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The (dys)functional extracellular matrix☆

Benjamin R. Freedman[a,1], **Nathan D. Bade**[b,1], **Corinne N. Riggin**[a,1], **Sijia Zhang**[a,1], **Philip G. Haines**[c,1], **Katy L. Ong**[d,1], and **Paul A. Janmey**[a,e,1,*]

[a]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA

[b]Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA, USA

[c]Division of Cardiovascular Medicine, University of Pennsylvania, Philadelphia, PA, USA

[d]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA

[e]Department of Physiology, University of Pennsylvania, Philadelphia, PA, USA

Abstract

The extracellular matrix (ECM) is a major component of the biomechanical environment with which cells interact, and it plays important roles in both normal development and disease progression. Mechanical and biochemical factors alter the biomechanical properties of tissues by driving cellular remodeling of the ECM. This review provides an overview of the structural, compositional, and mechanical properties of the ECM that instruct cell behaviors. Case studies are reviewed that highlight mechanotransduction in the context of two distinct tissues: tendons and the heart. Although these two tissues demonstrate differences in relative cell–ECM composition and mechanical environment, they share similar mechanisms underlying ECM dysfunction and cell mechanotransduction. Together, these topics provide a framework for a fundamental understanding of the ECM and how it may vary across normal and diseased tissues in response to mechanical and biochemical cues. This article is part of a Special Issue entitled: Mechanobiology.

Keywords

Mechanotransduction; Cytoskeleton; Biomechanics; Cell mechanics; Tendinopathy; Diastolic dysfunction

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*Corresponding author at: Institute for Medicine and Engineering, University of Pennsylvania, 1010 Vagelos Research Laboratories, 3340 Smith Walk, Philadelphia, PA 19104-6383, USA. Tel.: +1 215 573 7380 (Office); fax: +1 215 573 6815. janmey@mail.med.upenn.edu (P.A. Janmey).

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Conflicts of interest

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1. Introduction

1.1. Mechanical properties of the extracellular matrix instruct cell behaviors

The local microenvironment of cells plays important roles in both normal development and disease progression. A major component of the biomechanical environment of cells is the dense meshwork of fibrous proteins and other biopolymers called the extracellular matrix (ECM). This intricate biomaterial provides structural support for many tissues in vivo and provides cells with mechanical cues that modulate both cell morphology and physiology. Numerous cell types possess ATP-driven molecular machinery that applies forces and responds to the ECM.

Cell responses to the ECM are driven by intrinsic properties that include adhesive affinity, matrix stiffness, fiber alignment, and matrix density [1]. In mature tissues, cell adhesion to the ECM is primarily, but not exclusively, mediated by integrins, which are transmembrane heterodimeric receptors that interact with a range of ECM components and cluster into different kinds of adhesion sites with an array of intra-cellular components [2–4]. Integrins interact with the force-producing actin cytoskeleton, where changes in force alter the assembly of adhesion complexes and activate adhesion-mediated cell signaling, such as RhoA-induced actin stress fiber formation (Fig. 1) [5,6]. Integrin type and density determine the cell adhesion affinity for, and mechanical sensing of, the ECM [3,7,8]. The most commonly studied ECM mechanical properties are substrate stiffness (a structural property) and elastic modulus (a material property) (Table 1) [4,5,9]. Cells sense substrate stiffness primarily via integrin-dependent actomyosin contraction (Fig. 1). Human tissues have various collagen compositions and a wide range of moduli varying from 100 Pa in brain tissue to over 1 MPa in bone (Fig. 2A, B) [10,11]. Substrate stiffness has been shown to guide stem cell lineage specification in vitro and affect proliferation, motility, contractility, and many other cell functions by changing both acute signaling and transcriptional programs [12–14]. Fiber orientation in the matrix is also critical for various cell behaviors, including stem cell differentiation [15], cell alignment [16–18], and migration [19].

In cell-dense tissues such as the heart, cells are bound together by adherens junctions, and mechanotransduction occurs via calcium-dependent transmembrane proteins (termed cadherins) (Fig. 1) [20, 21]. Much like integrins, cadherins form complex adhesions, connect to the actin cytoskeleton via numerous proteins including catenins, and participate in various intracellular signaling pathways [22,23]. Thus, both cell–ECM and cell–cell contacts play important roles in the detection of tissue mechanical properties.

1.2. Effects of internal and external mechanical stimuli on cell responses

Cell- and environment-generated mechanical loads on the ECM can induce a variety of cell responses (Fig. 1). The types of stimuli include tensile, compressive, and shear stresses and strains (Table 1). Transformation of internal and external mechanical cues into cellular responses occurs via collagen fiber kinematics [24], focal adhesions (macromolecular assemblies of integrins and proteoglycans) [25], and cell–cell contacts [20,22, 23,18]. An internal source of ECM loading is cell traction force. Cells achieve tensional homeostasis with their ECM by balancing traction forces with matrix stiffness [26–28]. Maintenance of

tensional homeostasis plays an important role in the regulation of critical cell functions. For example, during wound healing, fibroblasts reorganize the collagen matrix by exerting traction forces [18]. The increased stresses within the ECM cause the cells to generate a stiffer matrix by producing collagen, become more contractile in response to the increased stiffness, and differentiate into myofibroblasts that propagate this feedback loop and ultimately contract the wound [29]. Normally, this positive feedback is countered by other signals that limit the extent of contraction and matrix deposition, but when left unchecked can lead to scar formation and other tissue defects. Forces transferred through the ECM have a wide range of magnitudes. Although macroscale orthopaedic tissues may experience up to 3500 N of loading [30,31], cell–ECM force interactions are nearly 12 orders of magnitude smaller (nN) (e.g., [32]), and subcellular interactions between myosin and actin crossbridges can balance forces of less than 1 pN [33].

Cells remodel the ECM in response to external mechanical signals as well as the biomechanical properties of the matrix [34–40]. Through tensional homeostasis, cells demonstrate reduced cell-mediated contraction with increased externally applied loads [26]. External stress- and strain-induced changes in cell behavior have been extensively studied in tissue injury. For example, acute mechanical compression of articular cartilage enhances chondrocyte proliferation and decreases proteoglycan synthesis [35]. Also, the production of various ECM components by cardiac fibroblasts in response to cyclic loads is implicated in pathological fibrosis of the heart [36]. Fluid flow through the ECM can also significantly impact cell behaviors [37–40]. For example, interstitial fluid flow has been demonstrated to regulate fibroblast alignment and lymphatic and vascular endothelial functions in three-dimensional cell cultures [39–41].

1.3. Mechanotransduction in the context of diseases and injury

Alterations in ECM composition, stiffness, and loading environment affect cell behaviors, which feed back to ECM dysregulation and disease progression. For example, in pulmonary and cardiac fibrosis, enhanced myofibroblast proliferation and collagen production increase tissue stiffness (Fig. 2C) [42–44]. In addition, abnormal mechanical stimulation can aberrantly activate signaling pathways, such as TGF-β signaling associated with osteoarthritis [45] and β-catenin signaling in cancer progression (Table 2) [46]. In light of these examples, it is important to understand the underlying mechanism of mechanotransduction in order to target ECM or cell mechanosensing to ameliorate the disease condition.

1.4. Overview for the rest of the paper

This review highlights the effects of ECM function and dysfunction on cellular responses in different tissues. Specifically, the remainder of this review examines the musculoskeletal and cardiovascular organ systems, with a focus on the tendon and the heart ECM. Although these two tissues demonstrate differences in relative cell–ECM composition and mechanical environment, they share similar mechanisms underlying ECM dysfunction and cell mechanotransduction. In each case study, we discuss the techniques and models used to investigate cell and ECM responses to injury and disease at tissue, cellular, and molecular levels.

2. Case study 1: the extracellular matrix in the tendon

2.1. Structure–function relationships in tendon ECM

Tendons connect and transmit forces from muscle to bone [47] and are composed of tenocytes (tendon fibroblasts) and tendon-derived stem cells (TSCs) [48]. These cells are embedded in a heterogeneous matrix of collagens (types I, II, V, IX, and X) [49,50], proteoglycans, elastin, fibronectin, and fluid (70% wet weight) [51,52]. Together, these molecules form a hierarchical network from the macro(“fascicle”) to nano- (“fibril”) scales (Fig. 3A) [53]. While collagen is generally thought to be the main mechanical element in tendons, removal of non-collagenous molecules, such as elastin or glycosaminoglycans, reduces the whole tendon mechanical response, emphasizing their role in development, homeostasis, and load transfer [54–56].

Tendons exhibit enormous variation in material properties as they anatomically originate from muscle (compliant material) and insert into bone (stiff material). This variation in tendon stiffness changes based on anatomical location and species [57], as well as the amount of strain applied [58]. Disorganized ECM becomes more aligned and less wavy (termed “crimp”) [59] with mechanical loading, giving way to a distinct “toe-region” or strain stiffening mechanism (Table 1; Fig. 3B). In addition, tendons are viscoelastic and poroelastic (Table 1), which results in rate- and time-dependent properties and fluid flow within the ECM [60], similar to other biological tissues (e.g., cartilage, bone, liver, heart, and lung).

During normal human motion, the stresses and strains that tendons experience vary based on anatomical position and activity level. Although many tendons operate in the toe-region of tissue's stress–strain curve where resistance to deformation begins to increase (less than 5% of their load until rupture) [61], higher load bearing tendons, such as the Achilles, can experience forces nearly 70% of their maximum load and stress before rupture (~3500 N or ~55 MPa) [30,31]. The primary direction of loading in tendons is tensile, yet compressive, biaxial, and shear stresses may be present [62,63]. The wide variation in mechanical properties and loading environments across tendons emphasizes their dynamic role in the musculoskeletal system and the complexity of research necessary to understand their basic mechanisms of homeostasis and injury.

2.2. Effects of external and internal mechanical stresses on the ECM and cell response in tendinopathy

Maintenance of mechanical, structural, and compositional properties is heavily influenced by mechanical loading and biochemical factors. Aberrant mechanical loading [64–68] can cause pathological changes resulting in tendinopathy, a degenerative condition characterized by an imbalance between degradation and synthesis of the ECM. For example, rotator cuff (Table 2) tendinopathy affects approximately 4–6% of the population between the ages of 25–64, with a much higher percentage in laborers (19%) [64] and athletes, such as elite swimmers (69%) [69, 70]. Tendinopathy can affect several tendons, especially those highly load bearing including the Achilles [53], patellar [71], and rotator cuff [72]. Individuals with tendinopathy present with tendon thickening and increased vascularization, as evaluated

with ultrasound [73]. While the pathogenesis of tendinopathy is poorly understood, it is suggested that aberrant mechanical stimuli may drive tenocytes and TSCs towards pathologic changes [48,74]. Although biochemical mechanisms driving tendinopathy may be present, we focus on the mechanical mechanisms at play from whole tendon biomechanics down to the ECM, cellular, and sub-cellular levels.

2.2.1. The ECM and cell response to in vivo loading in tendinopathy—The effect of in vivo joint loading on the ECM and the corresponding cell response in the tendon has been evaluated in both humans and animal models. External loading may generate interstitial pressures surrounding the tendon, fluid shift, and alterations in blood flow that activate mechanotransductive pathways [75]. Human studies have assessed changes in tendon stiffness using ultrasound [76,77], biochemical changes via tissue biopsy (e.g., collagen content and crosslinking) [78], or serum levels (e.g., TNF-α, IL-10, and CTGF) [79]. Without loading, tendon structural organization and dynamic elastic and viscous properties decreased [80], which may have been caused by increased matrix degradation [81] and increased expression of inflammatory cytokines [82]. In contrast, a single session of activity modulated the expression of ECM proteins and upstream cell signaling markers [83]. A model of repetitive loading increased tensile modulus (52%), stress to rupture (69%) [84], and tendon thickness (~9–20%) [85], which may be due to increased collagen synthesis [84,86]. However, it is noted that some studies have demonstrated no differences in tendon material properties following various bouts of moderate, repetitive loading [87,88], suggesting that the specific moderate-load protocols that create an adaptive response are not fully defined.

Excessive loading promoted tendon matrix synthesis through increased growth factor production, proliferation of TSCs, and expression of type I collagen, as well as cartilage and bone phenotypes [89]. Histopathological characterization of tendinopathy in humans demonstrated altered collagen content, decreased fiber organization, aberrant ECM deposition (calcification, ossification, lipid accumulation), and accumulation of proteoglycans between degenerated collagen fibers (i.e., mucoid degeneration) [90]. Rodent models of shoulder overuse (Table 2) induced similar tendinopathic conditions [91]. Specifically, overuse loading in the rat supraspinatus tendon increased inflammation, angiogenesis, the production of cartilage markers and proteoglycans, and type III/I collagen ratio (Table 2) [92–94]. Similarly, high-cycle fatigue loading produced a degenerative, microstructural damage response [95]. In addition to overuse, abnormal loading, such as disuse, compression or shear from contact with neighboring structures, or change in loading direction caused by injury can initiate a pathologic response and contribute to the development of tendinopathy [96]. For example, tendon impingement is a leading cause of rotator cuff tendinopathy. Additionally, rotator cuff tears can cause a force imbalance in the shoulder joint, which results in tendinopathy in adjacent rotator cuff tendons [97–99]. Taken together, these data suggest that overuse and abnormal loading may disrupt the homeostatic balance between synthesis and degradation, creating an overall catabolic response and development of disease.

2.2.2. The ECM and cell response to ex vivo tissue-level loading in tendinopathy—While in vivo models of tendinopathy provide the most clinically relevant information about the disease, these models have a limited ability to evaluate how the mechanical loads are transmitted at a smaller scale, and therefore their mechanistic effects on the cells and ECM. Thus, alternative methods apply tensile loads to ex vivo tendon explants to preserve the native architecture of the ECM, while also providing more controlled experimental conditions than are possible in vivo. Experiments on non-living tendon explants have investigated structural and mechanical alterations that occur to the ECM with repeated induction of micro-damage via overloading and fatigue loading models. When tendons are subjected to high dynamic loading, fatigue damage accumulation occurs [100] in concert with alterations in crimp properties [101]. Repeated subrupture loading results in collagen fibril kinking [102], which can affect cell morphology and matrix degradation [103]. These mechanisms of microdamage accumulation may be tendon type [104] and age specific [105].

To further investigate the effects of tissue-level loading on biological response, in vitro bioreactor model systems are used. Typically, fascicles derived from tail [106–109], flexor [110], extensor [111], or patellar tendons [112,113] are cultured in standard media conditions under various amounts of applied load, duration, and frequency. In vitro stress deprivation flattened and elongated fibroblasts, decreased cell number, and decreased tensile modulus [110]. Although low and moderate loading had no effect on water content [110] or glycosaminoglycan production [109], higher loading increased glycosaminoglycan content [114], lowered mechanical strength, and caused the release of pro-inflammatory cytokines and vasodilators, such as prostaglandin E_2 (PGE_2) and nitric oxide (NO) [115]. Certain loading regimes may promote optimal mechanical properties [113], potentially through collagen synthesis [109,111] as the molecular mechanisms switch from a catabolic to anabolic response [106]. Such mechanisms could depend on the frequency and duration of the loading [112].

Taken together, these studies suggest that ex vivo loading of tendon explants provides a controlled method for evaluating the specific response of tendon to loading, thus removing confounding variables present in whole tissue model systems. Many of the same mechanical and biological mechanisms are conserved, demonstrating the validity of these models.

2.2.3. ECM and cell response to cellular-level loading in tendinopathy—Loading applied directly to cells in vitro can provide more direct information on the cell level response to mechanical stimuli. Scaffolds seeded with cells under cyclic strains have been used to investigate cell behavior in response to loading. Tenocytes isolated from patellar and Achilles tendons subjected to physiological strain levels upregulated only tenocyte markers (type I collagen and tenomodulin), whereas higher levels of strain upregulated biomarkers found in cartilage [89]. Cyclically stretched human patellar tendon fibroblasts responded with a load-dependent increase in inflammatory cytokines, which could reduce cell proliferation and collagen synthesis and lead to the development of tendinopathy [116,117]. Over-tensioning TSCs caused osteogenic differentiation and upregulation of BMP-2 [118,119]. This response was regulated through the mechanosensitive activation of RhoA, which plays a role in cell proliferation, differentiation, and adhesion formation [119].

Microfluidics and modeling approaches [120,121] have provided further insight into the response of cells under fluid shear stresses. Fluid shear stresses have been implicated in gene expression changes in degradation [122], collagen remodeling [123], anti-fibrosis [124], ecto-ATPase activity [125], NO production [126], and calcium signaling [127] in the tendon. In addition to the application of fluid shear stresses to modulate cell behavior, biochemical cues activated by mechanical stimulation [128,129] might also drive phenotypic behaviors. In particular, the effect of cyclic strain has been shown to mirror that of TGF-β stimulation [130]. Primary tendon fibroblasts treated with TGF-β demonstrated increased expression of miRNAs that regulate cell proliferation, ECM synthesis, and scleraxis (a tendon marker) [83].

Observed changes in gene expression, cytokine release, and nontenogenic differentiation following high loading may be due to upstream mechanosensing in the cytoskeleton. Application of strains on cells can create an adaptive response to the cytoskeleton and adherens junctions [131]. Specifically, N-cadherin and vinculin levels increased in strained cultures, and cells organized their actin into stress fibers along the axis of principal strains (Table 1) [131]. In addition, tenocyte communication via gap junctions may be altered with loading [132,133]. For example, when tenocytes were subjected to cyclic strain, their gap junctions became disrupted and apoptosis was induced [134]. Cell–matrix adhesions allow tenocytes to sense and respond to their mechanical environment while also allowing them to act on the ECM through actomyosin mediated contractile forces [135]. Altering the tensile forces on tendons can elicit changes in integrin receptors, as well as in downstream ECM proteins [135]. Specifically, de-tensioning tenocytes in vitro caused a decrease in collagen binding integrin α11β 1, which is associated with the organization of collagen in the ECM, and an increase in collagen binding integrin α2β 1 and fibronectin integrin receptor α5β 1, which are associated with the transmission of cytoskeletal forces through collagen fibrils causing contraction and adhesion strength, respectively [135]. Additionally, de-tensioning decreased expression of tenomodulin and Mohawk homeobox, which are associated with tendon differentiation, as well as decreased collagens, decorin, and matrix organization and increased pro-inflammatory markers [135]. These results demonstrate that mechanical loading alters mechanosensitive proteins and therefore plays a large role in the maintenance of a normal tendon phenotype as well as the development of pathology.

2.3. Summary and future work

Since the primary function of tendons is to transmit forces from muscle to bone, its ability to adapt and respond to loads is essential to prevent injury. Previous work has shown the sensitivity of the tendon to alterations in mechanical stimuli (Fig. 4). Establishing changes in gene and protein levels following various mechanical protocols is necessary to confirm that models accurately represent the human condition. Once these model systems have been optimized, a more mechanistic evaluation of alterations in the cell and ECM that elicit tendinopathic responses is necessary.

Animal models can help to elucidate the underlying in vivo mechanisms of tendinopathy in humans, but they have limitations. Although bioreactor studies may overcome some limitations, they likely oversimplify true in vivo biological complexity. Additional

knowledge may be gained from other, genetically-tractable model systems that focus on cell–ECM interactions. *Drosophila* tendon cells have adopted a compact microtubule [136] and F-actin [137] array as cytoskeletal structures to withstand high mechanical loads, and may be used to study the muscle–tendon junction. In addition, zebrafish craniofacial tendons, which connect cartilage and muscle, contain parallel arrays of collagen fibrils, suggesting that they are structurally similar to mammalian tendons. These tendons are derived from neural crest cells, specified by muscle-induced expression of tendon-differentiation markers, and upregulate tenomodulin and type I collagen, as in mammals [138]. Therefore, zebrafish may provide an additional model system for elucidating mechanisms of tendinopathy.

3. Case study 2: the extracellular matrix in the heart

3.1. Structure–function relationships in the heart ECM

The heart is a muscular pump that circulates blood throughout the body composed of four major chambers (two atria and two ventricles), each containing several tissue compartments. First, the parenchyma is composed of specialized cardiac muscle cells called cardiomyocytes. These cells are further subdivided into atrial, ventricular, and conductive system cardiomyocytes. Cardiomyocytes are terminally differentiated, non-proliferating, excitable cells, which generate electrical signals that induce a coordinated contractile behavior allowing the heart to eject blood into the systemic and pulmonary circulations. The coronary vasculature represents a second tissue compartment that comprises arterial and venous tissue (Table 2) and oxygenates and facilitates removal of waste products. The cardiomyocytes and coronary vessels are tethered to an ECM comprising the endomysium, perimysium, and epimysium, which surround the myofibers and coronary vessels. The main component of the heart ECM is fibrillar type I collagen, with types III and V contributing 10–15% and <5%, respectively [139]; proteoglycans and glycoproteins are also present. Cardiac fibroblasts reside in the ECM and form the largest population of cells in the heart (two-thirds) whereas cardiomyocytes occupy two-thirds of the total tissue volume [140]. Further, these fibroblasts mediate a constant homeostatic state of synthesis and degradation of ECM.

During pumping, the heart undergoes continuous cycles of systole and diastole. Systole involves muscular contraction and the ejection of blood into the systemic and pulmonary circulations, whereas diastole involves relaxation and filling of the left and right ventricles (LV, RV) [141]. The heart ECM contributes to contractility, compliance, relaxation, and electrophysiology (Table 2). During stress states (e.g., hypoxia/infarction and pressure overload), fibroblasts adopt a phenotypic change into alpha smooth muscle actin- (α-SMA) positive myofibroblasts (activated fibroblasts able to promote ECM overexpansion) (Table 2). The interactions among the cardiomyocytes, fibroblasts, coronary vasculature, and ECM provide the structure necessary for mediating biomechanical cross talk, mechanotransduction, and the development of cardiac stress, stretch, and stiffness (Fig. 5) [139,142].

3.2. Introduction to heart failure pathophysiology

Abnormalities in heart biomechanics cause several common and highly morbid cardiovascular diseases including heart failure (HF), which is associated with 50% mortality at 5 years following diagnosis [143]. Aberrant changes in the cellular and ECM compartments of the myocardium (Table 2) lead to increases in tissue and cellular stiffness and wall stress [142,144–148]. These changes induce systolic and/or diastolic dysfunction, which has been strongly associated with the development of HF [149,150].

HF is a pathophysiological state mediated by myocardial (systolic and diastolic dysfunction) and extramyocardial (e.g. vascular stiffness, endothelial dysfunction, skeletal muscle metabolic derangements) abnormalities that either (1) undermine the ability of the heart to pump sufficient blood to meet the body's metabolic demands, or (2) allow it to meet these demands only when ventricular filling pressures are significantly elevated as a result of increased chamber stiffness and slowed active relaxation [141,151,152]. Two major subtypes of the HF syndrome are HF with reduced ejection fraction (HFrEF) (i.e., systolic dysfunction) and HF with preserved ejection fraction (HFpEF) (i.e., diastolic dysfunction) (Table 2) [153]. Although therapies targeting systolic dysfunction have improved the outcomes of many subjects with HFrEF [143,154], no therapeutic interventions in the HFpEF population have improved clinical outcomes. Furthermore, diastolic dysfunction is usually present in patients with HFrEF, and subclinical abnormalities in systolic function (detected non-invasively through assessment of systolic strain) are often present in patients with HFpEF.

3.3. Effects of HF on ECM remodeling and biomechanics

Abnormal diastolic biomechanics play a central role in the pathophysiology of HF. Severity of abnormalities correlates with worsening clinical outcomes. Furthermore, even the presence of abnormal diastolic biomechanics in asymptomatic individuals associates with a higher risk of developing HF, underscoring the importance of biomechanics in heart function [143,152,155–160].

Although these echocardiography-based studies introduced the concepts of abnormal diastolic biomechanics (e.g., slowed relaxation, increased stiffness, increased filling pressures), the mechanistic basis for these abnormalities (in humans) remained elusive until the advent of magnetic resonance imaging (MRI) to noninvasively characterize cardiac tissue properties in humans. In vivo cardiac MRI measures of myocardial fibrosis (Table 2) have demonstrated that expansion of the ECM compartment is associated with increased risk of sudden cardiac death, HF, mortality, and preclinical myocardial dysfunction [161–163]. Furthermore, increased fibrosis in HFpEF patients correlates with worse clinical outcomes [164]. In vivo measures of both focal and diffuse myocardial fibrosis have been strongly associated with major cardiac disease states and clinical events [165]. Remodeling in both the ECM and the cardiomyocyte myocardial compartments including fibrosis, deposition of advanced glycosylation end products, cardiomyocyte hypertrophy, myocardial thickening, and increased stiffness has been confirmed in myocardial tissue from humans with HFpEF and HFrEF [166–168].

Studies examining tissue-level rheology have also demonstrated increased stiffness of myocardial tissue in pathological stress states. Increased elastic modulus of infarcted versus non-infarcted tissue and the border zone myocardium were found in a rat model of ischemic cardiomyopathy (Table 2). Increased stiffness was also observed in the peri-infarct zone in a mouse infarction model [169]. The extension of adverse biomechanical remodeling beyond the infarct zone is well known to be central to the development of HF. Furthermore, tissue rheological changes occur in the presence of increased fibrosis and correlate to in vivo measures of systolic and diastolic dysfunction [170].

Changes in the cardiomyocyte compartments, in addition to the ECM, can also lead to abnormal global cardiac biomechanics, function (systolic and diastolic), and HF. Reduced cardiac strain assessed with echocardiography correlated with cardiomyocyte stiffening, disorganization of T-tubule structure, and abnormalities in intracellular calcium handling in a hypertensive rat model [171]. Interestingly, adverse cardiomyocyte remodeling preceded increased ECM stiffness in the transition from hypertensive heart disease to HF. This demonstrates that one source of increased stiffness may originate from the cardio-myocyte tissue compartment.

In summary, these strong associations of diastolic dysfunction, ECM, and cardiomyocyte remodeling with cardiovascular events and death in humans with HF underscore the importance of biomechanical derangements in human cardiovascular diseases. The potential molecular mechanisms mediating increased cardiomyocyte stiffness are addressed in the next section.

3.4. Cellular and molecular mechanisms underlying cardiac mechanotransduction and mechanics

The molecular composition of the ECM impacts the stiffness of cardiomyocytes, as demonstrated in in vitro cultures of cardiomyocytes on different matrices [172]. Integrins, hyaluronic acid content, and matrix stiffness are suggested to be tightly regulated for normal development of structure and function of cardiomyocytes, highlighting the importance of ECM remodeling and regulation in development [173]. The importance of cell–matrix interactions in the heart and of integrin-mediated matrix stiffness sensing by cardiomyocytes has also been demonstrated. Integrin-mediated (i.e., fibronectin–cardiomyocyte) interactions are critical for mediating normal ventricular contraction and relaxation [174]. The costamere, which couples the sarcomere (basic unit of muscle) with the sarcolemma (cell membrane of a striated muscle fiber cell), contains integrins that connect to the Z-disk (line forming sarcomere boundaries) and cytoskeleton via proteins including talin, vinculin, desmin, FAK, and α-actinin [175]. This forms the cardiomyocyte focal adhesion and mediates mechanotransduction between the cardiomyocyte and its surrounding matrix. In one study, fibronectin–integrin adhesion force and adhesion probability increased during isolated cardiomyocyte contraction and decreased during relaxation [174]. Specific cardiac integrins (e.g., α5-β 1, αV-β 3) are essential for the heart's response to stress states and overall survival [176]. Integrin β 1 and FAK mediate mechanotransduction in the heart in response to mechanical stretch. Integrin β 1 also mediates the phosphorylation of important intracellular MAP kinase signaling pathways including phosphorylation of ERK, p38, and

JNK following mechanical stretch of neonatal rat ventricular myocytes. Inhibiting FAK activity reduced resting and stretch-induced phosphorylation of ERK and integrin β 1 was required for stretch-induced phosphorylation of FAK [177]. Integrin β 1 may also be involved in age-related myocardial stiffening and diastolic diameter decrease [178].

Cell–cell interactions through cadherins have also been shown to be important in the response of cardiomyocytes to changes in their mechanical environment (Fig. 5). Matrices coated with N-cadherin elicited changes in myofibrillar organization, myocyte shape, and cell stiffness, demonstrating the importance of cell–cell mediated force perception via N-cadherin [173].

Mechanosensing molecules downstream from integrins and cadherins in cardiac tissue (including FAK, vinculin, and catenins) also play a role in cardiac remodeling, biomechanics, function, and mechanotransduction. Mechanical stretching of cardiomyocytes increased phosphorylation of FAK, migration of FAK from the perinuclear region of neonatal rat ventricular myocytes to the myofilaments, and activation of the ANF gene [179]. Cyclical mechanical stretch- induced FAK phosphorylation in rat cardiac fibroblasts and resulted in mTOR-dependent proliferation and activation of these fibroblasts into myofibroblasts [180]. This fibroblast activation to myofibroblast is believed to play a central role in adverse cardiac ECM remodeling in response to stress states resulting in HF [139]. FAK signaling in response to mechanical pulsatile stretch is also present in cardiomyocytes and involves MAPK and SPAK signaling [181]. Cardiac-specific FAK knockout mice developed significant hypertrophy, increased LV mass, increased myocardial fibrosis, increased markers of fibrosis, and increased collagen expression in response to vasoconstrictor or transaortic constriction, two pressure overload models [182]. Furthermore, isolation of cardiomyocytes from these mice revealed disorganized myofibrils and decreased phosphorylation of p130Cas (docking protein involved in tyrosine-kinase signaling in cell adhesion) and paxillin (protein contributing to a focal adhesion), implicating these additional known mechanosensing proteins in this process. The importance of FAK in the cardiac remodeling response has also been demonstrated in a model of pressure overload [183]. An important role for vinculin in cardiac tissue and cardiomyocytes was demonstrated in cardiac-specific vinculin knockout mice whereby loss of vinculin caused abnormal myocardial biomechanics resulting in the development of HF [184]. Additional molecules, such as β-catenin, mediate the hypertrophic response of cardiomyocytes and the activation response of cardiac fibroblasts to myofibroblasts during post-infarct healing [185].

As mentioned above, a major source of increased stiffness in the heart, in addition to the ECM compartment, is the cardiomyocyte compartment. Studies demonstrated that the large intracellular cardio-myocyte protein, titin, mediated diastolic biomechanics and acted as a source of increased stiffness in HF (Fig. 5). Passive force occurs in the myocardium during diastole and increases as the LV chamber expands and fills [186]. Titin, located in the sarcomere of the cardiomyocyte, is a major contributor to passive force and stiffness of the cardiomyocyte during diastole [187]. Titin exists in various isoforms based on its phosphorylation status, and myocardium co-expresses varying amounts of both isoforms. Cardiomyocytes from pigs expressing higher levels of the N2BA isoform were less stiff

compared to cardiomyocytes from rats and mice that expressed higher levels of the N2B isoform [188]. Increased expression of the stiffer titin isoform has been demonstrated in cardiomyocytes from failing myocardium [189]. Furthermore, increased expression of a stiffer titin isoform has been demonstrated in human HFpEF, suggesting that a component of the increased diastolic stiffness seen in HFpEF may be attributed to isoform expression shifts favoring the increased expression of the stiffer titin isoform by cardiomyocytes [166,190]. Therefore, therapeutic strategies targeting abnormal cardiac biomechanics to treat HF may need to target both ECM and cardiomyocyte tissue compartments.

Changes in the biomechanics and composition of myocardial tissue have also been demonstrated during development whereby the myocardial tissue and ECM become stiffer from the embryonic to neonatal period through to adult periods. Optimum matrix stiffness is required for neonatal cardiomyocytes to develop into structurally, functionally, and biomechanically normal adult cardiomyocytes, underscoring the importance of changes in cardiac biomechanics for normal cardiovascular development [191–193].

3.5. Summary and future work

Therapies demonstrated to improve systolic function have improved the outcomes of many subjects with HFrEF [143]. Unfortunately, no therapeutic interventions in the HFpEF population have demonstrated improved clinical outcomes [154]. Therefore, there is an urgent need to further characterize the cardiac and extracardiac phenotypes of this syndrome, identify and target novel risk factors, and propel the discovery of new therapeutic interventions.

Since myocardial infarction results in scar formation at the infarct site and deleterious remodeling in the infarct border zones and at distant sites, prevention and attenuation of these mechanisms are of clinical interest. To overcome these defects, valuable lessons may be learned in the zebrafish and newt since these animals regrow cardiac tissues with no scar tissue [42,43]. Injection of human mesenchymal stem cells into the acutely ischemic myocardium attenuated fibrosis, improved cardiac biomechanics, and reduced stiffness [170]. Furthermore, *Drosophila* may prove to be a powerful model for investigating the molecular basis of age-related cardiomyopathy. LV diastolic dysfunction is associated with aging [143], and senescent fruit flies experience decreased diastolic diameters, impaired myocardial relaxation, and increased myocardial stiffness, as measured in vivo by atomic force microscopy [178,194]. Such a model is genetically tractable, amenable to in vivo stiffness measurements, and recapitulates phenotypes observed in higher mammals [195]. This phenotype is dependent on integrin-linked kinase and β-integrin, implicating cell–ECM adhesion as a novel therapeutic target [178].

4. Conclusions and future directions

This review integrates the essential mechanisms of mechanotransduction underlying cell response to ECM dysfunction in the context of injury and disease by examining two specific cases, tendinopathy and HF. Tenocytes, TSCs, cardiomyocytes, and cardiac fibroblasts all sense ECM stiffness, one of the most profound and well-studied mechanical properties

influencing cell behavior, using integrin-based adhesion complexes that couple the ECM to the actin cytoskeleton.

Although the tendon and the heart are both soft tissues, they demonstrate distinct composition, structure, and function in both normal and diseased states. Tenocytes are present in lower cell densities in the tendon than cardiomyocytes in the heart. In HF, the stiffening of the heart tissue is a result of the production of fibrotic ECM by myofibroblasts as well as the stiffening of cardiomyocytes themselves in response to an altered microenvironment. Although the ECM in the tendon also adapts, changes in tenocyte biomechanics during disease progression remain unknown. The diseased state in tendinopathy is decreased stiffening of the ECM, whereas the diseased state in HF is increased stiffening.

Excessive loading from repetitive overuse activity causes TSCs to differentiate into non-tenocyte lineages which produce aberrant ECM components, further altering the mechanical environment and perpetuating the degeneration of native tendon properties. In the case of cardiac tissue, increased ECM stiffness causes fibroblasts to differentiate into myofibroblasts, which produce more ECM components and stiffen the local environment. Although the ECMs in the diseased tendon and heart are reorganized in dramatically different ways in response to their respective sources of disease onset, cells in these two tissues respond to changes in the same ECM mechanical properties using similar mechanotransduction machinery.

Despite this wealth of knowledge that highlights the central interaction between the ECM and cell mechanotransduction, the current treatments ameliorating dysfunctional ECM and cell mechanosensing are limited. In addition to continued work using human and animal model systems, additional knowledge may be gained from newt and zebrafish models that undergo unique ECM remodeling to regenerate heart and limb tissues without scarring [196–200]. Given that altered matrix stiffness often underlies tendinopathy and HF, understanding how this remodeling supports regeneration biochemically and mechanically may lead to novel therapeutic strategies for targeting the dysfunctional ECM.

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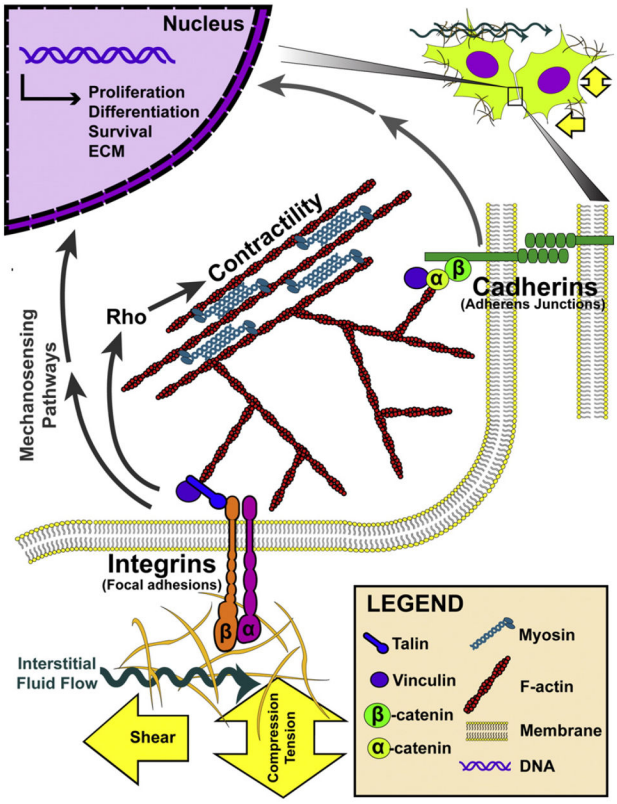

Fig. 1.

Model of cell–ECM and cell-cell mechanotransduction. Cells sense ECM stiffness and external loading by balancing the force between the actomyosin machinery and integrin adhesions. Important molecules involved in the generation of cytoskeletal tension include F-actin, myosin, and the Rho signaling cascade. It is noted that this illustration highlights only the basic subunits of these adhesion complexes, and that these components come together into higher order assemblies in vivo.

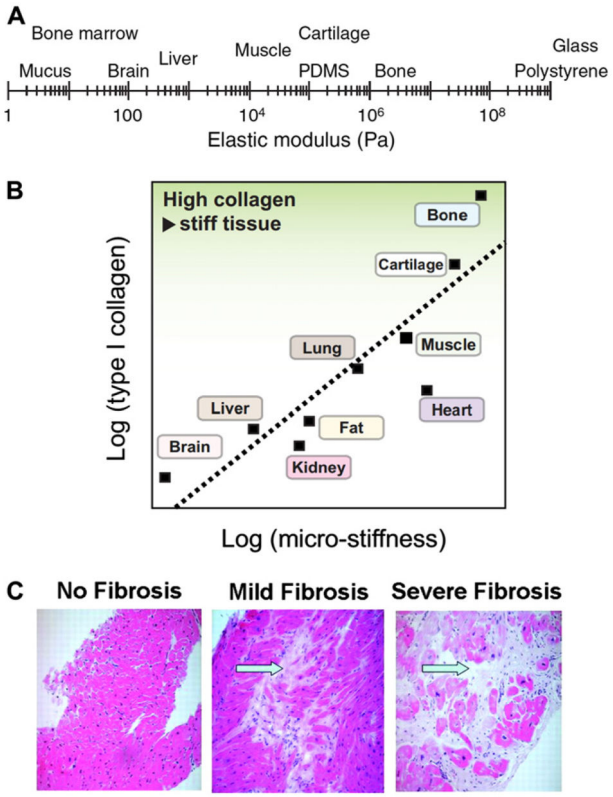

Fig. 2.

Tissue stiffness and its relationship to collagen content. (A) Tissues exhibit a wide range of stiffnesses as measured by the elastic modulus. (B) Tissue stiffness relates to the quantity of type I collagen. As the most prevalent protein in many tissues, collagen modulates mechanical properties of tissue. (C) Hematoxylin and eosin staining shows increasing severity of cardiac fibrosis with elevated collagen content (arrows) between muscle fibers (red), leading to tissue stiffening. Panel A: Reproduced with permission from Janmey and Miller [11]. Panel B: Reproduced with permission from Swift and Discher [10]. Panel C: Reproduced with permission from Weidemann et al. [44].

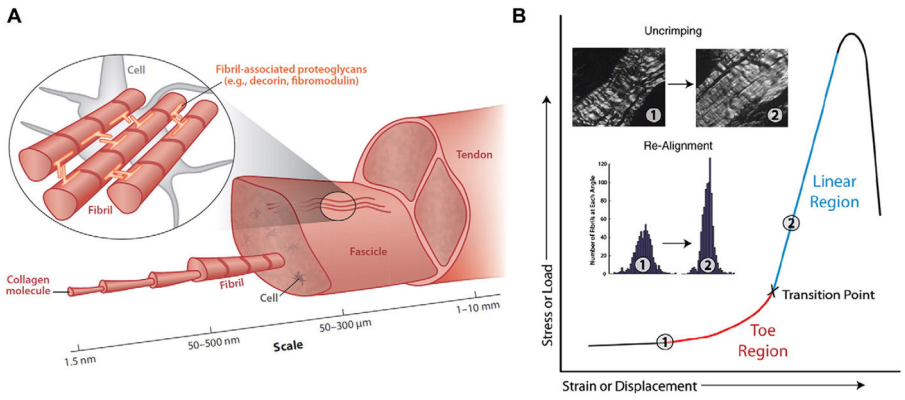

Fig. 3.

Tendon hierarchical structure and a typical stress-strain curve. (A) Tendons contain tenocytes (tendon fibroblasts) embedded in a hierarchical matrix of collagenous and noncollagenous components. Tendons are composed of fascicles, fibers, and fibrils that form from collagen molecules. (B) During mechanical loading, tendons exhibit nonlinearity in their stress strain curve, containing a toe region prior to a transition to a linear region. With increases in stress and strain, collagen fibers uncrimp and re-align. Panels A and B: Reproduced with permission from Connizzo et al. [201].

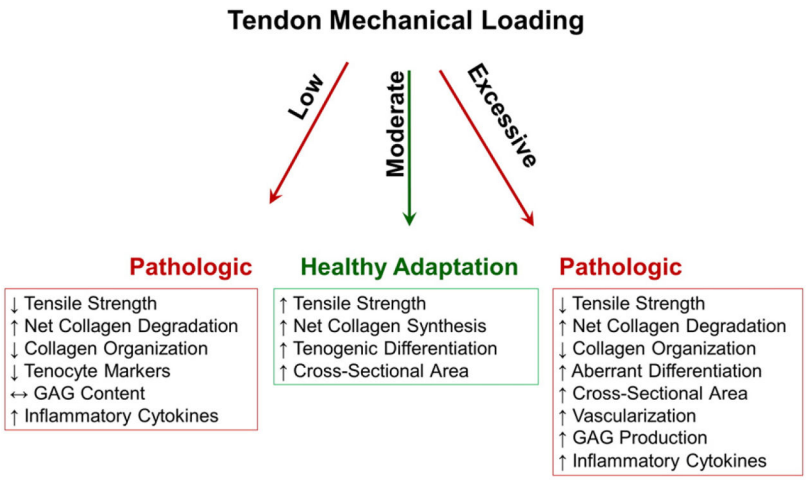

Fig. 4.

Various pathways that may result following mechanical loading in tendon. Low loading decreased tensile strength [80,82,110], collagen organization [80,110], and tenocyte markers [135]; net collagen degradation and inflammatory cytokines increased [82,135], and GAG content was unchanged [109]. Moderate loading increased tensile strength [84], net collagen synthesis [83,84,86,109,111], tenogenic differentiation [89], and cross-sectional area [84,85]. Knowledge regarding collagen organization, vascularization, GAG production, and inflammatory cytokines with moderate loading remains limited. Excessive loading decreased tensile strength [77,115] and collagen organization [91,95], and increased net collagen degradation [78,90], aberrant differentiation [89,92–94,118,119], cross-sectional area [73], vascularization [73,91,115], GAG production [114], and inflammatory cytokines [79,115].

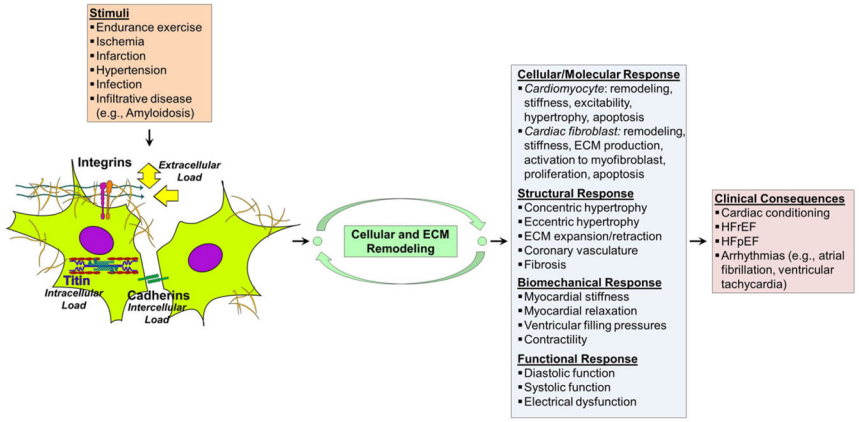

Fig. 5.

Feedback mechanisms of loading on cell–ECM, cell–cell, and intracellular proteins that regulate cytoskeletal architecture, remodeling, and functional response. Myocardial remodeling represents changes in the cell (fibroblasts and cardiomyocyte) and ECM compartments of the heart in response to physiologic (e.g., endurance exercise) and pathologic (e.g., ischemia, infarction, infection, infiltration, and hypertension) stimuli. This leads to changes in cardiac biomechanics (stiffness), electrophysiology, and function (systole and diastole). Adverse myocardial remodeling represents a major mechanism and endpoint leading to the development of HF. HFrEF — Heart Failure with Reduced Ejection Fraction, HFpEF — Heart Failure with Preserved Ejection Fraction.

Table 1

Engineering terms and definitions.

Term	Definition
Stress (σ)	Force (F) applied over an area (A), either perpendicular (e.g., pulling (tensile) and pushing (compressive)) or parallel (shear) to the material's axis; σ= F/A
Ultimate stress	Maximum stress before material failure
Strain (ε)	Unitless parameter quantifying the extent of deformation after application of stress. Strains are also categorized as tensile, compressive, or shear.
Principal strain	Maximum or minimum strain along the direction of zero shear strains
Strain stiffening	Increase in elastic modulus with applied strain
Stiffness (k)	Rigidity of a material defined as force (F) per resultant displacement (δ) (structural property); k = F/δ
Elasticity	Material property characterizing the ability of a deformed material to recover completely to its original state when the force is removed
Elastic modulus (E)	Resistance of a material to elastic deformation, measured by the ratio of stress and strain (material property); For linearly elastic materials, E = σ/ε
Viscoelastic	Combination of viscous and elastic mechanical properties. Viscosity refers to the resistance of fluid to flow, measured by the ratio of stress to the rate of strain or flow rate.
Poroelastic	Property of a porous material consisting of an elastic solid matrix and viscous fluid in which mechanical relaxations arise from fluid flow through the matrix.

Table 2

Medical terms and definitions.

Term	Definition
Angiogenesis	Formation of new blood vessels from existing ones
Arterial and venous tissue	Tissues pertaining to arteries and veins
Artery and veins	Blood vessels that carry blood away from and towards the heart, respectively
Ejection fraction	The fraction of total blood pumped from the heart with each heartbeat, typically measured by echocardiogram
Electrophysiology	Study of the electrical characteristics of cells and tissues
Infarction	Tissue death caused by a lack of oxygen supply (hypoxia)
Ischemic cardiomyopathy	Inferior ability of the heart to pump blood due to poor oxygen supply to the myocardium
Myocardium	Muscular tissue of the heart
Myocardial/cardiac or pulmonary fibrosis	Abnormal scarring of the muscular heart and lung tissues, respectively
Osteoarthritis	Degenerative musculoskeletal joint disease affecting articular cartilage and bone
Overuse	High repetitive loading such as labor-intensive work that may result in injury
Pressure overload	Pathological state of cardiac muscle whereby the heart contracts while experiencing excessive stress
Rotator cuff	Group of muscles and tendons that stabilize the shoulder joint. These include the supraspinatus, infraspinatus, teres minor, and subscapularis.