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Matrix mechanics and regulation of the fibroblast phenotype

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Fibroblasts are pivotal cells for ensuring normal connective tissue turnover and homeostasis and the repair of tissues after injury or insult. Deregulation of these activities leads to of fibrocontractive diseases. The term 'fibroblast' generally encompasses stromal cells that do not express markers specific for any other mesenchymal cell lineage, such as smooth muscle cells, pericytes and multipotent progenitor cells. This rather loose definition and the lack of molecular-defined fibroblast markers often leads to the misconception that fibroblasts are all equal. However, in skin dermis, for instance, different layers host different fibroblast populations (167, 203, 224). Another good example of fibroblast heterogeneity is found in the periodontium, where gingival and periodontal ligament fibroblasts are the most prominent fibroblast populations (138, 152, 162, 176). Heterogeneity exists also within the populations of gingival fibroblasts (20, 244) and periodontal ligament fibroblasts (193, 211). Both populations have been reported to contain a fraction of cells with stem-cell characteristics and self-renewal capacity (60, 106, 243). These multipotent cells may comprise the fibroblast progenitor cells reported previously, but are not necessarily identical (137).

Periodontal ligament fibroblasts are oriented along collagen fibers throughout the whole ligament and are responsible for the unusually high collagen turnover in the periodontal ligament compared with other connective tissues (153, 201). They preserve collagen homeostasis in the periodontium according to mechanical challenge. Cells perceive mechanical signals by forming intimate contacts with collagen fibrils and intracellular stress fibers (153). The formation of *in-vivo* stress fibers is limited to a very small number of fibroblast populations, such as alveolar septa fibroblasts in the lung (121), heart valve fibroblasts (207) and pericryptal fibroblasts in the gut (1), that play active roles in mechanotransduction or are exposed to an active mechanical environment. Formation of stress fibers is part of a cell-protection response to mechanically loaded tissue, but the development of contractile features is also pivotal for fibroblasts to mechanosense through cell–extracellular matrix linkages; this function will be discussed later in the text. Gingival fibroblasts preserve the architecture of the connective tissue underlying the gingival epithelium and attaching to the bone of the jaw. Dysregulation of gingival fibroblast activities in secreting and remodeling collagen is associated with gingival overgrowth, including that observed in hereditary gingival fibromatosis (34, 198, 212). Consistent with these functional differences, periodontal ligament and gingival fibroblasts exhibit different biologic reactions in response to environmental factors, including infection (195) and mechanical challenge (175) to give only two examples.

Mechanical challenges for fibroblasts

Stress forms character

In addition to distinct embryological origins (61) and inherent molecular differences (64), periodontal fibroblast populations of different phenotypes and with different functions are generated by their chemical and mechanical environments or, to use a more popular term, 'niches'. Mechanical challenges are among the most dominant factors determining cell character (101, 206). Gingival fibroblasts live in a rather loose collagen extracellular matrix that is surrounded by cementum, epithelium and bone, whereas the collagen arrangement around periodontal ligament fibroblasts is more organized and denser and is confined by bone and cementum (162). Fibroblasts in the normal periodontium are exposed to a variety of mechanical stimuli. They are strained and

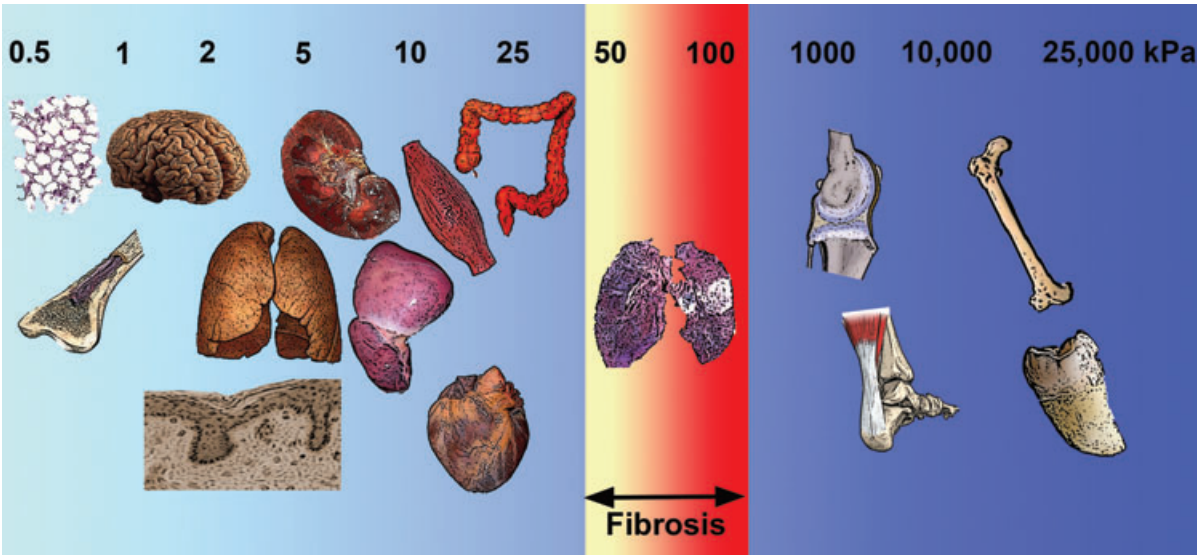

Fig. 1. The stiffness of our body. The stiffness range of different organs and tissues, determined by atomic force microscopy, is measured using Young's elastic modulus and presented in kPa. Note that fibrotic tissue (here stylized for a fibrotic lung) is always stiffer than normal tissue. References supporting this scheme are given in the text.

compressed during mastication and tooth movements (85, 133). These mechanical signals are important in adopting the ligament's collagen structure to the levels of applied stress (74, 84, 153). Gingival fibroblasts are frequently exposed to small injuries and are responsible for regenerating the connective tissue structure (e.g. in the wound-healing response around implants) (80, 175). Mechanical signals from the extracellular matrix are crucial for controlling the onset and termination of the repair process. The effects of various mechanical stimuli on fibroblasts in different organs, including the periodontium, has been discussed in excellent reviews (12, 26, 28, 45, 101, 108, 113, 114, 170, 221, 225). In the present article I will concentrate on the influence of tissue stiffening on fibroblast function and phenotype.

How stiff is our body?

Tissue stiffness is measured using Young's elastic modulus with the dimension Pascal (Pa). Over the last decade, the elastic modulus of a great number of tissues and organs has been assessed at the cellular perception level using atomic force microscopy indentation (24, 115) (Fig. 1). Very soft organs, not surprisingly, are bone marrow (230), brain (0.1–0.5 kPa) (55) and fat (1–3 kPa) (23). Soft organs are liver (1–2 kPa) (70) and lung parenchyma (2–4 kPa) (144), whereas muscular tissues are of medium stiffness (10–15 kPa) (16, 49, 50). Bone and teeth, again not surprisingly, are the stiffest structures in our body (100). The mechanical properties of periodontal structures, including the periodontal ligament and gingiva, have been measured and delivered elastic moduli spanning a wide range from tens of kPa to GPa (54, 188, 239). This high variation is partly explained by the use of various different methods to quantify tissue elasticity and partly by the heterogeneity of periodontal tissues. Few studies that used the atomic force microscope to measure the stiffness of collagen structures in the periodontium delivered elastic moduli in the GPa range (100) and the elastic modulus of the periodontal ligament was 10–50 MPa (99).

The stiffness of the extracellular matrix influences cell behavior at different levels, including proliferation, differentiation, migration and gene expression (44, 45, 53, 91, 113, 115, 149). When cultured on substrates matching the stiffness of brain, muscle or pre-bone (osteoid), mesenchymal stromal cells neoexpress early markers of the corresponding cell lineages (51). Substrate stiffness similarly affects early differentiation events in cultured embryonic stem cells (53), epidermal stem cells (213) and muscle precursor cells (49, 63, 185). Cardiomyocytes differentiate into striated muscle cells on synthetic gel substrates with the same stiffness as cardiac muscle (112, 241). Endothelial cells adapt their phenotype to the stiffness of soft culture substrates (25), and neuronal cells exhibit preferential growth on brain-soft substrates that eliminate contaminating glial cells from the tissue preparation (55, 71). Fibroblastic cells, including gingival and periodontal ligament fibroblasts, generally become activated upon exposure to substrates that are stiffer than their physiological environment (91), as discussed in the next section.

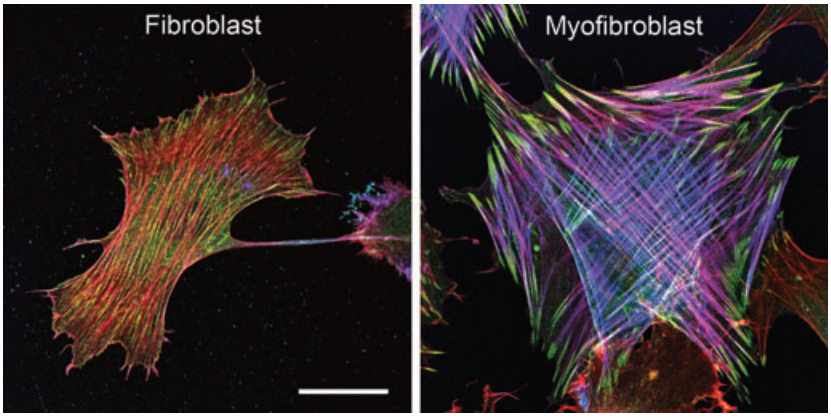

Fig. 2. Myofibroblast features *in vitro*. After 4 days of culture in the absence (fibroblasts) or the presence (myofibroblasts) of transforming growth factor beta-1, primary fibroblasts were immunostained. Compared with fibroblasts, myofibroblasts exhibit more prominent stress fibers (F-actin: red, purple in overlay) that are positive for alpha smooth muscle actin (blue, purple in overlay). Myofibroblast focal adhesions (vinculin: green) at the termini of stress fibers are larger than are fibroblast cell–extracellular matrix adhesions. Scale bar: 20 μm.

When tissue stiffness changes: injury, repair and regeneration

Fibroblasts are stress-shielded by the collagen architecture of intact connective tissues, which is most evident in tendon, ligaments and dermis (209). Consistently, culturing fibroblastic cells on substrates with the physiological stiffness of the normal organ often preserves a comparably quiescent cell phenotype. Mouse embryonic fibroblasts remain quiescent on normal organ-stiff polymer substrate culture (128). Human bone marrow-derived mesenchymal stromal cells grown on culture substrates with a stiffness similar to that of the soft bone marrow extracellular matrix remain quiescent but are viable in culture (230). Activated valvular fibroblasts can be silenced by dynamically changing substrate stiffness from high to low (223), and culture on soft substrates is employed to prevent hepatic stellate cell activation (168). Conversely, exposing fibroblastic cells to pathologically stiff culture conditions mechanically activates a 'myofibroblast program'. *In vivo*, loss of the protective tissue collagen structure as a result of injury directly exposes fibroblasts to mechanical stress and initiates a repair program aiming to restore the mechanical tissue integrity (209). Tissue stiffening occurs as a consequence of collagen remodeling during wound healing and/or pathological accumulation of collagen during fibrosis (98, 234). Similarly to other tissues, injury of the periodontium activates precursor cells to become reparative myofibroblasts (163). However, unlike most other adult tissues, but similarly to embryonic tissues, oral gingiva and oral mucosa scar only a little upon reasonable injury (196). This property, together with the progenitor character of the stromal cell subpopulations, renders oral gingiva, mucosa and periodontal ligament as attractive sources of regenerative cells (37, 60). Such cells, with regenerating and nonfibrotic properties, are being considered for restoring diseased arteries and large-area skin wounds (47, 52), where tissue contraction and stiffening caused by the action of myofibroblasts jeopardize the positive outcome of cell therapy (68).

Myofibroblasts – it takes a stressed cell to get a dirty job done

A myofibroblast warrant

Myofibroblasts were discovered 41 years ago, in wound granulation tissue, as fibroblastic cells that exhibit prominent endoplasmatic reticulum and contractile microfilament bundles (66). These ultrastructural features, indicating simultaneous high extracellular matrix secretion and contractile activity, coined their name, and the functional predictions deduced from their phenotype have been confirmed over the last four decades by hundreds of studies. Another hallmark feature, and the molecular basis for the high contractile activity of the myofibroblast, is the neo-expression of alpha smooth muscle actin in stress fibers (209) (Fig. 2). By strict definition, alpha smooth muscle actin-positive cells that do not form microfilament bundles are not myofibroblasts because they lack their defining contractile element (91). Conversely, alpha smooth muscle actin-negative fibroblastic cells that display microfilament bundles are functional contractile myofibroblasts, at least *in vivo*. As fibroblastic cells almost inevitably form microfilament bundles (stress fibers) in standard

cell-culture conditions, the term 'myofibroblast' is usually used to describe alpha smooth muscle actin-positive stress fiber-forming cells. The percentage of fibroblasts that will spontaneously become alpha smooth muscle actin-positive myofibroblasts in standard culture is variable and often characteristic for the species and tissue origin (91, 191).

Myofibroblast heterogeneity

Myofibroblast activation from a variety of different precursor cells is a key event in physiological and pathological tissue repair. Myofibroblasts are primarily extracellular matrix-secreting cells and are largely responsible for the contractility of scar tissue as it matures over time (98, 229, 234, 236). The contribution of myofibroblasts to normal tissue repair (89), and their specific implications in different fibrotic conditions, has been discussed for the lung (5, 81, 166, 233), liver (86, 109, 111), kidney (77, 154), skeletal muscle (197), systemic sclerosis (9, 18, 19), heart (132, 190, 218), the stroma reaction to tumors (38, 171) and fibrosis in the oral cavity (34, 198, 212).

Similarly to 'fibroblast', 'myofibroblast' comprises an equally heterogeneous collection of cells and describes a phenotype rather than a cell type. A number of recent reviews have considered the nature of myofibroblast progenitors in different organs (97), which include local fibroblasts (32, 38, 41, 46, 89, 179), fibrocytes (15, 87, 124), smooth muscle cells (33), pericytes (6, 105, 126, 143), endothelial and epithelial cells undergoing endothelial or epithelial-to-mesenchymal transition (21, 27, 136, 177, 184), mesenchymal stromal cells (90, 156) and hepatic stellate cells (41), to name only the most prominent. Both gingival fibroblasts and periodontal fibroblasts can be activated to become myofibroblasts during chronic inflammation, injury or fibrosis of the periodontium (153, 163).

Regulation of myofibroblast contraction

Because myofibroblasts share properties of fibroblasts (e.g. collagen secretion) and smooth muscle cells (e.g. alpha smooth muscle actin expression), the question has been raised regarding how myofibroblasts regulate the contractile activity that ultimately produces tissue contractures. Myofibroblasts, like smooth muscle cells and fibroblasts, develop contractile force upon phosphorylation of myosin light chain, which allows the myosin head to interact with actin filaments. This action is terminated by dephosphorylation of myosin light chain via the action of the myosin light chain phosphatase. In smooth muscle cells, increased levels of cytosolic Ca^{2+} regulate contraction by regulating the activity of myosin light chain kinase via Ca^{2+}/calmodulin. In fibroblastic cells, RhoA regulates contraction by regulating the myosin light chain phosphatase. Active RhoA activates the Rho-(associated) kinase, leading to inactivation of myosin light chain phosphatase and thus to continued contraction (122, 127). In a recent review, we reported that the current literature supports myofibroblast contraction regulation by both cytosolic Ca^{2+} and Rho/Rho-(associated) kinase (58) (Fig. 3).

Experiments with myofibroblast-populated wound granulation tissue strips suggest RhoA as the chief regulator of myofibroblast contractile activity. Inhibition of myosin light chain phosphatase using calyculin was shown to be sufficient to induce strip contraction (210), whereas increasing cytosolic Ca^{2+} by membrane depolarization and the addition of Ca^{2+} ionophore had little effect on myofibroblast contraction of tissue strips and collagen gels (210). Corresponding data were produced using three-dimensional myofibroblast-populated collagen gels (48, 165, 173, 237). Using two-dimensional deformable culture substrates that allow visualization of cell contractile forces, inhibition of Rho was shown to block myofibroblast contraction, whereas stimulation with lysophosphatic acid, an upstream effector of Rho, enhanced force development (57, 232). Conversely, other studies support that the level of cytosolic Ca^{2+} mainly regulates myofibroblast contraction. Administration of calmodulin inhibitors impaired closure of full-thickness rat skin wounds (140) by myofibroblast contraction. Stimulation of myofibroblast-populated tissue strips with agonists that induce increases of Ca^{2+} in smooth muscle cells results in the induction of contraction (65, 76, 93, 148, 210). Contraction of three-dimensional collagen gel cultures of various different myofibroblast populations was regulated by cytosolic Ca^{2+} dynamics rather than by RhoA activity (30, 125, 135, 186). Similar results were obtained by treating two-dimensional myofibroblast cultures with agonists that induce cytosolic Ca^{2+} transients (123, 178).

One possible explanation for these seemingly contradictory observations is that myofibroblasts use both contraction regulation pathways rather than relying on either one (Fig. 3). We have addressed this hypothesis in a recent study by simultaneously assessing local and global contraction events of cultured cardiac myofibroblasts at the single-cell level (57). A key finding of this study was that contractions of dorsal stress fibers engaged with extracellular matrix-coated microbeads were mediated by varia-

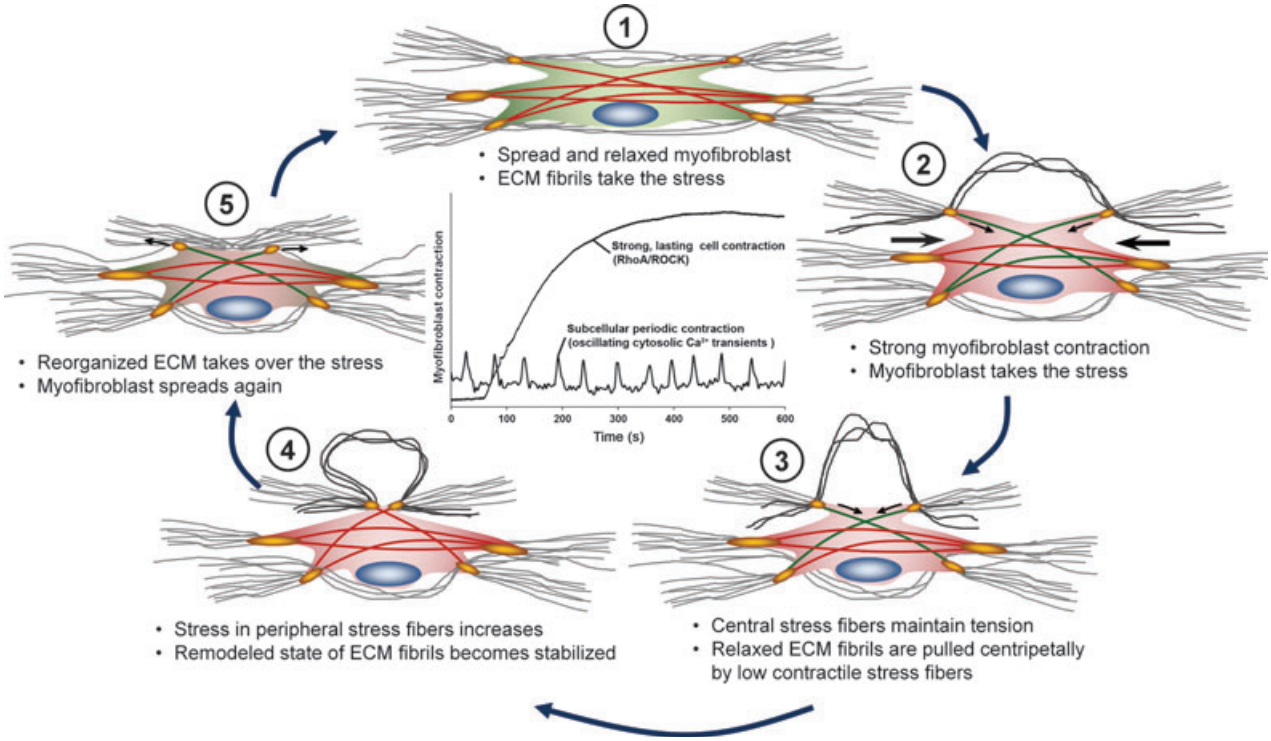

Fig. 3. Lock step ratchet model of myofibroblast extracellular matrix (ECM) remodeling. (1) In a three-dimensional environment, myofibroblast stress fibers are connected to extracellular matrix fibrils (light gray) through cell–extracellular matrix adhesions (orange). Locally intact extracellular matrix architecture protects the populating cells from strain/stress. (2) Rho-(associated) kinase (ROCK)-mediated global myofibroblast contraction (red stress fibers) shortens the bulk extracellular matrix and generates slack in individual extracellular matrix fibrils (dark-gray loops). (3) Such locally relaxed fibrils are now free to be contracted in a $[Ca^{2+}]_i$-dependent manner by low-contractile-stress fibers (green). (4) Incremental pulling events gradually shorten the extracellular matrix fibrils, gradually leading to stress build-up. (5) Local proteolytic processing and stabilization of the fibrils by new extracellular matrix material and/or cross-linking mechanically stabilize the remodeled extracellular matrix. Then, the cell can spread again (outwards pointing arrows at adhesion sites) to start a new cycle, while the extracellular matrix remains shortened. The central graph is a schematic of the coexistence of long-lasting overall cell contraction and weak, but periodic, subcellular contractile events.

tions of cytosolic Ca^{2+}, whereas overall isometric cell tension was maintained through RhoA/Rho-(associated) kinase-mediated contraction of ventral stress fibers engaged with an elastic rubber substrate. Contractile events following periodic cytosolic Ca^{2+} increases (approximately one contraction/100 s) were acting on a short range (approximately 400 nm/contraction) and were comparably weak (approximately 100 pN/contraction), as measured by atomic force microscopy (57). This was in contrast to isometric contraction of the rubber substrate over hours with a force development of several μN per cell (57, 93). We developed a lock-step or ratchet model to explain how global tissue remodeling results from the contraction of single myofibroblasts and subsequent stabilization of tissues by secreted extracellular matrix molecules (57, 209). Long-lasting and strong isometric cell contractions [Rho/Rho-(associated) kinase] generate slack in individual collagen fibrils, whereas weak and short-ranged but periodic microcontractions (Ca^{2+}) remodel such relaxed fibrils. When the gradually rising tension in locally pulled fibrils resists further local translocation, the new fibril configuration has to be stabilized, possibly by digestion of local collagen, deposition of new collagen fibrils and cross-linking (Fig. 3). Although the details of this putative remodeling step are not explored, it has been shown that collagen remodeling by matrix metalloproteinases can be regulated by mechanical tension (2, 56). The stabilized extracellular matrix can then sustain tissue stress and myofibroblasts are able to respread (57, 58). The outcome of this process is persistently remodeled tissue (209).

Substrate stiffness activates myofibroblasts

The function of myofibroblasts to rapidly re-establish tissue integrity by secreting and organizing new extracellular matrix is at least partly controlled by a

mechanical feedback from the extracellular matrix. Compared with the collagenous extracellular matrix of intact soft connective tissues, the provisional extracellular matrix produced after acute tissue injury (e.g. the fibrin clot of dermal wounds) is even softer (91). Culturing fibroblasts on two-dimensional polyacrylamide gels and in three-dimensional collagen gels with a similar softness suppresses the development of stress fibers. It is the spreading of these non-contractile cells and the *de-novo* secretion of collagen that gradually compacts and stiffens the provisional extracellular matrix (79, 88). During this early remodeling process, fibroblasts develop *in-vivo* stress fibers and higher contractile forces. One such example is the neo-formation of stress fibers by granulation tissue fibroblasts in healing and gradually stiffening dermal wounds (75, 96). Contraction and migratory activities of such 'proto-myofibroblasts' can initiate wound closure, but the high forces required to pull the wound edges together are only produced by differentiated, alpha smooth muscle actin-positive myofibroblasts (93, 96). Differentiation of myofibroblasts is controlled by high extracellular matrix stiffness and the presence of transforming growth factor beta 1 (209). A plethora of studies have demonstrated the spontaneous activation of various precursor cells into myofibroblasts on super-physiologically stiff tissue-culture plastic or scar-stiff polymer culture substrates (10, 57, 75, 94, 104, 142, 168, 228, 233).

How fibroblasts 'feel' mechanical stress and translate into gene expression

Stress-activated ion channels

Intracellular Ca^{2+} not only controls cell contraction but serves as a universal second messenger in fibroblasts, including mechanotransduction (13, 43, 120, 130, 204, 217). Mechanical stimuli and contractile forces applied to cell-membrane receptors induce Ca^{2+} entry through the plasma membrane of locally and globally stretched fibroblasts (8, 26, 57–59, 113, 119, 134). The molecular identity of mechanosensitive, cation-permeable channels in the plasma membrane is currently not defined. Candidate mechanosensors belong to the family of transient receptor potential channels (7, 134). In addition to regulating Ca^{2+} entry in response to stretch, mechanosensitive ion channels appear to be involved in the active probing of the mechanical environment by fibroblasts. As discussed earlier in this review, increasing substrate stiffness leads to the formation of contractile stress fibers in fibroblastic cells (10, 57, 75, 144, 174, 238). Consequently, development of intracellular force is greater on stiff than on soft culture substrates (31, 36, 45, 115, 150, 181, 202, 214). High levels of stress fiber formation and contraction are suggested to result in a higher probability for the opening of mechanosensitive Ca^{2+} membrane channels (82, 102, 129, 206). More recently, the termini of stress fibers at cell–extracellular matrix adhesions and cell–cell adherens junctions have been identified as preferred sites of Ca^{2+} entry through mechanosensitive channels in the plasma membrane (82). In migrating fibroblasts, localized Ca^{2+} influx at the leading edge, a region of concentrated adhesion sites, directs cells towards higher substrate stress, a phenomenon called durotaxis (110, 129, 145, 159, 214–216, 227). Myofibroblast contraction induces stress-dependent Ca^{2+} entry in contacting cells at sites at cell–cell adherens junctions; this mechanism has been shown to coordinate the contractile activities of physically connected cells (59).

Mechanosensing at sites of extracellular matrix adhesions

Cells 'feel' tissue stiffness by adhering to the extracellular matrix and actively probing the deformability using cell–extracellular matrix adhesions and actin/myosin pulling forces. In most normal connective tissues, fibroblasts do not form extensive cell–extracellular matrix contacts and are entangled in the collagen architecture. Some reports have demonstrated expression of extracellular matrix adhesion protein in periodontal ligament fibroblasts *in vivo*, which may be attributed to their important role in mechanosensing (205). Extracellular matrix adhesions are best studied in cultured fibroblasts, where the *in-vitro* analogs are focal adhesions (69, 157, 183, 226) (Fig. 2). The size or maturity state of fibroblast focal adhesions correlates with the stiffness of the culture substrate and/or the level of intracellular stress. On soft substrates and in relaxed cells, cell–extracellular matrix adhesions remain as small focal complexes (11, 174, 238). The maturation of focal complexes into larger focal adhesions is stress-dependent and involves the recruitment of new components into the adhesion plaque (17, 29, 141, 189, 194).

Stress perception at focal adhesions starts at the single transmembrane integrin, which connects extracellular matrix ligands to the intracellular cytoskeleton. Computational simulations imply that forces applied to the αvβ3 integrin via the 10th type

III module of fibronectin can shift the integrin to its active conformation (182). The switch from weak to strong integrin interaction with fibronectin upon application of force has been experimentally tested for α5β1 integrin. Inhibition of cell contraction or lowering extracellular resistance by cell growth on soft substrates suppresses the switch to the high-affinity integrin state (62). Direct application of force to single α5β1 integrins increased the lifetime of the adhesion bond, as assessed using atomic force microscopy (131). A similar mechanism of binding reinforcement by mechanical stress occurs at the cytoplasmic side of integrins. Pulling single integrins with optical tweezers generates a 2-pN force-resisting slip bond with the actin cytoskeleton (118), which is mediated by the cytoplasmic protein talin-1. Forces measured in the same range can unfold the talin-1 molecule and reveal cryptic binding sites for another focal adhesion protein, vinculin (39, 172). Recruitment of vinculin to nascent adhesions further strengthens and matures the adhesion structure, possibly involving force-dependent conformational changes in vinculin itself (67, 78). Force-induced conformational changes in focal adhesion protein components is one possible mechanism of how mechanical signals are translated into chemical signals. Central roles in this conformation-dependent translation step have been attributed to tyrosine phosphatases and kinases (73, 157, 222). Other stress-dependent focal adhesion proteins are vinculin (189), paxillin (194, 242), zyxin (139, 240) and the actin filaments themselves (83).

The level of actin polymerization regulates gene transcription via the myocardin-related transcription factor

The recruitment of monomeric alpha smooth muscle actin to stress fibers augments fibroblast contractile activity, whereas loss of alpha smooth muscle actin from stress fibers acutely reduces myofibroblast contraction (93, 95). Long-term treatment of myofibroblasts with a peptide that selectively depolymerizes alpha smooth muscle actin reduces transcription of collagen and alpha smooth muscle actin (95). This effect on specific genes may be explained, in part, by the increased pool of monomeric actin over polymerized F-actin. G-actin controls gene transcription by interacting with the myocardin-related transcription factor (MRTF/MAL/MKL) (155, 158, 180, 220). Enhanced formation of actin filaments and stress fibers leads to reduced levels of G-actin and increased liberation of myocardin-related transcription factor-A, which is then free to travel from the cytoplasm into the nucleus. Myocardin-related transcription factor-A directly controls transcription of the alpha smooth muscle actin gene by binding to CArG elements in the alpha smooth muscle actin promoter and enhancing the transcriptional activity of the serum response factor (26, 151, 200). Myocardin-related transcription factors A and B also mediate transforming growth factor beta-1-induced myofibroblast differentiation and transcription of smooth muscle genes in fibroblasts (35).

Transforming growth factor beta-1 activation is a consequence of extracellular stress

Fibroblast contraction and extracellular matrix stiffening have a profound effect on the activation of transforming growth factor beta-1 from latent stores in the extracellular matrix. Transforming growth factor beta-1 is the most potent cytokine known to induce myofibroblast activation (42, 192). Myofibroblasts are able produce and activate their own transforming growth factor beta-1, which partly contributes to the persistence of myofibroblasts in fibrosis (231, 234). Transforming growth factor beta-1 and its latency-associated pro-peptide are intracellularly cleaved but remain noncovalently associated. This small latent complex is secreted with the latent transforming growth factor beta-1-binding protein as a large latent complex. Latent transforming growth factor beta-1-binding protein-1 is an extracellular matrix protein that stores latent transforming growth factor beta-1 extracellularly (3, 40, 107, 116, 146, 169, 187, 231). Dissociation from latency-associated pro-peptide activates transforming growth factor beta-1, which is physiologically promoted by proteolysis of the latent complex, action of thrombospondin or binding of integrins (3, 4, 116, 160, 199, 208, 231). Integrin αvβ8 supports transforming growth factor beta-1 activation by proteases that are possibly guided to the latent complex (116, 164). Integrins αvβ3, αvβ5 and αvβ6 activate transforming growth factor beta-1 by transmitting cell forces to latency-associated pro-peptide independently from proteolysis. The epithelial integrin αvβ6 was shown to promote the onset of fibrosis by activating transforming growth factor beta-1 via traction (3, 72, 103, 117, 161, 235). Mechanical activation of transforming growth factor beta-1 by the myofibroblast integrins αvβ3 and αvβ5s was suggested to dominate in progressive fibrosis (22, 147, 231, 232). Because activation of transforming growth factor beta-1 by cell traction requires

physical anchoring of the latent complex (3), the stiffness of the extracellular matrix is an important control element in the activation process (232). It is conceivable that activation of transforming growth factor beta-1 by fibroblast contraction only occurs when the extracellular matrix is sufficiently remodeled and that further reorganization requires the action of higher contractile myofibroblasts (92).

Conclusion

Understanding when and why physiological remodeling to preserve tissue homeostasis and repair injuries turns into pathological tissue remodeling and overhealing is a major challenge. The mechanical state of the extracellular matrix is central in regulating fibroblast function and phenotype at several levels and through different pathways. Therapeutic approaches are beginning to emerge that directly target cellular processes resulting in extracellular matrix stiffening, such as collagen cross-linking through the actions of lysyl oxidases and related enzymes (14, 219). Another approach is to interfere with the adhesion of extracellular matrix to fibroblastic cells. Inhibition of specific myofibroblast integrins *in vivo* is expected to reduce force transmission, stress sensing and even activation of profibrotic growth factors, all of which culminate in harmful tissue contractures.

Acknowledgments

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