Pa; the liver, while also soft, is slightly stiffer at 400–600 Pa, and the muscle and bone are stiffer still (10⁴ and 10⁵ Pa, respectively) [1]. It is clear from clinical practice that tissue stiffness changes in disease states. In the same way that we can easily tell by touch that a steel bar is stiffer than gelatin, palpation as part of the routine physical exam enables detection of differences in skin or liver stiffness and suggests that fibrotic tissues are stiffer than normal tissues. Multiple studies have shown that fibrotic lungs become stiffer in fibrosis, with elastic modulus values ranging from approximately 2 kPa for normal tissue to approximately 17 kPa for fibrotic tissue [11–13]. We have found normal livers ex vivo to have a shear modulus less than 1 kPa, while fibrotic livers range from 3 kPa to 22 kPa [14].

Transient elastography, which measures the elastic modulus, is widely used in clinical practice outside of the U.S. to assess the liver stiffness in patients with liver disease; although values vary from one study to the next, elastic moduli (measured at time scales that are shorter than those generally used for ex vivo studies) are typically less than 5 kPa for normal livers and greater than 12 kPa for cirrhotic livers [15].

Organs with established fibrosis are thought to be stiffer as a result of their increased quantity of extracellular matrix, in particular fibrillar collagens. It appears, however, that increased matrix alone is unlikely to account for increases in tissue stiffness in fibrosis and that stiffness and matrix quantity are not linearly related. Our studies suggest that increases in collagen and elastin cross-linking account for some of the increase in elastic modulus in liver fibrosis and that the mechanical properties of the injured liver change significantly early after injury, before significant matrix deposition has occurred [14,16]. This crosslinking appears to be initiated by lysyl oxidase family crosslinking enzymes; the changes in stiffness are consistent with the effects of lysyl oxidases in isolated collagen cushions, the vasculature, and different cancers (see below) [17–21]. The contribution of altered cell (as opposed to matrix) stiffness to tissue mechanics in fibrosis has not been established but may be considerable [12].

4. Other forces acting on tissues

Forces other than tension generated in response to tissue stiffness may also contribute to the development and progression of fibrosis. Tissues are subject to shear stress caused by fluid flow through the vasculature, ducts, and interstitium. Of these, vascular flow and the effects of shear stress on the vascular endothelium are best understood, in particular in the context of cardiovascular disease and remodeling (a form of injury and fibrosis, although not strictly speaking tissue fibrosis). Alterations in vessel geometry, flow rate, and fluid viscosity contribute to changes in shear stress, regulating the release by endothelial cells of growth factors, vasodilators like nitric oxide, and other soluble factors, and leading to long-term changes in gene and protein expression [22]. Mechanotransduction results from cell surface deformation (affecting ion channel function, cell surface receptors, the glyocalyx, the primary cilia, and the physical properties of the membrane) as well as the transmission of signals from the cell surface to distant regions of the cell, affecting cell-substratum and cell–cell interactions which in turn lead to changes in chemical signals [22,23]. Tissues such as the kidney, liver, and lung have significant amounts of flow through specialized vessels including the glomerulus in the kidney, sinusoids in the liver, and pulmonary vessels in the lung. Altered flow through these vessels may be both the cause and the result of tissue remodeling and fibrosis, and may also result in pathologic angiogenesis [24].

*Fig. 1. Forces affecting tissues. A) Forces acting on tissues. B) The elastic modulus of a material is the slope of the stress (force per unit area) plotted against the strain (deformation). The diagram demonstrates linear elasticity, where the stress/strain relationship is constant. In reality, most biological materials demonstrate non-linear elasticity, such that the elastic modulus changes as strain increases.*
Fluid flow through ducts such as the bile duct, pancreatic duct, and renal tubules represents another source of shear stress in tissues that may be relevant to fibrosis [25]. The primary cilia appear to be particularly important for mechanotransduction in these settings [26] with genetic disorders of the primary cilia (including polycystic kidney disease and nephronophthisis) leading to epithelial pathology and fibrosis in the liver, kidney, and pancreas. Experimental data suggest that altered shear stress in the renal tubules increases inflammation [27], while altered shear stress in the glomerulus alters the actin cytoskeleton [28], suggesting that the effects of shear stress in tissue fibrosis may be complex.

Interstitial fluid flow, the extremely slow flow between capillaries and lymphatics (0.1–2 μm/s, compared to 10–20 cm/s for flow in blood vessels), is another important source of shear stress in tissue fibrosis. Interstitial flow varies with inflammation and edema, the amount and quality of the matrix (especially collagens and glycosaminoglycans), the composition of the fluid, and the size of lymphatics [29]; altered flow results in changes in growth factor release (including TGF-β), collagen alignment, and myofibroblast differentiation [30].

Other forces potentially operative in fibrosis include hydrostatic pressure, osmotic pressure, and stretch. These forces may be related to flow (pulsatile flow, for example, causes stretch; elevated hydrostatic pressure results from obstructed flow; and interstitial flow is driven by gradients in hydrostatic and osmotic pressure). Obstruction of the bile ducts, pancreatic duct, and ureters leads to fibrosis, likely due at least in part to changes in shear stress (stasis) and hydrostatic pressure [31]. The development of cardiac cirrhosis in response to high central venous pressures suggests that elevations in hydrostatic pressure are highly relevant to fibrosis in vivo. Stretch is particularly important in the lung, which is subject to cyclical stretch during respiration [32]. The pathways responsible for transducing these different forces are still under investigation, although there is evidence that cation channels in the transient receptor potential (TRP) family, the actin-interacting protein zyxin, and G protein-coupled receptors are activated in response to stretch [33,34] while ion channel activation and alterations in cytoskeletal stability are part of the response to hydrostatic pressure [35].

5. Integration of soluble and mechanical signals

Mechanical and soluble signals are often interdependent. Mechanical forces can act both directly and indirectly on soluble factors. TGF-β, which is arguably the most important soluble factor in fibrosis, undergoes activation as the direct result of mechanical tension (Fig. 2). TGF-β is secreted as part of a latent complex and stored in the ECM; one component of this latent complex, the latency-associated peptide (LAP), binds directly to certain integrins, linking it to cells. Hinz and co-workers, in a series of elegant experiments, demonstrated that cells exert tension on the LAP through this integrin attachment. If the matrix to which the cells and TGF-β complex are attached is soft, it deforms in response to tension and the complex remains intact. If the matrix is stiff, however, resistance to cell-generated tension results in deformation of the LAP and release of active TGF-β [36,37]. For myofibroblasts, this increased TGF-β results in increased α-smooth muscle actin (α-SMA), which interacts with cellular myosin to contract and produce increased tension — effectively a feed-forward loop incorporating both soluble and mechanical signals [37]. Thus, the stiffness of injured and fibrotic tissues may perpetuate fibrosis via mechanically-regulated increases in the amount of active TGF-β present. TGF-β is a common factor downstream of many mechanical forces: in addition to tension, other forces including interstitial fluid flow and stretch have been implicated in TGF-β activation and release [30,38]. Similar mechanisms have not yet been identified for other growth factors but many are also stored in the matrix and may be activated or released in response to cell-generated tension and matrix deformation [39].

In addition to acting directly on growth factors, mechanical signals can be converted to biochemical signals that intersect with or are part of soluble factor signaling pathways [40–42]. In lung fibroblasts, increased stiffness causes inhibition of prostaglandin E(2), which promotes fibrosis [11]. Stiffness also enhances the response to exogenous TGF-β [43]. NF-κB, which is downstream of many critical soluble factor pathways in fibrosis [44,45], is also downstream of some mechanical forces. Wnt/β-catenin [46,47], interleukins, and (as discussed above) G protein-coupled receptors and ion channels are common to both mechanical and soluble signaling pathways [41,42]. Integrins and their downstream effector FAK are part of the cellular mechanotransduction apparatus but they also transduce signals from soluble factors. In some cases, integrins interact directly with soluble factors (for example, vascular endothelial growth factor (VEGF)) and growth factor receptors [48,49]. Specific interactions between mechanical and soluble factors in fibrosis need to be defined.

6. The effects of mechanical forces on myofibroblasts

Myofibroblasts are the major fibrogenic cells in all forms of tissue fibrosis. These cells, which are derived from precursor cells including pericytes and fibroblasts, express α-SMA de novo after injury, develop stress fibers, and generate contractile force, exerting tension on the surrounding matrix. The importance of mechanical forces to myofibroblast activation and matrix deposition was demonstrated first for skin and mucosal wounds [50–52], and has since been shown for myofibroblasts in multiple tissues including the heart [53], lung [11,54,55], liver [56–58], and kidney [59,60], although different amounts of force may be required in different tissues and different contexts. In the case of matrix stiffness, increased stiffness results in increased α-SMA expression and matrix deposition, potentially as part of a positive feedback loop. Whether the reverse occurs is not clear: some in vitro studies, including studies with fibroblasts from patients with idiopathic pulmonary fibrosis, show that substrate softening results in the reversion of myofibroblasts to non-fibrogenic cells [61,62], while other work suggests that myofibroblasts have a “mechanical memory” and retain their phenotype even after changes in the stiffness of their surroundings [54]. Most studies of the role of stiffness in myofibroblast differentiation have been carried out using artificial two-dimensional substrates; neither the effects of using three-dimensional systems nor the effects of viscoelastic or non-ideal elastic substrates are well characterized. A recent intriguing study of mesenchymal stem cells in culture showed that, when the elastic modulus was held constant, changes in substrate viscosity had a significant impact on cell behavior, including morphology, proliferation, and α-SMA expression [5]. Given that tissues are viscoelastic, it will be important to carry out similar studies exploring the effects of viscosity on myofibroblasts and their precursors.

Tension in response to matrix stiffness is not the only force that causes precursor cells to become myofibroblastic and fibrogenic. Hydrostatic pressure, which can occur within a tumor or edematous tissue, for example, enhances myofibroblast differentiation. Relevant to fibrosis, increased hydrostatic pressure resulted in the myofibroblastic activation of pancreatic and hepatic stellate cells in vitro [63,64].

Stretch also regulates myofibroblast behavior [51]. Mouse skin, which was stretched, showed increased numbers of myofibroblasts [51]. In lung fibroblasts, stretch induced production of hyaluronic acid, which led to activation of the innate immune response [65]. A new method enabling the simultaneous study of stiffness and stretch suggested that stretch could overcome the effects of softness and that stiffness and stretch sensing employ similar mechanotransduction pathways to similar effect [66]. Interestingly, however, cyclic stretch, which is typical of the normal lung and which is decreased in fibrosis, inhibited the differentiation of lung myofibroblasts [67].

Finally, fluid flow, including vascular flow and interstitial fluid flow, can also regulate the myofibroblast phenotype [23,30,68]. The time course over which flow-related shear forces act on myofibroblast
forces. Chief among these are cells of the vasculature. Angiogenesis progression of in tissue sample the mechanics of their environment and may be sensitive to in vitro large-scale architectural remodeling associated with renal disease. I n changes in podosomes, which regulate adhesion and migration, liver sinusoidal endothelial cells, which are key cells in the development of liver fibrosis, demonstrate stiffness-dependent changes in podosomes, which regulate adhesion and migration, and alterations in the stiffness of glomerular podocytes are associated with renal disease. In in vitro models, vascular endothelial cell contractility and permeability increased with increasing matrix stiffness, enhancing leukocyte extravasation; this could be important to fibrosis-associated inflammation (although it was not studied directly). Mechanical forces regulate other cell types as well. Macrophages, which mediate fibrosis in multiple organs, demonstrate phenotypic, transcriptional, and functional changes in response to altered matrix stiffness. Stem cells, which are an important part of the response to injury, are also increasingly recognized as being mechanosensitive.

7. The effects of mechanical forces on non-fibrogenic cells in fibrosis

Although myofibroblasts are the primary matrix-depositing cells in tissue fibrosis, other cells also participate in the development and progression of fibrosis and are similarly responsive to mechanical forces. Chief among these are cells of the vasculature. Angiogenesis and fibrosis often progress in parallel, and may positivity regulate each other. Two- and three-dimensional angiogenesis assays show that stiffness regulates the dynamics of tube formation and more specifically regulates transcriptional pathways that control angiogenesis. Liver sinusoidal endothelial cells, which are key cells in the development of liver fibrosis, demonstrate stiffness-dependent changes in podosomes, which regulate adhesion and migration, and alterations in the stiffness of glomerular podocytes are associated with renal disease. In in vitro models, vascular endothelial cell contractility and permeability increased with increasing matrix stiffness, enhancing leukocyte extravasation; this could be important to fibrosis-associated inflammation (although it was not studied directly).

Mechanical forces regulate other cell types as well. Macrophages, which mediate fibrosis in multiple organs, demonstrate phenotypic, transcriptional, and functional changes in response to altered matrix stiffness. Stem cells, which are an important part of the response to injury, are also increasingly recognized as being mechanosensitive.

8. The effects of forces on architectural remodeling

Most work on mechanics in fibrosis has focused on the effects of forces on single cells. Not yet studied in detail is the role of mechanics in the large-scale architectural remodeling associated with fibrosis. Pioneering in vitro work by Harris and Grinnell provides potential mechanical explanations for large-scale architectural arrangements in tissues and may be applicable to fibrosis — for example, to explain the development of bridging fibrosis in the liver or of the similarly complex reticular pattern of fibrosis in idiopathic pulmonary fibrosis. Harris and Grinnell found that embedding stiff, fibroblast-containing implants in soft collagen gel resulted in realignment of collagen fibrils along the axes connecting implants, with shortening of the axes and migration of cells across the newly aligned collagen fibril bridges. Mechanistically, the investigators suggested that the stiffness of the implants led to enhanced contractility of embedded fibroblasts, and that this cellular contractility enabled alignment of intervening collagen fibrils, with cell-generated tension leading to shortening of the implant-to-implant distance. More recently, Janney's group has shown that the non-linear elastic properties of certain matrix proteins (including fibrin) enable cells to influence neighboring cells hundreds of microns away. Collectively, these observations suggest that extracellular matrix (ECM) remodeling and reciprocal interactions between cells and the remodeled ECM, even over long ranges, may be central to the progression of fibrotic disease.

Key features of these models are that myofibroblasts are contractile in stiff environments and that tissue stiffness is heterogeneous. This has been shown experimentally for the lung and liver. Myofibroblasts from both tissues demonstrate increased contractility on stiffer substrates in vitro and unpublished work, and both tissues are mechanically heterogeneous in the normal and fibrotic states. Atomic force microscopy measurements of bleomycin-treated lung tissue demonstrated marked overall increases in stiffness after injury, focal areas of significantly higher stiffness, and increased heterogeneity; while microindentation methods demonstrated similar increases in mechanical heterogeneity in carbon tetrachloride-treated livers. A challenge of future research will be to incorporate these observations into regional and tissue-scale models of fibrosis and to similarly incorporate other forces (including those from fluid flow and hydrostatic pressure) into the models.

In one approach to using mechanics to model tissue behavior in fibrosis, Bates and Suki proposed that the concept of “percolation” — transmission of events across networks — might be important in understanding lung fibrosis. They highlight the concept of a “percolation threshold,” which occurs when isolated fibrotic lesions connect to form a contiguous septum, resulting in a sudden increase in macroscopic stiffness (and in symptoms). Although this work was based on computational models and was highly simplified, examination of human tissues provided support for the model, which, akin to the work of Harris and Grinnell, emphasizes the importance of geographical variation and heterogeneity in fibrosis progression. The authors also proposed that limited but targeted antifibrotic therapy might be an effective treatment for tissue fibrosis, a so-called “reverse percolation” effect.

9. Matrix proteins and tissue mechanics

The matrix and mechanics are inextricably intertwined in injured and fibrotic tissues. The matrix determines the mechanical tension and stretch sensed by cells, regulates tissue resistance to hydrostatic pressure, and mediates interstitial fluid flow. Specific matrix molecules, in particular the load-bearing matrix proteins, have specific roles in the mechanical environment. The collagens (with non-linear stress-strain...
properties) provide strength, the elastins (with linear stress–strain properties) resilience, and the proteoglycans, resistance to compression and shear [6,89]. Importantly, however, we do not yet understand what specifically causes altered mechanics (especially altered stiffness) in diseased tissue — whether cells, specific matrix proteins, or specific protein modifications are responsible.

Increased deposition of the fibrillar elastogens (especially collagens I and III) is typical of fibrosis, and these collagens add stiffness to tissues. Given their rigid, rod-like shape, they can also undergo alignment (to form parallel arrays) when subject to various forces; this may be important to cell migration, angiogenesis, and the exposure of cells to flow, and may permit long-distance transmission of forces [2,30,90]. Data from the cancer literature suggest that aligned collagen fibrils serve as “tracks” for cell metatases [91], and it is possible that in fibrosis these fibrils have a similar role in facilitating migration of myofibroblasts or vascular cells.

As noted above, collagen cross-linking enzymes appear to play a critical role in fibrosis, likely through their effects on mechanics. In vitro analyses demonstrate that collagen cross-linked through the actions of lysyl oxidase is stiffer than non-cross-linked collagen [17]. Increased liver stiffness early after injury is associated with increases in lysyl oxidase-mediated collagen cross-linking and with tissue mechanical properties typical of a cross-linked matrix; inhibiting lysyl oxidase activity blunts changes in stiffness, reduces myofibroblast differentiation, and partially prevents fibrosis [14,16,92,93]. Tissue transglutaminases also cross-link collagens. Although some investigators suggest that tissue transglutaminases are responsible for the protease-resistant cross-links of liver cirrhosis [94], others have found no role for these cross-linking enzymes in the progression of advanced liver fibrosis [95]. Thus, the role of transglutaminase-mediated cross-links in tissue mechanics and fibrosis remains to be determined. [Lysyl oxidases in fibrosis are discussed in detail in the previous chapter.]

The other major structural proteins upregulated in fibrosis, the elastins, contribute resilience, as opposed to rigidity, to tissues [96,97]. Like the collagens, elastins undergo cross-linking initiated by lysyl oxidases, although the mechanical ramifications of this are not known. The relevance of elastin cross-linking is likely to be particularly important in lung fibrosis.

Proteoglycans, which make up most of the “ground substance” between cells, also influence the mechanical properties of normal and fibrotic tissues. The glycosaminoglycan chains attached to the core proteins of proteoglycans are characterized by closely-packed negative charges, enabling them to generate electrostatic repulsive forces and to increase their hydration. In the lung (and likely other tissues as well), the resulting resistance to compression contributes to tissue mechanics by stabilizing the network of collagen and elastin fibrils [98]. The small leucine-rich proteoglycans, including lumican, fibromodulin, and decorin, regulate the assembly and alignment of collagen fibrils [99,100] and may alter collagen fibril mechanics [99,101,102]. Expression of the small leucine-rich proteoglycan biglycan was significantly correlated with changes in lung mechanics, including resistance and compliance, in a bleomycin model of lung fibrosis, with mechanical changes identified before collagen deposition [103]. Liver fibrosis in response to injury was reduced in both lumican and fibromodulin null mice, although the mechanics of the knockout livers were not examined [104,105]. Larger proteoglycans such as versican, as well as the glycosaminoglycan hyaluronic acid, resist compressive forces and regulate interstitial flow. Additionally, the glycolalyx, of which proteoglycans are a part, may mediate mechanotransduction [23].

The fibronectins are a final major component of the fibrotic matrix. Fibronectins are among the first matrix proteins upregulated after injury and are highly mechanosensitive. Fibronectin is remarkably tenacious, with a highly non-linear stress–strain curve, and is able to tolerate very high strains without breaking [106]. On stiff substrates, it becomes very rigid, with the expression of cryptic epitopes [107]; rigidity may therefore lead to changes in integrin binding and signaling.

Cell-generated tension, which increases in fibrosis, is critical to fibroblast unfolding and self assembly [108]. Additionally, cellular fibronectin splice variants, which have insertions, are dramatically upregulated in fibrosis [109]. Although these have not been studied in detail, the insertion of these extra domains may alter the mechanical properties of the fibronectins and thereby the mechanical milieu of a given tissue.

10. Summary

Mechanical forces are increasingly appreciated to play a role in fibrosis on a par with soluble factors. Matrix stiffness is so far the best appreciated mechanical stimulus in fibrosis, and the liver and lung are the tissues best studied. Even for these tissues and stimuli, our understanding of forces, their effects, and mechanotransduction in fibrosis is rudimentary. Future work will need to expand our understanding of the variety and magnitude of forces, the characteristics of those forces in biological tissues, and their impact on fibrosis in multiple tissues. Ultimately, the mechanical features of fibrosis may prove to be attractive targets for antifibrotic therapies.

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References