

Chapter 5

The Role of Mechanical Forces in Guiding Tissue Differentiation

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Abstract Stem cell differentiation is regulated by a diverse array of extracellular cues. Recent evidence suggests that mechanical interactions between extracellular matrix (ECM) and cell surface receptors as well as physical interactions between neighboring cells play important roles in stem cell self-renewal and differentiation. It is also becoming clear that the ECM effects cellular behavior through many physical mechanisms, such as ECM geometry, elasticity, and the propagation of mechanical signals to intracellular compartments. Considerable effort is being targeted at developing biomaterials that exploit cellular microenvironments in guiding cells to desired phenotypes and organizing these into functional tissues. Improved understanding of the interactions between stem cells and their physical environment should yield new insight into the mechanisms governing their activity and allow the fabrication of artificial ECM to promote tissue development.

Abbreviations

CAD	Computer-aided design
ECM	Extracellular matrix
LINC	Linker of nucleoskeleton and cytoskeleton
MRTFs	Myocardin-related transcription factors
MSCs	Mesenchymal stem cells
SRF	Serum response factor
STARS	Striated muscle activator of Rho signaling

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5.1 Mechanotransduction

Embryonic development is marked by dynamic, adaptive self-assembly, and self-organizational processes over the course of gastrulation and subsequently, during the formation of nascent organs. While chemical gradients and genetic regulatory networks certainly play important roles in morphogenesis, it is clear that the expression of genetic markers is necessary, but not sufficient, to explain differentiation. Microenvironmental chemistry and genetic synchrony are choreographed with mechanical signaling cues to drive development [1]. Increasing evidence suggests that epigenetic factors include mechanical and structural cues that play essential roles in embryogenesis and organogenesis [1, 2]. For example, mechanical tension in the cytoskeleton arising from physical interactions between neighboring cells and adhesion of cells to the ECM has been shown experimentally to contribute to epithelial branching and angiogenesis during lung development [3]. Moreover, branching morphogenesis during angio- and vasculogenesis arises from a complex interplay between tension exerted by epithelial cells on the ECM and regional differentials in ECM turnover by matrix metalloproteinases that creates localized fluctuations in ECM rigidity [4].

Direct physical interactions between cells play a vital role in development as well. Regulation of transcriptional programs via the Wnt/ β -catenin signaling pathway mediated by cadherins junctions has been found to play a key role in the epithelial budding that gives rise to structures such as hair follicles [5]. This process of converting physical forces into intracellular biochemical responses is referred to as mechanotransduction [2]. As research in the field of stem cell biology has progressed, an increasing interest has arisen in evaluating the role of mechanotransduction in stem cell lineage commitment and its potential for exploitation in the development of regenerative therapies [6, 7]. Coordinated interactions with soluble factors, other cells, and extracellular matrices define a local biochemical and mechanical niche that stem cells occupy in vivo [8]. The ECM in this niche influences stem cell behavior both by providing mechanical signals and by physically trapping growth factors, limiting their diffusion, and regulating the temporal dynamics of paracrine signaling within the niche. A better understanding of the mechanisms of mechanical interaction between stem cells and the niche microenvironment will be important for directing the development of synthetic niches for therapeutic stem cell delivery [9].

5.1.1 *The Role of Cell–Extracellular Matrix Interactions in Differentiation*

Cellular interactions with the ECM play an essential role in tissue formation, as shown in the heart where coordinated expression of specific ECM and integrin isoforms direct the proliferation and differentiation of early myocytes [10]. During fetal

development, the ECM undergoes rapid changes in its composition and this change is associated with alterations in the expression of α -integrin isoforms that specifically recognize various ECM components [11]. Stem cells play an important role in tissue homeostasis and injury repair throughout the lifetime of an individual and thus must reside in an environment that maintains a balance between self-renewal, quiescence, and cell fate commitment. The mechanisms through which the stem cell niche maintains a population of self-renewing undifferentiated cells while simultaneously expelling differentiating daughter cells have been studied extensively, such as in bone marrow and intestinal crypts, where stem cell niches have been found to reside and participate in tissue development [12]. Niche localization and asymmetric division of stem cells is widely regarded to be a product of the specific intercellular and cell–ECM interactions that are characteristic of the stem cell compartment (Fig. 5.1a) [12, 13]. Uncommitted stem cells have been observed to express high levels of β 1-integrins in the niches of a number of tissue types [13]. Thus, transmission of mechanical signals from the ECM to intracellular signaling pathways via transmembrane integrin receptors may play a prominent role in regulating cell cycle entry and stem cell fate decisions.

5.1.1.1 Signaling Through the Integrin–ECM Interphase

Magnetic twisting cytometry experiments have shown that the transmembrane integrin receptors form a direct mechanical linkage between the ECM and the cytoskeleton [14]. Since this report, integrins have been demonstrated to serve as the primary conduit of bi-directional signaling between cells and the ECM, despite the fact that they do not possess intrinsic kinase activity [15]. Rather, integrins transmit information, encoded as mechanical forces, to the cytoskeleton that in turn activate mechanosensitive signal transducers, such as focal adhesion kinase that are able to translate the mechanical signal into a biochemical response. Integrin-mediated mechanotransduction has been shown to activate a myriad of chemical signaling pathways, including the Rho kinase, PI3K, ILK, Src, ERK, and MAP kinase pathways that modulate gene expression and direct important cellular activities, such as cell cycle progression and the induction of apoptosis (Fig. 5.1b) [2, 15, 16]. Many of these signaling molecules, along with biochemical mediators of transcription and protein synthesis, do not freely diffuse throughout the cytoplasm. Rather, they are immobilized on the cytoskeleton, and are thus subject to mechanical perturbations of the cytoskeleton, modulating their activity and translocation to cellular compartments, such as the nucleus [13].

5.1.1.2 Mechanical Force Balance and ECM Stiffness in Mechanotransduction

Mechanotransduction may be mediated simultaneously at multiple locations inside the cell through force-induced rearrangements within a tensionally integrated

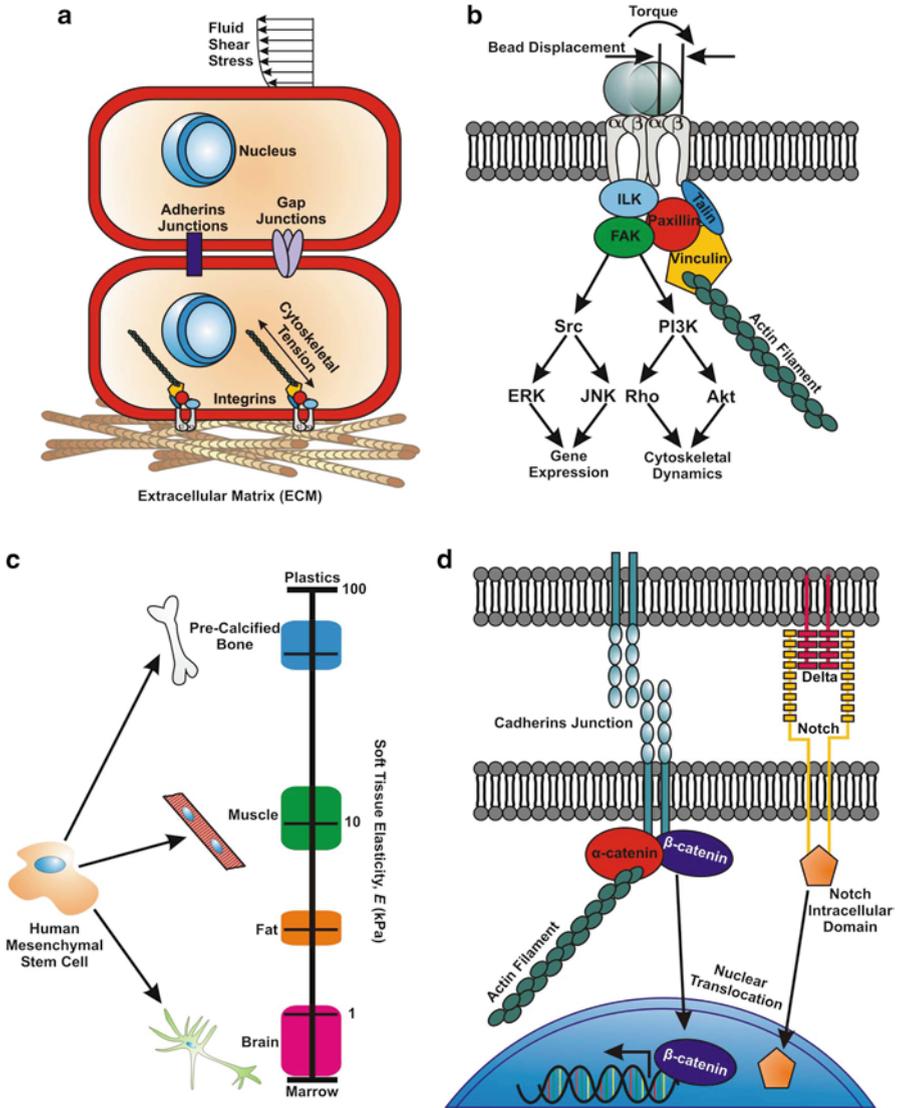


Fig. 5.1 Mechanotransduction in the stem cell niche. (a) Mechanical interactions between neighboring cells and with the ECM govern the response of stem cells to physical signals, such as tensile, compressive, and fluid stresses present within their local microenvironment. (b) Magnetic twisting cytometry experiments show that transmembrane integrin receptors form a direct mechanical linkage between the ECM and the cytoskeleton that can activate a number of intracellular signaling pathways. (c) The force balance between the ECM and the cytoskeleton allow naïve mesenchymal stem cells to adopt different fates depending on physical properties of the ECM, such as elastic modulus. (d) In addition to mechanotransduction through the integrin–ECM interface, stem cells also respond to mechanical signals from neighboring cells through intercellular junctions and direct transmembrane ligand–receptor interactions

cytoskeleton [14]. This force balance between the cytoskeleton and the ECM allows cells to respond to variations in matrix compliance in a distinctive manner [17, 18]. Physical properties, such as elastic modulus, can vary considerably between, and within, organs. The elastic modulus of brain tissue has been measured to be on the order of 1 kPa, while those of muscle and bone are approximately 10 and 100 kPa, respectively [19]. These variations in stiffness are as a result of variety of factors, including cell demographics, extracellular heterogeneities such as ECM, sinuses, and the extent of the interstitial space. Cells have developed a variety of intra- and intercellular mechanisms to optimize these material properties for physiological function. For example, myosin-II motors play an essential role in force-feedback response of stem cells to matrix elasticity [19, 20]. Phenomena observed in vitro, such as durotaxis where cells crawl up stiffness gradients, have lead many researchers to postulate that the mechanical microenvironment can influence tissue morphogenesis and stem cell fate choices [17]. Studies on embryonic cardiomyocytes reveal that changes in matrix rigidity associated with heart morphogenesis and fibrotic ECM remodeling caused by myocardial infarction dramatically affect rhythmic contraction of the cells [21]. In the case of marrow-derived MSCs, studies have shown that their lineage commitment is influenced by the elastic modulus of the substrate they are grown on. Culturing naïve MSCs on elastic substrates with a modulus of around 1 kPa promoted neurogenic differentiation, whereas growth on stiffer substrates, 10 kPa modulus, induced myogenic differentiation, and 100 kPa modulus substrates resulted in osteogenic lineage commitment (Fig. 5.1c). Further, experiments with the myosin II ATPase inhibitor blebbistatin showed that the elasticity-dependence of stem cell fate specification could be ameliorated by the inhibition of nonmuscle myosin II activity [19].

5.1.2 Intercellular Contact-Based (Juxtacrine) Mechanotransduction

In addition to force transmission across the integrin–ECM interface, cells also receive mechanical signals from their neighbors through intercellular junctions and through direct transmembrane ligand–receptor interactions (Fig. 5.1d). The specification and proper arrangements of new cell types during tissue differentiation require the coordinated regulation of gene expression and precise interactions between neighboring cells, interactions that target transmembrane Notch receptors and the Wnt signaling intermediates localized to adherens junctions [22]. Cytoskeletal tension plays a key role in the formation and maintenance of adherens junctions during embryogenesis. Studies quantifying force transmission between endothelial cells across adherens junctions showed that this “intercellular tugging force” was associated with increases in the size and strength of adherens junctions, and in turn, regulated tissue architecture [23].

The synthesis of gap junction channels is closely tied to the formation of adherens junctions [24]. Gap junctions are intercellular channels that allow the direct exchange of ions and biomolecules smaller than 1 kDa between the cytoplasm of adjacent cells. In cardiomyocytes, it has been found that N-cadherin and connexin 43 share a temporal relationship in their expression and spatial co-localization during adherens junction formation [25]. Further, mechanical forces acting on myocytes during contraction *in vivo* and pulsatile stretch *in vitro* were found to cause a dramatic increase in the expression of connexin 43 and a concomitant increase in conduction velocity due to increased electrical coupling between myocytes [24]. Mechanical loads placed on myocytes by contraction and pulsatile stretch were found to induce mechanotransductive signaling events through the $\beta 1$ -integrin-ECM interface that were responsible for the upregulation of N-cadherin and connexin 43 observed [26]. Studies of human embryonic stem cells reveal that they express both connexin 43 and connexin 45 that are assembled into functional gap junction channels [27]. Altogether, these results reveal that mechanical interactions between cells can influence chemical signaling by providing alternative pathways for signal transmission that potentially act on faster time scales than paracrine signaling through extracellular diffusion gradients.

5.1.2.1 Notch Signaling Pathway

The Notch family of transmembrane receptors participates in an evolutionarily conserved signal transduction pathway that has been found to affect stem cell differentiation in a time- and context-dependent manner. Notch receptors mediate cell fate decision in multiple organs, including the skin, brain, and heart [8, 28]. Neighboring lineage committed cells present a transmembrane ligand known as Delta that interacts with and activates the extracellular domain of Notch receptors presented by an uncommitted stem cell when the cells come into physical contact with one another. Thus, the Notch receptor acts as a “touch sensor” for cells sharing the same tissue compartment, allowing them to sense and respond to the developmental activity of their neighbors. The spatial localization of Notch receptors and ligands in the cell membrane has also been found to affect the signaling response initiated upon Notch activation, although the mechanisms of Notch trafficking are still largely unknown [29]. Upon activation, an intracellular fragment of the Notch receptor is proteolytically cleaved and subsequently translocates to the nucleus where it initiates transcription to promote either proliferation or lineage commitment in a context-dependent manner [29]. Activation of Notch in neural stem cells has been associated with expansion of the uncommitted cell population both during development and in response to ischemic injury [30]. Notch1 activation in cardiac progenitor cells gives rise to a population of Nkx2-5 expressing transit amplifying myocytes that mediate postnatal growth of the myocardium [28]. Taken together, the results of these studies suggest that Notch serves as a mechanical signaling relay between cells within the stem cell niche that provides greater spatial and temporal precision than soluble cytokine gradients.

5.1.2.2 Wnt/ β -Catenin Signaling Pathway

Wnts are secreted lipid-modified signaling peptides that play a ubiquitous role in development. Canonical Wnt signaling involves translocation of β -catenin from cadherins junctions to the nucleus where it interacts with a number of transcription factors to mediate transcription [22]. During embryonic development, Wnt signaling is necessary for the establishment and maintenance of cell polarity during gastrulation by modulation of actin cytoskeletal organization and contraction via its activation of the Rho signaling pathway. It is speculated that mechanical regulation of Wnt activity during embryonic development could serve as “mechanical checkpoints” that ensure certain structural criteria are met before the next stage of development proceeds [31]. Maintenance of the hematopoietic stem cell niche in bone marrow has been shown to depend on N-cadherin intercellular junctions with osteoblasts cells that serve to regulate β -catenin activation by Wnt [22]. The activity of the Notch and Wnt/ β -catenin signaling pathways has been found to have reciprocal effects in cardiac progenitor cells. Notch1 signaling promotes differentiation of cardiac progenitor cells and negatively regulates the activity of β -catenin. Activation of β -catenin by the canonical Wnt pathway inhibits differentiation by negatively regulating cardiac transcription factors and instead promotes proliferation of cardiac progenitor cells [32]. Altogether, these studies reveal that cells possess signaling modalities beyond just the traditional chemical signaling pathways associated with development and that these mechanical signaling intermediates play important roles in tissue formation.

5.2 Role of Cell Geometry and Cytoskeletal Dynamics in Differentiation

The ECM provides a number of contextual signaling cues during tissue formation that act by exerting tension on the cytoskeleton [13]. Cells respond to these signals from the ECM to “tune” their mechanical properties through cytoskeletal remodeling. Human MSCs cultured on micropost arrays adopted either an adipogenic or osteogenic phenotype depending on the stiffness of the microposts, with stiffer microposts promoting the osteogenic lineage and softer microposts promoting adipogenesis. It was postulated that the observed dependence of lineage commitment was due to changes in Rho-mediated cytoskeletal contractility in response to matrix elasticity and that the cytoskeletal architecture of naïve MSCs could be used to predict the fate they will ultimately adopt [7, 33]. Several studies have examined the influence of specific physical stimuli, such as tension, compression, and fluid shear stress on stem cell behavior to characterize the biophysical mechanisms that govern lineage commitment [34]. Experiments on *Drosophila melanogaster* embryos showed that acto-myosin-mediated tensional forces promoted proliferation, while compression suppressed it. These opposing physical forces are transmitted throughout the developing tissue and continually feed back to regulate tissue shape and organization [31].

5.2.1 Effects of Mechanical Microenvironment on Cellular Organization

Local variations in ECM mechanics act as motility cues that direct local cell growth differentials critical in organogenesis and wound healing [35]. The orientation of the mitotic spindle in dividing cells, and thus the division plane and spatial arrangement of daughter cells is affected by the spatial distribution of ECM proteins [36]. When grown on planar substrates *in vitro*, mammalian cells exhibit random walk motion. However, observations of pattern formation in epithelial and endothelial tissues revealed that cells migrated in a coordinated fashion [37]. Haptotaxis is widely regarded to be responsible for cohort migration at the macroscopic tissue scale, but at the scale of the cell's local microenvironment, boundary conditions imposed by ECM topology, adjacent cells, and heterogeneities in the interstitial space provide the symmetry breaking cues that initiate the formation of specialized tissue patterns (Fig. 5.2a) [37]. Evaluation of the motility of cells grown on isolated ECM islands reveals that the direction of cell motility is defined by the topological organization of the cytoskeleton with respect to geometric cues in the ECM, the resulting tractional forces exerted by cells on the substrate, and the subsequent, spatially segregated activation of Rac, Rho, and cdc42 [35]. Cells grown on polygonal ECM islands exerted the greatest tension forces at the corners of the islands and this localization of mechanical force was associated with the localization of lamellipodia and filopodia to the corners as well. Taken together, the results of these studies indicate that the spatial organization of cells during tissue morphogenesis is the product of a complex interplay between mechanical guidance cues imposed by the ECM and tractional forces exerted on the ECM by cells mediated by dynamic rearrangement of the cytoskeleton and focal adhesions that serve to "steer" the direction of cell movement in response to the force-balance between cells and the ECM (Fig. 5.2b). Examination of these mechanisms in differentiating stem cells could prove useful in linking multicellular organization to spatial differentials of cell differentiation within a tissue [38, 39].

5.2.2 Effects of Mechanical Microenvironment on Cellular Shape and Function

During embryonic development, changes in the mechanical microenvironment exert tensile and compressive forces that alter cell shape. Alterations to cell shape have been associated with stem cell fate decisions, as in the differentiation of embryonic stem cells into vascular endothelial cells [1]. Studies of human MSCs *in vitro* showed that they adopt an osteogenic phenotype when they were allowed to flatten and spread out, whereas they became adipocytes when they were restricted from spreading and maintained a rounded morphology [40]. In the heart, interactions between myocytes and the ECM give rise to changes in cell shape that direct actin filament orientation, sarcomere organization, and

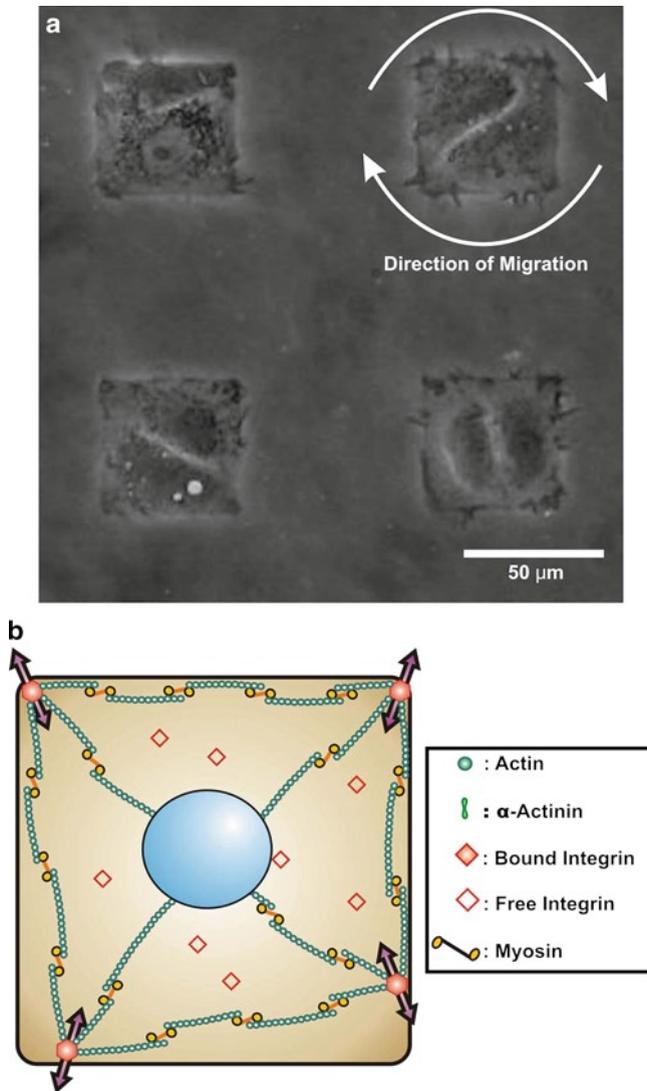


Fig. 5.2 Effects of the mechanical environment on tissue morphogenesis. **(a)** Localized boundary conditions imposed on cells by the ECM and the degree of mechanical coupling between neighboring cells provide cues for the coordinated migration of vascular smooth muscle cells on micropatterned square fibronectin islands. **(b)** Studies of cells grown on square ECM islands have shown that tractional forces imparted on the cell at focal adhesions cause dynamic rearrangement of the cytoskeleton in response to geometric constraints

myofibrillogenesis [41]. Changes in cell shape are the product of Rho-mediated rearrangement of the cytoskeleton. Examination of capillary network formation by human microvascular endothelial cells *in vitro* and retinal angiogenesis *in vivo* using the Rho inhibitor p190RhoGAP revealed that Rho-induced changes in

cytoskeletal architecture regulated angiogenesis by modulating the activities of two antagonistic transcription factors, TFII-1, and GATA2, that govern the expression of the VEGF receptor. Moreover, the activity of p190RhoGAP was found to be sensitive to ECM elasticity [42]. Dynamic assembly and disassembly of cytoskeletal elements generate directed forces that perturb cell shape and guide the organization of cellular components. This mechanical force-balance influences cellular behavior by modulating gene expression activity and could serve as an important factor in cell fate decisions made by stem cells during tissue morphogenesis.

5.2.3 Actin Cytoskeletal Remodeling and Transcriptional Regulation

The mechanical stiffness of the local microenvironment and the contractile activity of cells influence gene expression during embryogenesis [31]. In particular, genes encoding proteins involved in tissue remodeling processes have been found to be susceptible to changes in cellular morphology induced as a consequence of direct perturbation of cytoskeletal structure with actin and microtubule disrupting agents, such as cytochalasin D and colchicine [43]. Indeed, coordination between protein synthesis and cell motility is necessary for the timely generation of the structural components that support remodeling of the cytoskeleton. Examination of the link between cytoskeletal dynamics, motility, and gene expression revealed that MRTFs are physically bound to globular actin monomers until they are incorporated into actin filaments. Upon release from actin monomers, the MRTFs are free to translocate to the nucleus where they interact with the transcription factor SRF to promote the expression of genes under its control (Fig. 5.3a) [44]. This actin–MRTF–SRF mechanotransduction pathway may be particularly important in striated muscle development, as studies have identified a muscle-specific actin binding protein known as STARS that activates SRF through a Rho-dependent mechanism [45]. It is postulated that the upregulation of STARS during myogenesis provides a feed-forward mechanism for driving the expression of genes regulated by MRTF and SRF and reinforcing the differentiation process during the formation of skeletal and cardiac muscle tissues [45]. RhoA-dependent regulation of the actin cytoskeleton also plays a central role in regulating transcription during smooth muscle differentiation as well. Most smooth muscle-specific differentiation marker genes code for proteins associated with contractility, suggesting that Rho-dependent changes in smooth muscle contractility may be coupled to long-term regulation of smooth muscle-specific gene expression [46].

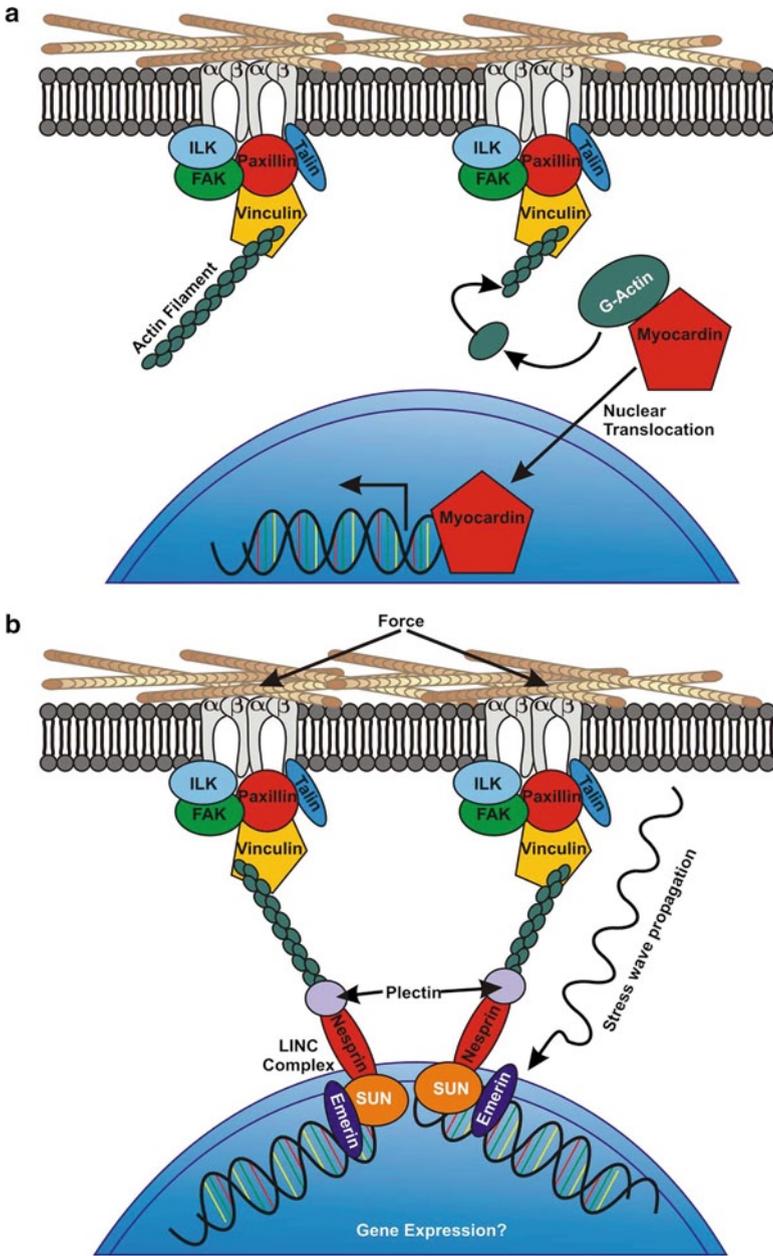


Fig. 5.3 Mechanical regulation of gene expression. (a) Myocardin-related transcription factors associates with globular actin in the cytoplasm translocate to the nucleus and alter gene expression as globular actin is incorporated into actin filaments during cytoskeletal remodeling. (b) Mechanical continuity between integrins, the cytoskeleton, and nuclear scaffolds could provide a path for mechanical signal transfer between the ECM and the nucleus

5.3 Nuclear Mechanics and Regulation of Gene Expression

The induction of gene expression by mechanotransduction has traditionally been assumed to occur via activation of established transcriptional regulatory pathways through biochemical signaling molecules localized to the surface of the plasma membrane. Experimental data suggest that individual filaments of the cytoskeleton bear tensile and compressive loads and give rise to a mechanical network under isometric tension that propagate physical signals throughout the cell at a velocity far exceeding the limits of chemical diffusion [14, 47]. An intriguing alternative signaling paradigm is the transduction of mechanical signals through the ECM–cytoskeletal network to structures deep within the cytoplasm, such as the nucleus, where they can alter enzymatic activity or gene expression by altering nuclear shape or physically deforming genomic structures within the nuclear compartment.

5.3.1 *Mechanical Continuity Between ECM and Nucleus*

It is widely recognized that focal adhesions serve as a mechanical conduit between the ECM and the cytoskeleton. However, much speculation remains about the physical continuity between the cytoskeleton and the nucleus and whether this mechanical linkage serves as an epigenetic regulator of gene expression [13]. Molecular connections between integrins, cytoskeletal filaments, and nuclear scaffolds may therefore provide a discrete path for mechanical signal transfer through cells as well as a mechanism for producing integrated changes in cell and nuclear structure in response to changes in the ECM. Studies involving the application of force to focal adhesions using micropipettes and RGD-coated microbeads provided evidence of mechanical continuity between membrane-localized integrin receptors and the nucleus via the actin cytoskeleton [48]. Interactions between nesprins, SUN, and lamins form a specialized nuclear anchoring structure for cytoskeletal filaments referred to as the LINC complex [47]. Emerin proteins within the nucleus provide a physical connection between the LINC complex and many proteins involved in chromatin modification. Chromosomes are traditionally regarded as discrete, physically separate entities, but microsurgery experiments revealed that isolation of one chromosome from living cells under isotonic conditions resulted in the removal of all of the chromosomes within the nucleus. Analysis of chromosome positioning and movement suggested that different chromosomes often behave as if they were physically connected during interphase and this mechanical coupling may coordinate dynamic alterations in chromatin structure [49]. Taken together, the results of these studies provide strong evidence that a direct physical linkage between the ECM and genome exists, and raises the question of whether this mechanical continuity provides a mechanotransduction pathway for modulating gene expression by directly altering chromatin architecture (Fig. 5.3b).

5.3.2 Modulation of Nuclear Shape and Its Effect on Gene Expression

Nuclear shape, structure, and stiffness strongly correlate to cellular function and phenotype in physiological and pathological situations where force is involved. The nucleus of most cells is roughly ellipsoid, or spheroidal, in shape and is regarded as the stiffest of the organelles. Studies of differentiating human embryonic stem cells noted that uncommitted cells possessed large, round nuclei with little lamin A and highly mobile chromatin [50–52]. As the cells adopted a particular lineage, researchers found that the nuclei demonstrated concomitant changes in nuclear shape and structure, revealing a strong correlation between nuclear shape change and changes in cellular phenotype [53]. Forces applied directly to the surface of cells, such as shear forces during fluid flow, can increase the load on the cytoskeleton and subsequently deform the physically connected nucleus. Examination of neonatal cardiomyocytes in vitro showed that the spatial organization of myofibrils in response to geometric cues provided by the ECM caused the aspect ratio of the nucleus to increase as the aspect ratio of the myocytes increased [54]. Measurements of gene and protein expression in primary osteogenic cells cultured on micropatterned islands of ECM protein revealed that changes in nuclear shape affected the activity of transcription factors that govern the expression of collagen I and osteocalcin, markers for the osteogenic phenotype [55]. Together, the results of these studies provide strong evidence for a possible role for mechanotransductive regulation of gene expression through alterations in the transfer of mechanical forces from the cytoskeleton to the nucleus.

Recent experiments confirm that gross epigenetic modifications that occur during stem cell differentiation can be detected as changes in the shape and stiffness of the nucleus, clearly demonstrating a relationship between nuclear architecture, chromatin organization, and transcription [52]. The intriguing, recently proposed concept of cytoskeletal epigenetics raises the question of whether the continued reorganization of long-lived cytoskeletal structures in a cell can serve as an epigenetic mechanism to record the “mechanical history” of a cell and influence the behavior of its daughter cells. The implications of this hypothesis are that stable cytoskeletal structures could potentiate variability in cell behavior and guide cell fate decisions toward certain phenotypes across generations of cells [36]. Nuclear shape is emerging as an important indicator of mechanical continuity between the nucleus, cytoskeleton, and ECM that has been implicated in providing an alternative pathway for regulating gene expression in response to the mechanical microenvironment of the cell.

5.4 Utilization of Mechanical Cues to Guide Engineered Tissue Formation

Advances in the field of cellular biomechanics are beginning to explain how physical forces and mechanical structures impact information processing and cellular decision-making [9]. Increased understanding of the relationship between cellular behavior

and the mechanical characteristics of their environment is motivating the development of new biomaterials that take advantage of this phenomenon to drive stem cell differentiation and tissue morphogenesis with greater precision [56]. One example of this is efforts to fabricate functional myocardial tissue grafts to repair damaged areas of the heart after myocardial infarction. Current efforts aim to derive cardiac progenitor cells that can be expanded *in vitro* and then selectively differentiated into the muscular, vascular, and conduction system cells that comprise the myocardium. Of equal importance is the development of ECM scaffolds that provide appropriate mechanical cues to guide the differentiation and organization of cardiac progenitor cells into a functional tissue structure that can be incorporated into highly complex structure of the native myocardium [57]. In addition to the structural guidance cues provided by the ECM, the behavior of cells during embryonic development is also influenced by tractional forces created by contracting cells and propagated through the ECM to neighboring cells. The application of a 10% static stretch to mouse embryonic stem cells was found to increase the number of contracting cells, whereas application of 10% cyclic stretch to human embryonic stem cells was found to decrease differentiation and maintain them in a pluripotent state [58]. The results of these studies clearly indicate that a better understanding of the influence of the mechanical environment on stem cell activity and the development of novel biomaterials that take advantage of this knowledge is required to advance the field of regenerative medicine.

5.4.1 Computational Modeling of Mechanotransductive Effects

With refinements in our understanding of mechanobiology and the *in vitro* experimental platforms used to study mechanotransduction, mathematical models of force distribution in tissues and the parameters that dictate mechanosensing are starting to emerge. The development of *in vitro* techniques to regulate ECM composition and geometry has made it possible to explore the effects of cell–ECM interactions on specific parameters of cell behavior [59]. Such techniques have been used to develop a computational model of the relationship between cell shape and calcium dynamics in developing cardiomyocytes [60]. It has also been used to develop a computation model to forecast the fate specification of human MSCs based on the early cytoskeletal arrangement imposed on the cells by the geometry of the ECM [7]. As techniques to fabricate free-form engineered tissues emerge, mathematical models are being developed that attempt to describe their behavior and predict their performance characteristics given some change to tissue architecture. For example, a finite element model was recently developed to simulate the performance characteristics of engineered myocardial constructs and provide predictions about the effects of changing myofibrillar orientation on their contractile function [61]. In addition to mathematical descriptions of *in vitro* model systems, researchers have also begun to develop computation simulations of the injured *in vivo* tissue environments for which engineered tissues are being developed to repair. A multiscale mathematical

model of strain-driven eccentric growth and stress-driven concentric growth of the myocardium during ischemic injury has recently been developed that allows researchers to explore the effects of local changes in cardiomyocyte morphology due to alterations in stress/strain distribution caused by fibrosis on cardiomyocyte function from the multicellular tissue scale down to the molecular scale of the sarcomere [62]. These reports are the first effort to develop CAD tools for engineered tissues. While CAD tools are routinely used in many engineering disciplines, in tissue engineering these tools, combined with medical imaging data, will require understanding of biotic–abiotic interface physics and a hierarchal understanding of self-organizing biological systems.

5.4.2 Fabrication of ECM Substrates That Promote Functional Maturation

Artificial tissues suitable for regenerative applications will require scaffolds that can promote controlled differentiation of a stem cell population and impose precise cellular organization. A number of synthetic polymer compounds have been evaluated for their ability to support the efficient clonal expansion and differentiation of stem cells based on structure–function relationships between cell behavior and substrate material properties [63]. By mimicking the physicochemical properties and self-assembly fabrication of natural materials, artificial scaffolds are beginning to be developed that incorporate peptide motifs that support the engagement of specific pairs of integrins and allow remodeling of the synthetic matrix by proteases secreted by cells [56, 63, 64]. As our understanding of the influence of mechanical cues on stem cell fate decisions matures, this information can be used to direct the development of cell substrates that utilize these mechanical cues to create stem-cell derived tissue constructs with desirable functional properties.

5.4.2.1 Control of Cell Shape and Organization

The intercellular and cell–ECM interactions within a tissue govern the shape and organization that the cells comprising that tissue will ultimately adopt, and these interactions clearly play an important role in regulating the survival and functionality of those cells [16]. Microcontact printing is a well-established technique for fabricating planar cell growth substrates with precisely defined ECM geometry (Fig. 5.4). An elastomeric stamp with micrometer-scale features can be “inked” with an ECM protein of choice, such as fibronectin, laminin, or collagen, and transferred to a flat substrate that promotes protein adsorption. Cells seeded onto these substrates preferentially bind to the portions of the substrate coated with the patterned ECM protein, giving rise to a large population of cells with shapes defined by the ECM pattern [65]. This technique has been used extensively to study the relationship between shape and behavior and a number of cell types, including differentiating

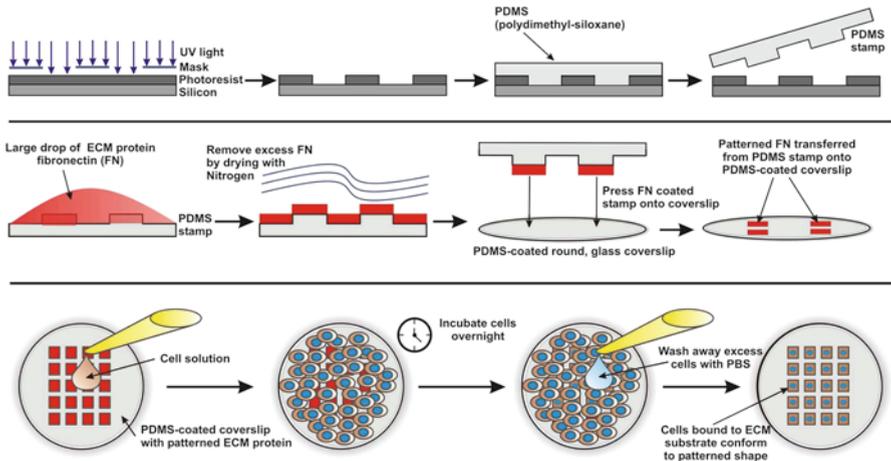


Fig. 5.4 Utilization of mechanical cues to guide differentiation. Microcontact printing allows the fabrication of ECM substrates with defined microscale geometry using photolithographic templates. This technique has been used extensively to study the contribution of cell geometry and tissue organization on stem behavior in vitro

stem cells. The results of these studies indicate that cell geometry is indeed an important factor in directing the lineage commitment of stem cells and continues to influence their behavior throughout their lifetime. A critical limitation of this technique is the fact that it can only be used with rigid planar substrates that do not mimic the mechanical properties of natural tissues and give rise to monolayers of cells. Three-dimensional scaffolds with natural tissue-like mechanical properties need to be developed that incorporate precise ECM cues for controlling cell shape in a nonplanar substrate.

5.4.2.2 Evolution of Biomaterials for Regenerative Medicine

Biomaterials made today are routinely information rich and incorporate biologically active components inspired by natural analogs [66]. Researchers have begun to design materials that combine synthetic polymer compounds with peptide motifs that can be proteolytically cleaved by matrix metalloproteinases secreted by cells to create scaffolds that can be sculpted by cells during tissue formation [64]. Advances in the construction of three-dimensional polymeric scaffolds are also starting to make the fabrication of therapeutically relevant artificial tissue constructs a reality [56, 67, 68]. A recently developed method derived from the microcontact printing approach to fabricating two-dimensional tissues in vitro allows the fabrication of free-standing protein nanofabrics. These protein nanofabrics are constructed by microcontact printing successive layers of ECM protein onto a rigid substrate coated with a thermosensitive polymer. These nanofabrics can be comprised of a heterogeneous

composition of ECM proteins and the microcontact printing technique provides control over the shape, size, and orientation of the protein “threads” with respect to one another. Further, cells will readily adhere to these ECM fabrics and stacking of these nanofabrics may allow the construction of ECM scaffolds with precise organizational cues throughout the volume of the scaffold [69]. Another promising approach for fabricating three-dimensional ECM tissue scaffolds with precise geometry is the recently developed rotary jet spinning technique for generating fibrous tissue scaffolds [70]. This technique overcomes the limitations of the traditional electrospinning technique to produce highly aligned nanoscale fibers using a nozzle rotating at high speed to produce a jet of polymer solution that undergoes extensive stretching before polymerization. The primary advantage of this technique over other methods of three-dimensional scaffold production is its ability to quickly produce large quantities of tissue scaffolds of arbitrary size composed of precisely aligned protein nanofibers. The focus of future biomaterials design will likely be focused on the development of “smart” materials that integrate multiple inputs from both chemical and mechanical stimuli to direct their behavior [56]. Such materials could simplify and optimize engineered tissue fabrication by more closely reproducing the dynamic microenvironment presented to differentiating cells during development, allowing researchers to take advantage of the natural interactions between cells and their environment during tissue morphogenesis to reproducibly drive the fate commitment of cells without the need for complex experimental manipulations.

5.4.3 Measurement of Maturation and Tissue Function

An important final consideration in the fabrication of engineered tissues from uncommitted stem cells is the evaluation of functional performance characteristics of the artificial tissue. Traditionally, differentiation has been assessed by measuring the expression of specific marker genes. However, this metric requires destruction of the tissue to isolate mRNA for measurement and is not informative for cells and tissues that require the precise assembly and organization of macromolecular structures, such as the sarcomeres of striated muscle for their functionality. Biomimetic microfluidic devices are emerging as a promising platform for measuring the performance characteristics of engineered tissues *in vitro*. A recent study provided the first proof of principle demonstration of this approach to model the structural, functional, and mechanical properties of the alveolar–capillary interface of the human lung. This microfluidic device was not only able to reproduce the functionality of an alveoli, but it also allowed the identification of novel mechanosensitive responses of the lungs to nanoparticulates [71]. Application of these organ-on-chip devices to the fabrication of tissues using stem cells could provide a powerful tool for the quantitative analysis of stem cell-derived artificial tissues. Evaluation of the functional characteristics of muscle tissue is especially challenging, as traditional assays are not able to provide direct measurements of their contractile performance.

A novel muscular thin film assay was recently developed that allows direct measurement of the contractile force of engineered muscle tissues [72]. This assay has been successfully used to demonstrate the myogenic potential of mouse cardiac progenitor cells isolated from the primary and secondary heart fields during various stages of cardiogenesis [73]. Subsequent modifications to the muscular thin film assay have made it amenable to the evaluation of smooth muscle cell contractility, in addition to striated muscle contractility, and allow the simultaneous measurement of multiple engineered muscle constructs in the same dish [74]. As the field of regenerative medicine advances, and the complexity of engineered tissues increases, new approaches will be needed to evaluate the utility of these tissues for therapeutic applications. Cell-based biochips represent an attractive test system that negate the need for costly animal models and allow quantitative analyses of tissue function that are not possible in traditional cell culture systems.

5.5 Opportunities and Challenges for Utilizing Mechanical Cues to Guide Tissue Formation

It is now commonly accepted that mechanotransduction plays an important role in stem cell differentiation and tissue morphogenesis. However, much remains to be discovered about the cellular mechanisms that provide the interface between mechanosensation and activation of biochemical processes, such as gene expression. Much evidence points to the cytoskeleton as this nexus, since it provides the mechanical continuity between the ECM and intracellular structures, and dictates the shape and spatial organization of a cell. As the biomechanics of mechanotransduction are elucidated, these findings must be incorporated into next-generation, multiscale biomaterials to provide stem cells with a mechanical microenvironment that directs their behavior in a predictable and reproducible manner.

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