Chapter 2
Matrix Stiffness: A Regulator of Cellular Behavior and Tissue Formation

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Abstract The extracellular environment is an essential mediator of cell health and provides both chemical and mechanical stimuli to influence single and collective cell behaviors. While historically there has been significant emphasis placed on chemical regulators within the extracellular matrix, the role of the mechanical environment is less well known. Here, we review the role of matrix mechanics on cell function and tissue integrity. Cellular responses to mechanical signals include differentiation, migration, proliferation, and alterations in cell–cell and cell–matrix adhesion. Interestingly, the mechanical properties of tissues are altered in many disease states, leading to cellular dysfunction and further disease progression. Successful regenerative medicine strategies must consider the native mechanical environment so that they are able to elicit a favorable cellular response and integrate into the native tissue structure.

Matrix Mechanics Are Essential Design Parameters for Regenerative Medicine

Tissue engineering (TE) was defined in the late 1980s as a field concerned with "the application of the principles and methods of engineering and life sciences toward. . .the development of biological substitutes to restore, maintain, or improve functions" [111]. Motivated by a clinical need to restore normal physiologic function to tissues and organs that malfunction due to injury and disease, TE approaches may provide an avenue of treatment for patients with organ and tissue failure additionally plagued by increasing costs of care and donor shortages [63].

Significant numbers of investigations into biomaterials have confirmed that surface chemistry is a critical parameter contributing to the clinical success of

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implanted devices or TE constructs [118]. Surgery and implantation of biomaterial or TE constructs induces biochemical cascades that mediate the normal wound healing and foreign body responses that ultimately result in the success (functional integration into the tissue) or failure (rejection from the tissue, mechanical failure) of the implant. While the need to tailor the surface chemistry of an implant has been given significant attention for decades, the need to also consider the mechanical properties of an implant and its effects on cells has only been gaining momentum in recent years. Similar to surface chemistry, the mechanical properties affect the local behaviors of tissues and cells and contribute to the success of biomaterial and tissue-engineered implants.

While TE and regenerative medicine have recently focused on the micromechanical properties of a construct and its effects on cells, the notion that mechanical forces act as critical regulators of physiological processes at the cell and tissue level is not a new paradigm. Physical forces were known to contribute to the development of brain morphology [46] and bone remodeling [103, 128] as early as the late nineteenth century. Since then, elucidating the relationship between force and biological responses has spanned a variety of mechanical settings and length scales from probing the role of weightlessness on the musculoskeletal system during spaceflight [56] to understanding how shear stress in the vascular tree specifies endothelial cell phenotype [22]. These studies drew attention to the role of the physical environment as an important regulator of biological responses in living systems.

This chapter describes the role of the mechanical properties of the extracellular matrix (ECM) as a mediator of cellular responses and tissue formation. An overview of the nature of the mechanical properties of the cellular microenvironment and how it affects cellular function and tissue formation are discussed. Lastly, the role of matrix mechanics in disease states is presented.

The Cellular Response to Matrix Mechanics: Cellular Function Is Modulated by Local Matrix Stiffness

The Mechanical Environment of Cells

Cells *in vivo* are organized into tissues and organs that reside in complex mechanical environments. At the cellular level, the mechanical environment consists of endogenous (generated *by* cells) and exogenous (applied *to* cells) forces. Endogenous forces generated by cells on their ECM and neighboring cells largely result from cytoskeletal contractility (discussed below; [13, 76]). Examples of exogenous forces include gravity and tissue-specific interactions; for example, endothelial cells in the vasculature are subjected to pulsatile shear forces from blood flow [6] as well as migratory traction forces during leukocyte transmigration [94].

In addition to these actively imposed forces, the local stiffness of the ECM that serves as a biological scaffold is an important mechanical effector of cell function.

Stiffness is a measure of the ability of a material to resist deformation. In the body, tissue stiffness ranges several orders of magnitude, from adipose tissue (Young's Modulus E ~ several kPa) [106] to bone (E ~ GPa) [99]. In addition, tissue stiffness is not static, but changes during physiological processes including embryonic development, tissue remodeling during wound healing, and in pathological responses like tumorigenesis. Since there is an intimate association between cells and the ECM within tissues, and cells function in a variety of mechanical environments, many studies have investigated the mechanisms that cells use to sense and respond to their mechanical environment.

Biological Force Transducers

Tissue cells have an ability to sense and probe the stiffness of their surroundings as they adhere to and interact with the local ECM [28]. Mechanotransduction, where cells convert mechanical stimuli into chemical signals that affect cellular responses, occurs through a variety of mechanisms. Well-described mechanotransducers include stretch-mediated ion channels [74], primary cilia [8], and integrins [36, 100]. Additional mechanosensors, including G-protein receptors [70], cell–cell adhesions [57, 86], and the cytoskeleton [126] have been suggested. While these transducers sense the mechanical environment through a variety of mechanisms, they all share the ability to convert mechanical input into complex intracellular signaling cascades that ultimately regulate cellular responses including adhesion, spreading, migration, and proliferation [54]. The number and variety of mechanosensors identified in cells suggests that cells have a robust capacity to interact with their mechanical environment. This robustness is particularly important when considering that in addition to regulating normal physiological responses, abnormal mechanotransduction at the cellular level has been implicated in mediating a wide variety of prominent disease states including asthma [127], osteoporosis [2, 19], and cancer [51, 52, 115].

While it is likely that no single cell feature is responsible for driving all mechanobiological responses, the integrin family of proteins has emerged as a prominent and well-studied force transducer. The concept of a mechanical linkage between the ECM and the intracellular cytoskeleton was postulated in the mid-1970s [49], and the structure of integrins was determined in the next decade [116]. Composed of α and β subunits (18α and 8β subunits combine to form over 20 distinct integrin heterodimers to-date), integrin receptors are a family of transmembrane glycoproteins that serve as mechanical linkages between the ECM and the cytoskeleton [50]. On the exterior of the cell, integrins bind ECM protein ligands including collagen, laminin, and fibronectin [93]. Within the cell, the β subunit of integrin heterodimers binds to the actin cytoskeleton through a variety of adaptor proteins [66]. Integrins cluster into focal adhesions that spatially localize and

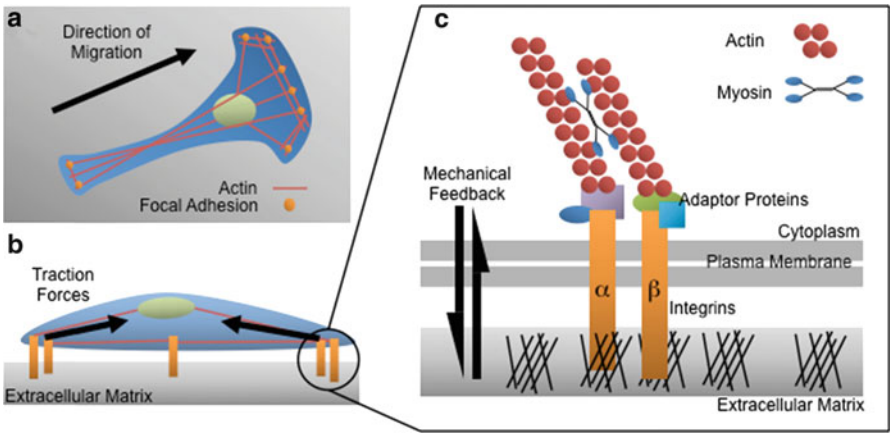

Fig. 2.1 (**a**) A typical cell migrating over a substrate utilizes actin stress fibers anchored to focal adhesions. (**b**) Together with the actin cytoskeleton, focal adhesions composed of integrins facilitate cell–substrate adhesion, contractility, and traction force generation. (**c**) A close-up depiction of a focal adhesion. Actin stress fibers are tensed by myosin motors and attach to integrin receptors via adaptor proteins within the cytoplasm. Integrin transmembrane receptors bind to the extracellular matrix outside the cell and participate in mechanosensing events

anchor actin stress fibers to the plasma membrane thus providing a mechanical linkage between the ECM and the cytoskeleton (Fig. 2.1a) [37]. Moreover, the integrin "adhesome" serves as a scaffold for a host of signaling proteins within the cell [132], suggesting that integrin receptors serve as prominent sensors and integrators of environmental signals.

Cells Sense Matrix Stiffness with Cellular Contractility and Traction Forces

"Stiffness sensing" means that cells have an ability to detect and respond to the mechanical resistivity of the extracellular environment. Stiffness sensing has been demonstrated in a variety of cell types including endothelial cells [17, 26, 96, 98], smooth muscle cells [31, 53], and transformed cells [67, 125]. The ability to sense stiffness is partly dependent on actomyosin-generated contractility that is transmitted to the extracellular environment through transmembrane integrin receptors that, with a number of intracellular signaling and scaffold proteins, organize into focal adhesions. Cells, in turn, respond to the stiffness of their substrate by altering cytoskeletal organization, cell–substrate adhesions, and other processes important for regulating cell behaviors.

Cellular contractility is generated in part by the actomyosin cytoskeleton. Actin stress fibers are tensed by myosin motors [61, 101], and cytoskeletal contractility is

transmitted to the ECM as traction forces (Fig. 2.1b, c) [65]. Cellular traction forces were first observed in landmark experiments as wrinkles or strains in flexible silicone rubber substrates [44]. Since then, methods have been developed to quantify traction forces generated by cells. Prominent techniques include traction force microscopy [25, 72] and the use of microfabricated post-array detectors [113, 117]. Other methods include the use of microfabricated cantilevers [35] and micropatterned silicone elastomeric substrates [4]. These techniques calculate traction forces based on strains created in the substrate by adherent cells. The ability of adherent cells to generate traction forces and cell–substrate adhesions facilitates sensing of the local extracellular environment and is involved in feedback mechanisms where matrix stiffness in turn modulates responses such as adhesion, spreading, and migration.

Matrix Stiffness Modulates Focal Adhesions, Cytoskeletal Assembly, and Traction Forces

The measurement of cell traction forces has helped to describe the role of force and focal adhesions as mediators of cell–substrate attachment and matrix stiffness. Experiments in real-time indicate that focal adhesion size is linearly dependent on the local force exerted by a cell [4]. Mature focal adhesions elongate and orient in the direction of actin stress fibers and applied force. However, the correlation of focal adhesion size with cell-generated forces may only hold for adhesions larger than 1 μm^2, as smaller adhesions are capable of exerting large traction forces that do not correlate with adhesion size [117]. Indeed, small nascent adhesions (focal complexes) at the leading edge of cells are capable of generating strong transient traction forces that drive cell migration [7]. Moreover, when cells on magnetic microposts are deflected by an external magnetic field, changes in traction force generation occur at sites of adhesion peripheral to the site of force application [112]. These data are indicative of a dynamic association between the actin cytoskeleton, cellular traction forces, and focal adhesions that mediates cell adhesion and migration.

Additional work has investigated focal adhesion organization with regard to matrix stiffness. Seminal experiments with fibroblasts and epithelial cells indicate that compliant ($E \sim 1$ kPa) substrates promote focal adhesions that are dynamic and irregular punctate structures [90]. In contrast, an increase in stiffness ($E \sim 30$–100 kPa) promotes the formation of stable arrays of elongated focal adhesions and an increase in tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin, suggesting that stiffness sensing involves intracellular signaling events. Such changes in focal adhesion organization suggest alterations in cell–substrate adhesivity. Accordingly, an increase in cell–substrate adhesion with increasing substrate stiffness has been demonstrated [32].

In general, stiff substrates increase both focal adhesion and cytoskeletal organization [31, 38, 41, 90, 130]. The formation of stable focal adhesions with increasing substrate stiffness is accompanied by changes in cell shape. For example, fibroblasts plated on compliant substrates are rounded with diffuse actin, while those plated on stiff substrates exhibit an increase in spread area and actin stress fiber organization [39, 130]. Similarly, endothelial cell spread area increases with increasing substrate stiffness [16, 97], where endothelial cells on compliant substrates adopt an elongated spindle-shaped morphology, while those on stiffer substrates exhibit more isotropic spreading [17]. Interestingly, endothelial cell stiffness is also modulated by matrix stiffness in 2D and 3D environments [15]. These data suggest an intimate association between substrate stiffness, cytoskeletal organization and cell shape, focal adhesions, and traction force generation.

The investigation of matrix stiffness as a mediator of cell shape has further elucidated the relationship between stiffness and force generation. It has been shown that matrix stiffness and cell shape help regulate the polarization and alignment of stress fibers within cells [134]. Indeed, matrix stiffness can alter cellular contractility [135]; traction force generation by fibroblasts and endothelial cells increases with increasing substrate stiffness [17, 41, 68]. Moreover, experiments with endothelial cells have demonstrated that both cell area and substrate stiffness are significant predictors of traction force generation [17]. In turn, the orientation and organization of the actin cytoskeleton helps determine cell shape; the ablation of a single stress fiber in a cell results in significant rearrangements in cell shape and cytoskeletal organization [61]. These data provide evidence for feedback mechanisms that relate matrix stiffness to cytoskeletal organization and traction force generation and provide a role for mechanotransduction as a contributor to cell shape.

The sensitivity of cellular traction force generation to matrix stiffness has implications for the organization of the local ECM. For example, the fibrillogenesis of the ECM protein fibronectin is mediated by endogenous cellular contractility [5]. Experiments with fibronectin-based native ECM scaffolds versus scaffolds stiffened by chemical crosslinking indicated differential scaffold remodeling by fibroblasts; native scaffolds were progressively remodeled over several days while cross-linked scaffolds were not [60]. These data indicate that there are feedback mechanisms that relate matrix stiffness to matrix remodeling and suggest that cellular responses to matrix stiffness may regulate ECM homeostasis.

Matrix Stiffness Modulates Cell–Cell Assembly, Migration, and Proliferation

In addition to modulating cellular contractility and force generation, matrix stiffness plays a role in mediating cell–cell interactions. Seminal work by Guo et al. established a relationship between matrix stiffness, cell–matrix, and cell–cell

interactions [43]. When heart tissue explants were plated on stiff matrices, cells from the tissue migrated out of the explant to cover the matrix. In contrast, cells in explants plated on compliant matrices did not migrate out of the explant. Separate studies with endothelial cells also indicate sensitivity of cell–cell interactions to matrix stiffness. On compliant substrates, endothelial cells prefer cell–cell interactions [98] and self-assemble into networks [16]. On stiffer substrates, ECs prefer cell–substrate interactions and fail to form network assemblies. In epithelial cells, cell–cell assembly is anisotropic along directions of stiff substrate and correlates with actin cytoskeletal organization and force generation [104]. These data suggest that matrix stiffness and traction forces modulate cell–cell organization.

Further work has investigated the role of matrix stiffness in mediating cell migration [55, 91]. For example, fibroblasts migrate toward substrates of increasing stiffness, a response termed durotaxis [68]. Smooth muscle cells also exhibit durotaxis with respect to the magnitude of substrate stiffness gradient [53]. These data indicate that substrate stiffness provides important cues that foster traction force organization responsible for cell migration. The sensitivity of cell migration to stiffness gradients may have important implications for disease states such as fibrosis or tumorigenesis that are accompanied in increases in ECM stiffness.

In addition to affecting migration, forces between contacting cells can also influence proliferation. Gray et al. found that the number of cell–cell contacts influences the proliferation of a cell in a bi-phasic manner [42]. Single cells are less proliferative than those with at least one cell–cell contact but increasing the number of neighbors inhibits proliferation. Interestingly, increasing the amount of cell–cell contacts may concurrently decrease the ability of cells to adhere to the ECM, thus decreasing proliferation. This response is essential for healthy tissue function where contractility, spreading, and proliferation are intricately regulated by cell–cell and cell–matrix adhesion and tension.

Collective Cell Responses to Matrix Mechanics: Implications for Tissue Development, Regeneration, and Repair

We have discussed the importance of matrix mechanics on individual cellular behavior and function. However, while single cell studies may be informative of cellular behavior, cells within tissues interact and respond collectively to stimuli. Similar to the influences on individual cells, mechanics are integral to overall tissue and organ physiology and mechanical alterations or disturbances can lead to disease and tissue malformation (discussed below). Interestingly, the earliest stages of embryonic development, tissue patterning, and organ formation are governed, in part, by mechanical interactions with the extracellular environment [21, 82, 110]. Studying these interactions can inform the design of tissue engineered and regenerative therapies.

Mechanical Stimuli Influence Embryonic Development

Throughout embryonic development, all tissues of the body are derived from a single-fertilized cell via a complex process of specification and differentiation. Cellular differentiation is the process whereby a cell with an unspecified fate is influenced by genetic, chemical, and mechanical [14] factors to become a specific cell type. A fully differentiated cell maintains its gene expression patterns through generations of proliferation and has a distinct role within an organized tissue. During embryogenesis, biochemical factors and pre-programmed genetic cues initially dictate the polarity of the embryo as well as the cell lineage specification of its progeny into the three germ layers: ectoderm, endoderm, and mesoderm [34, 92]. Concurrent with these chemical and genetic signals, mechanical stimuli reinforce and further specify cell fate and play a crucial role in the development of the unique tissues and organs of the body [34]. Specifically, mechanical signals such as pressure, fluid flow, shear stress, tension, and stiffness are important regulators of embryogenesis and have been shown to affect the development and tissue patterning of many major organs [71] including the eye [45, 82], heart [48, 89], vasculature [77], and neural tube [136].

Further investigations into developmental processes have indicated that matrix mechanics play a vital role in proper tissue development throughout the entire embryo. Recent work in *Xenopus* has confirmed a temporal and spatial distribution of mechanical stiffness within developing embryos due to the contraction of the actomyosin network [136]. This cytoskeletal contraction not only increases the stiffness of the surrounding tissue structures as much as 50-fold within 8 h, but may also drive the formation of the neural tube and allow for further cell patterning and differentiation [136]. Similarly, repeated and coordinated contractions of the actomyosin cortex in *Drosophila* embryos create tension between cells that facilitate cell invagination and formation of the ventral furrow [73]. These data indicate that intra- and inter-cellular contractility drive tissue morphogenesis.

In addition to the exogenous mechanical stimuli within developing tissues, differential adhesion and repulsion between cells and the surrounding matrix plays an integral role in embryonic tissue morphogenesis [114, 121]. It has been shown that the ectoderm–mesoderm boundary is not only maintained by self-sorting due to preferential adhesion of similar cells to each other, but is also a function of the active repulsion between unlike cells [102]. Interestingly, the development of structures within the retinal epithelium in *Drosophila* embryos mimics the formation of soap bubble aggregates, where the surface tension is minimized during aggregate formation [45]. This patterning occurs due to differential adhesion between cells with the most adhesive cells forming central aggregates surrounded by less-adhesive cells to minimize the "surface energies" of the cell contacts. Similarly, during a phase of embryogenesis known as epiboly, cell adhesion proteins are differentially expressed so that a group of cells can migrate toward the vegetal pole of the embryo and begin gastrulation [110]. These data indicate that tissue formation is influenced by the balance of cell–cell and cell–substrate adhesion.

The mechanical environment is intimately linked with collective cell behavior such as contractility, adhesion, and tissue patterning during embryogenesis. Importantly, matrix mechanics can regulate cellular specification and tissue formation. Regenerative strategies may exploit these responses to mechanical stimuli to produce organized cellular structures that mimic the original, healthy tissues.

Mechanical Control of Cellular Differentiation

In addition to embryogenesis, mechanical cues play an integral role in maintaining and influencing cell fate and tissue maintenance throughout life. While the process of differentiation is most obvious during embryonic development, some cells (e.g., stem cells) remain multipotent even in adult tissue [80]. These stem cells are essential for tissue maintenance and repair, may have important implications for disease progression, and have been the focus of many engineered tissue therapies. Importantly, each of these processes is influenced by the mechanical properties of the surrounding environment.

Although initial tissue engineering strategies were concerned primarily with maintaining the mechanical integrity of the implant, current therapies look to integrate mechanical cues to differentiate and pattern cells into complex tissues. Stem cells have been a popular choice for regenerative medicine research since they are capable of self-renewal and differentiating into multiple cell types [80]. The stem cell niche, the 3D microenvironment surrounding the cells, is a key factor in their maintenance and differentiation [9, 29, 124]. To further understand the factors that influence stem cell differentiation in 2D and 3D, synthetic and natural scaffolds have been used to probe the interactions of the cells with their extracellular environment [27]. Many groups have combined novel materials and chemical cues to encourage stem cell differentiation along a chosen lineage in the hopes of creating regenerative therapies [69].

Endogenous cellular stiffness is predominantly regulated by the actomyosin cytoskeleton and has been shown to change during differentiation [64]. Using AFM, Titushkin and Cho observed that mesenchymal stem cells stimulated with osteogenic medium became less stiff throughout their course of differentiation [119]. In contrast, cells differentiated from mouse embryonic stem cells are tenfold stiffer than their precursors [21]. Similarly, Pajerowski et al. found that the nucleus of human embryonic stem cells becomes sixfold stiffer when terminally differentiated (Fig. 2.2a) [87]. These results suggest that the mechanical properties of cells depend on both the origin and differentiation stage of the stem cells.

Matrix mechanics are also known to be independently capable of dictating stem cell differentiation into different lineages. In a seminal study, Engler and colleagues demonstrated that mesenchymal stem cells can be stimulated to differentiate into neurons and osteoblasts when plated on soft and stiff matrices, respectively, that were chemically similar (Fig. 2.2b) [33]. Recently, scientists have exploited the ability of stem cells to sense and respond to their mechanical environment to create

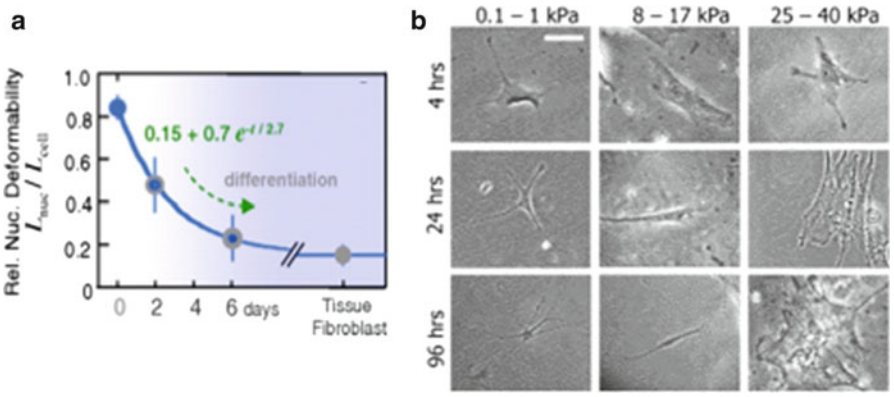

Fig. 2.2 (**a**) During differentiation, the nuclear compliance of human embryonic stem cells decreases (stiffness increases) relative to the cellular cytoplasm. Reprinted with permission from PNAS 104(40): Pajerowski et al.: Physical plasticity of the nucleus in stem cell differentiation, 15619–15624, Copyright 2007 National Academy of Sciences, U.S.A. [87]. (**b**) Mesenchymal stem cells sense and respond to substrate stiffness by changing differentiating to neural cells and myoblasts on soft and stiff substrates, respectively. Reprinted from Cell 126(4): Engler et al.: Matrix Elasticity Directs Stem Cell Lineage Specification, 677–689, Copyright 2006 [33], with permission from Elsevier

scaffolds that vary in stiffness spatially such that an entire tissue might be created by simply seeding the engineered matrix with stem cells [20, 59, 109, 123]. Very recent work indicates that mesenchymal stem cells plated on a stiffness gradient directionally migrate toward the stiffer portions of the substrate and subsequently differentiate [123]. Interestingly, the cells that migrate from soft to stiff regions of the substrate maintain neuronal markers similar to the cells that are plated on uniformly soft substrates [123]. Importantly, these results suggest that even though the cells in a specific lineage may become differentiated, they are able to retain a "memory" of the previous signals they have received. These data suggest that mechanical microenvironmental cues are essential to the promotion and preservation of stem cell lineage specification and, to produce a functional tissue replacement, will be required design parameters for regenerative therapeutics.

Matrix Mechanobiology Alterations in Disease and Injury

Altered tissue mechanics are a prominent feature of many injured diseased tissue states and are commonly a result of abnormal ECM deposition, matrix cross-linking and/or matrix degradation. Specifically, matrix stiffening accompanies aging [23], cardiovascular disease [105], wound healing [40], and tumor formation [85]. Native ECM mechanics can be modified by changes in protein deposition or cross-linking of preexisting matrix components. These changes in matrix mechanics can lead to aberrant cell behavior that can cause or exacerbate disease states [3, 62].

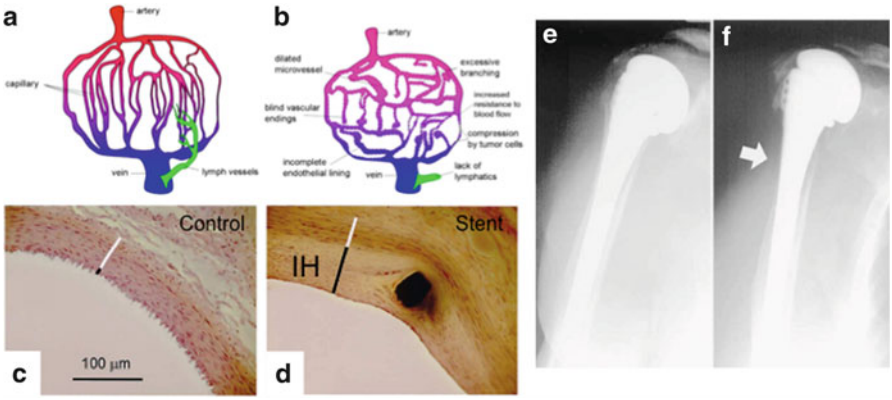

Fig. 2.3 (**a**, **b**) A cartoon depicting the vascular system in a normal tissue (**a**) and in a solid tumor (**b**). Reprinted with permission from Oxford University Press from Trédan et al.: Drug Resistance and the Solid Tumor Microenvironment. Journal of the National Cancer Institute 99(19):1441–54 [122]. (**c**, **d**) Measurements of intima (*black bars*) and media (*white bars*) in control (**c**) and stented (**d**) rabbit carotid arteries. *IH* intimal hyperplasia. Reprinted with permission from Oxford University Press from Alp et al.: Increased intimal hyperplasia in experimental vein graft stenting compared with arterial stenting: comparisons in a new rabbit model of stent injury. Cardiovascular Research 56(1):164–72, 2002 [1]. (**e**, **f**) Clinical radiograph taken immediately after shoulder prosthesis implantation (**e**) and after 7 years of follow-up (**f**). The arrow in (**f**) depicts a region of cortical bone resorption. Reprinted from the Journal of Shoulder and Elbow Surgery 12(1): Nagels et al.: Stress shielding and bone remodeling in shoulder arthroplasty, 35–39, 2003 [81], with permission from Elsevier

In general, tumor tissues have altered mechanical properties as compared to native, healthy tissue [83, 85, 108, 131]. In fact, breast cancer is often first detected by the patient or physician finding a palpable mass or lump that is stiffer than the surrounding tissue. Large tumors are associated with an increase in local ECM stiffness and angiogenesis, an in growth of newly sprouted blood vessels that facilitate increased tumor mass (Fig. 2.3a, b) [122]. The increase in ECM stiffness is primarily due to increased collagen deposition and cross-linking within the tumor stroma [85], but a disruption in the tensional homeostasis of the cells may also contribute [88]. As discussed previously, changes in the stiffness of the ECM can lead to phenotypic cellular changes such as increased proliferation and migration. Indeed, Paszek and colleagues found that increasing substrate stiffness correlated with changes in cytoskeletal tension, integrin expression, cellular proliferation, oncogene activity, and tissue formation in mammary epithelial cells [88]. Additionally, tumor cell migration was found to be modulated by the stiffness of the ECM [133]. These results indicate that the increased mechanical stiffness of the surrounding ECM that accompanies tumor progression may, in fact, drive malignancy.

ECM stiffening is also known to be a critical factor in the progression of cardiovascular disease. Vessel stiffening occurs through a number of mechanisms including glycation, the formation non-enzymatic cross-links (also known as

advanced glycation end products or AGEs) within the ECM [23]. These post-translational biochemical alterations cause tissue stiffening and prevent cellular remodeling of the existing tissue [79]. For example, the greater prevalence of reducing sugars such as glucose and ribose within the blood of diabetic patients leads to increased cross-link density of collagen and elastin, and consequently increased stiffness of the vasculature when compared with non-diabetics [12, 24]. These alterations in the mechanical environment cause changes in cellular behavior and result in an inability to maintain proper vascular tone and regulate blood pressure effectively [58]. Together, these changes contribute to the increased prevalence of cardiovascular disease in diabetic patients. These data indicate that changing the matrix mechanics of a tissue can lead to disease.

Tissue stiffening also accompanies wound healing. Unfortunately, most of the time the body is unable to perfectly replicate the native tissue structure and a scar is formed at the site of an injury. In some areas of the body, such as the skin, a small scar does not typically impair function. However, in other regions of the body such as the central nervous system, scar formation can cause the tissue to severely malfunction [75]. Specifically, within the brain and spinal cord, tissue injury leads to glial scar formation which acts as a mechanical barrier and inhibits signal transduction [47]. In a study that investigated the molecular changes that occur during glial scar maturation, Camand et al. found that fibronectin matrix deposition inhibits axonal growth and healing [18], but promotes astrocyte attachment as a mechanism of physically separating the injured site from the surrounding tissue [95]. To better understand how the mechanical cues from the glial scar affect cellular function, Georges and colleagues investigated the response of astrocytes and cortical neurons to matrix stiffness [40]. Interestingly, they found that while the cortical neurons were able to spread and extend neurites on both soft and stiff surfaces, the soft substrates were not conducive to astrocyte growth. These data suggest that the mechanical properties of the glial scar are promoting astrocyte recruitment and barrier formation, thus limiting axonal regeneration. These results suggest that matrix mechanics play a key role in wound healing and tissue regeneration.

Just as perturbations in native tissue mechanics can lead to disease states, regenerative tissue engineering therapies can also facilitate the formation and progression of disease when the mechanical properties of the native tissue are not recapitulated. One prominent example is intimal hyperplasia (IH), a response characterized by thickening of the blood vessel wall due in part to the proliferation and migration of smooth muscle cells from the medial layer of the vessel wall and increased ECM deposition (Fig. 2.3c, d) [84]. Notably, mechanical differences in the matrix have been shown to induce migration [129] and proliferation [11] of vascular smooth muscle cells, both hallmarks of IH. The causes of IH stem from mechanical damage to the endothelium due to compliance mismatch between synthetic vascular grafts and native vascular tissue at sites of anastomoses [105] and changes in blood flow characteristics or luminal diameter at the anastomosis [107]. IH is ultimately responsible for poor patency after bypass grafting [78, 120] that may require additional surgical intervention. Similarly, mechanical mismatch between implant and native tissue also occurs in orthopedic implants that reduce the

physical loading on nearby bone tissue. This phenomenon, known as stress shielding, results from the difference in stiffness between the orthopedic implant and the host tissue, and results in bone resorption and osteopenia (Fig. 2.3e, f) [30]. Such changes at the bone–implant interface may ultimately allow micromotion that facilitates implant loosening, osteolytic particle debris [10], and implant failure.

These examples demonstrate that matrix mechanobiology plays a significant role in promoting a diseased phenotype. Moreover, they illustrate that the mechanical properties of engineered regenerative therapies are a critical design consideration for implant success.

Conclusions

The mechanical properties of tissues are not only important for maintaining macroscale mechanical integrity but also essential regulators of cellular function. Cells sense stiffness using structures such as integrins to attach to the ECM and then respond and, oftentimes, remodel their environment by generating traction forces via actomyosin contractility. When alterations are made to the extracellular mechanical environment, cells can react to these mechanical stimuli by influencing tissue development, cellular differentiation, or disease progression. An understanding of how the mechanical properties of the ECM contribute to cell responses and tissue formation will ultimately further the understanding of disease states associated with aberrant mechanosensing and guide the design parameters of successful biomaterials and TE constructs. Future tissue engineering strategies should work to produce biomaterials and implants that are not only chemically favorable, but also integrate mechanical cues that dictate cellular behavior to aid in cellular differentiation and tissue regeneration.

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