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Stem cell mechanobiology: diverse lessons from bone marrow

Irena L. Ivanovska¹, Jae-Won Shin¹, Joe Swift¹, and Dennis E. Discher¹

¹Molecular and Cell Biophysics Lab, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

A stem cell niche is defined by diverse chemical and physical features that influence whether a stem cell remains quiescent, divides, or differentiates. Here we review mechanical determinants that affect cell fate through actomyosin forces, nucleoskeleton remodeling, and mechanosensitive translocation of transcription factors. Current methods for physical characterization of tissue microenvironments are summarized together with efforts to recapitulate niche mechanics in culture. We focus on mesenchymal stem cells, particularly in osteogenesis and adipogenesis, and on blood stem cells – both of which reside in mechanically diverse marrow microenvironments. Given the explosion of efforts with pluripotent stem cells, the evident mechanosensitivity of these clinically relevant, multipotent marrow cells underscores an increasing need to examine and understand *in vivo* and *in vitro* physical properties on length scales that cells sense.

Keywords

Extracellular-matrix; actomyosin; nucleus; mechanotransduction; osteogenesis; hematopoiesis

In vitro approaches to mimic and characterize ECM in the stem cell niche

Stem cells are plastic in that they possess a potential to differentiate into multiple lineages. The control of stem cell fate has been classically attributed to growth factors that regulate transcription factors. However, because stem cells do not exist in isolation *in vivo*, additional environmental factors are now being recognized as likely regulators of stem cell fate. Indeed, multipotent stem cells or progenitors present in many adult tissues [1] reside in an environment known as a niche that helps maintain the cells in a naïve state until prompted to differentiate. The niche combines tissue-specific matrix and nearby differentiated cells with key soluble molecules, all of which the stem cell probes and interprets when deciding to undergo differentiation [2]. Mechanotransduction is also now being studied as a differentiation mechanism in which cells physically probe their surroundings with mechanical forces that alter protein organization and ultimately gene expression.

A role for extracellular matrix (ECM) mechanics in determining cellular function – including stem cell differentiation – has been extensively studied, in part by using

Corresponding author: Discher, D.E. (discher@seas.upenn.edu).

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reductionist *in vitro* approaches that simplify the complex mechanical properties of tissue. For example, collagens are the most abundant proteins in metazoans, but they display complex mechanics; collagen fibrils are semi-flexible biopolymers with non-linear elastic behavior and, when cross-linked, form strain stiffening networks [3]. Development of biomimetic culture systems depends on methods to measure the mechanical properties of both biological and synthetic systems with high spatial resolution such as rheology, micro-pipette aspiration and atomic force microscopy (AFM) as described in Box 1. Here we discuss how these techniques provide insight into the roles of ECM, actomyosin contractility, nuclear mechanics and mechanosensitive pathways in determining stem cell commitment to specific lineages. We describe some of the mechanical properties of tissues that increasingly motivate the characterization and control of biomimetic platforms at nano-scales in order to understand the role of ECM and mechanotransduction in stem cell biology, with a particular focus on bone marrow stem and progenitor cells.

Influence of matrix mechanics on differentiation of bone marrow cells

Mesenchymal Stem Cells (MSCs) contribute to an osteo-progenitor population of cells, which differentiate into osteoblasts that produce the osteoid matrix at the interface between bone marrow and calcified collagen (Figure 1A) [4]. Osteoid contains fibrillar collagen, non-collagenous proteins and proteoglycans, all of which are cross-linked by enzymes secreted by osteoblasts. With time, the matrix thickens and mineralization is initiated through deposition of apatitic (calcium phosphate mineral) crystals [5]. The nanoscale composition and topology of the bone matrix (Figure 1B) defines how cells experience stresses at the sub-cellular level. Matrix fibrillar and non-fibrillar proteins, and apatite crystals have nanoscale dimensions and their structure is likely to affect matrix nano-mechanics. Calcium phosphate crystals grow between fibrils but can also be found embedded within the fibrils themselves [6]. Interestingly, it is the orientation of the apatite crystals, rather than the density of the mineral that correlates most strongly with the stiffness of this nascent bone [7].

Isolated from marrow, MSCs are adherent cell type that spreads on tissue culture plastic or glass. In culture with the proper soluble factors, MSCs are multi-potent and besides their osteogenic and chondrogenic potential, they certainly possess an adipogenic potential [8, 9]. Adipocytes are common in human bone marrow [10], and based on findings reviewed below, it is tempting to speculate that the soft marrow cavity is conducive to adipogenesis whereas the stiffer surface of bone influences osteogenesis. The latter is likely an important function of MSCs, and there is some evidence that marrow adipocytes – perhaps also differentiated from marrow MSCs – contribute to regulation of hematopoiesis [11, 12].

Hematopoietic stem and progenitor cells (HSPCs) isolated from bone marrow are non-adherent compared to MSCs, and HSPCs are also capable of growing in suspension *in vitro*. Nonetheless, interactions *in vivo* with the surrounding marrow cells and ECM seem unavoidable. Indeed, HSPCs express surface adhesion receptors, including integrins and cytoskeletal components such as actomyosin that, in principle, enable the sensing of physical forces and ECM stiffness from the microenvironment. Historically, HSPCs have been cultured in viscous methylcellulose media in combination with soluble factors to

control the growth and differentiation of progenitors. Unlike suspended cells that diffuse away and disperse freely as they divide, the viscous environment restricts cell motions, leading to colonies that have allowed investigation into how individual progenitor cells give rise to specific lineages. While the methylcellulose system might be viewed as recapitulating a viscous marrow environment, it is not clear what cells feel on a cellular level with this system. Recent studies have used better defined hydrogel systems that allow stiffness to be tuned to study how matrix mechanics can regulate MSCs and HSPCs either in isolation or in combination with other intrinsic and extrinsic factors and forces.

Importantly, MSCs have been differentiated from pluripotent stem cells, including induced pluripotent stem cells (iPSCs) [13, 14], and such cells exhibit the expected multi-lineage potential when given standard soluble factors. It is a long-term vision of the field to use easily accessible cells, such as skin cells (as opposed to marrow biopsies), to generate patient-specific iPSCs that could then be used to make well-defined lineages for tissue engineering applications. HSCPs have also been derived from pluripotent stem cells [14], but the efficiency is much lower and may reflect the fact that adult HSCPs seem to derive from a type of endothelial cell exposed to early blood flow [15, 16]. The mechanotransduction capacities of iPSC-derived MSCs and HSCPs have yet to be tested, thus further motivating this review.

Tissue mechanics: the case for nanoscale characterization

Solid tissues in general are flexible or elastic on the macro scale, and small deformations can be well described by a Young's modulus, E (modulus of elasticity, see Glossary). Tissues span a wide range of elasticities: with brain being the most flexible (<1 kPa) [17] and mineralized bone being the stiffest (GPa) [18]. Bone marrow is a softer tissue with an apparent elasticity measured *in situ* at 0.3 kPa [19, 20]. However, it is important to note that mechanical properties can vary depending on the resolution with which they are measured. The macro-scale elasticity of a given tissue reflects its structure at a larger scale than what a cell might feel at micron scales or smaller.

Many ECM proteins beyond fibrillar collagens can assemble into gels with measurable elasticity. Matrigel, for example, is a commercially extracted mixture of basement membrane proteins secreted from mouse sarcoma cells that also contains many growth factors [21]. Although such gels can be useful substrates for studies of cell behavior, modifications to these gels to control stiffness (e.g. density changes or crosslinking) generally change ligand density. The complex composition of naturally derived substances such as matrigel adds to the difficulty of separating mechanical effects from biochemical effects [21]. Synthetic hydrogels can simplify and clarify the biology. Polyacrylamide (PA) gels coated with non-fibrillar collagen ligand of a chosen density are a common system introduced by Pelham and Wang [22]; flexibility is easily controlled through crosslinking and/or density of acrylamide. Such systems and others have increasingly been used to mimic tissue microenvironments and to study cell responses.

Matrix mechanics and actomyosin contractility in differentiation

Most tissue cell types are anchorage dependent and must adhere to ECM in order to survive and function properly. Cell-matrix interactions are mediated by cell-adhesion receptors such as integrins that mediate transmembrane connections to the cytoskeleton, signaling across the membrane. Information provided by specific extracellular ligand binding sites to the outer integrin domains is transmitted to intracellular domains, a process dependent on long range conformational changes that are allosterically regulated [37]. Cells respond to spatial organization of the ECM through the formation of focal adhesions (FAs) -complex dynamic structures that link integrins and scaffold proteins to the actin cytoskeleton [38].

Once cells adhere, they are able to ‘feel’ the mechanical properties of their environment by pulling on their surroundings with the contractile forces generated by actin-myosin protein networks [39]. Non-muscle myosin-II is a filament-forming, actin crosslinking protein with force-generating contractile properties that are regulated by the phosphorylation of its light and heavy chains [40]. Forces that myosin-II exerts can cause the matrix to deform, but resistance in the matrix can feed back to cause stress inside the cell where the assembly and organization of stress fibers – cytoskeletal features composed of bundled actin filaments – can be modified. The same processes are found in many contractile cell types, including stem cells.

Cell fate can be influenced by a number of mechanical properties including elasticity, geometry, and adhesion, which ultimately impact on cytoskeletal tension (Figure 2A). Indeed, MSCs cultured on PA gels coated with collagen respond to matrix elasticity by specifying to a lineage. On matrix with osteoid-like stiffness (40 kPa), MSCs became osteogenic, whereas on softer matrix (10 kPa), MSCs became myogenic [33]. The same study showed that pharmacological inhibition of myosin-II contractility blocked lineage specification. Matrix stiffness favored actin-myosin assembly and contractile forces, evidenced by formation of stress fibers, as predicted by theory [41]. In particular, stress fiber alignment within cells was a non-monotonic function of matrix elasticity for which there was an optimal ratio of matrix to cell elasticity that governed cell polarization and stress fiber anisotropy.

In some of the original determinations of traction stresses (τ) exerted by cells on gels, the mean traction stresses increased with gel elastic modulus E such that the ratio of τ/E was ~ 3–4% over a range of physiological elasticity reviewed in [39]. Cells can only exert a finite stress τ (perhaps kPa), but the elastic modulus can be far higher (e.g. GPa for glass or plastic), so that τ/E approaches 0%. Tissues also have a non-zero viscosity, especially very soft tissues such as bone marrow, and so it is interesting to consider the contribution of the loss modulus to cell behavior. Recent work shows that when cells were cultured on soft substrates that exhibits stress relaxation, their spreading was increased compared with elastic substrates with the same elastic modulus [42]. In another study where the loss modulus was varied at fixed E , MSCs appeared sensitive to substrate creep and showed enhanced differentiation potential towards the lineages commensurate with elasticity [43].

Acto-myosin forces can also be modulated by constraining cell shape: geometric features that increase cell contractility at a constant spread area favor osteogenesis over adipogenesis,

independent of soluble factors [23]. Cell fate is also influenced by shaped clusters of ‘nano-pits’ – 120 nm in diameter and 100 nm deep – etched into polymethylmethacrylate substrates by electron beam lithography [24]. Osteogenesis of MSCs is maximal on nano-pit arrays with 50 nm spacing, where long FAs are observed and prolonged culture results in mineralization. A subsequent study showed that MSCs cultured on square arrays maintain multi-potency [25]. By culturing cells on elastic pillars that allow traction forces to be measured by tracking the bending of pillars, a strong correlation was observed between FA distribution, traction forces and cell spreading [26]. When MSCs were cultured in a bipotential, osteogenic-adipogenic media, the spring constant of the pillars was found to be a deciding factor for cell fate, with high stiffness favoring osteogenesis and low stiffness favoring adipogenesis. The study suggested that traction forces, and by implication cellular tension, can predict which cells will preferentially differentiate upon biochemical stimuli and hypothesized that cell tension or stiffness can be used as an early marker for predicting cell fate decisions in an heterogeneous MSC population.

Many ECM proteins contain adhesive sequences such as Arg-Gly-Asp (RGD), with over 20 integrins known to recognize this sequence [27]. Peptides immobilized on monolayers have generally proven useful for studying cell-matrix adhesion [28–31]. Substrates presenting high-affinity peptides promote osteogenesis, those with low-affinity peptides at high density promote myogenesis, and those with low-affinity peptides at low density promote neurogenesis [32]. These lineage specifications seem in rough agreement with matrix stiffness cues [33], suggesting a unifying physicochemical mechanism: the resistance of substrate engagement to cellular traction forces feeds back into cytoskeletal tension. However, it remains to be seen whether adhesive ligands are limiting *in vivo*, since tissue stiffness varies *in vivo*.

Beyond MSCs, hematopoietic differentiation to various lineages is also regulated by mechanical stresses. For example, platelets are generated from megakaryocytes (MKs) that undergo maturation by accumulating DNA content without division. This process, called polyploidization or multi-nucleation, occurs because weak adhesion to external interfaces limits cytokinesis. Interestingly, studies with non-mammalian cells have shown that adhesion to matrix provides traction forces to pull cells apart [44]. Consistent with this notion, MK polyploidization is inhibited on stiff matrix where stronger adhesion increases traction forces to drive cell division [45]. In contrast, soft matrix (typical of soft marrow [19]) inhibits myosin-II forces and maximizes MK maturation. High ploidy MKs have motile projections that migrate into the nearby bloodstream where shear causes fragmentation to pro-platelets [46] that then activate myosin-II to promote its division into small, familiar platelets [47].

Very early hematopoietic differentiation is also regulated by ECM mechanics in combination with other physical factors. The HSC:Progenitor (P) ratio is maintained or increased on highly flexible substrate of tropoelastin compared to stiff cross-linked tropoelastin [48]. As with MSCs, inhibition of myosin-II eliminates mechanosensing of HSCs and progenitors. Using mass spectrometry calibrated intracellular flow cytometry, the composition of myosin-II isoforms was observed to switch from the polarizable B isoform to the more homogeneous A isoform during hematopoietic differentiation [19]. External

stresses such as fluid shear and stiff matrix induced polarization of myosin-IIB, resulting in the switching of lamin isoforms. In contrast, soft matrix prevented myosin-IIB polarization. When polarization occurred during cell division, myosin-IIB was asymmetrically segregated to a daughter cell that maintained its stemness, while the cell with low myosin-IIB differentiated (Figure 2B). When myosin-IIB was down-regulated in differentiated cells, myosin-IIA was activated by dephosphorylation, inducing actin polymerization. Soft matrix also maximized myosin-IIA phosphorylation. Together, these findings suggest that soft matrix (away from bone) likely suppresses myosin-II isoform switching, while stiff matrix (near bone) drives asymmetric division (Figure 2B).

By understanding the matrix constraints that regulate cell differentiation, one can begin to rationalize how cells are organized within a tissue. In bone marrow, cells exist in an environment with a gradient in stiffness over length scales several times greater than cellular dimensions. Osteoblasts localize to the osteoid interface, which is relatively stiff but adjacent to cells such as pre-osteoblasts that are surrounded by softer matrix. MSCs reside in the interior of the bone marrow, but it is not clear how they reposition during commitment to lineages such as adipocytes or pre-osteoblasts. MSCs not only polarize toward and migrate into regions of greater stiffness [34] but also tend to differentiate according to ECM elasticity cues [35, 36]. It is notable that this ‘durotaxis’ process depends on myosin-II, and is evident in 3D matrix systems. Nonmuscle myosin II-B (NMII-B) is unpolarized on soft substrates but polarizes in the rear of the cell when the cell migrates from a soft to stiff substrate. Moreover, stiffer substrates promote the assembly of NMII-A into stress fibers, which are necessary for NMII-B polarization. [34].

Lamins: Regulating cell fate in the nucleus

Within a cell, the nucleus is caged by the cytoskeleton so that stresses in the cytoskeleton propagate to the nuclear envelope (Figure 3) [49] and possibly influence global organization of chromatin [50]. Key to maintaining the physical properties of the nucleus is the nuclear lamina, a structural network composed of intermediate filament proteins of A-type (lamins A and C, products of alternate splicing of the *LMNA* gene) and B-type lamins (lamins B1 and B2, encoded by *LMNB1* and *LMNB2* genes, respectively) located at the interface between the inner nuclear membrane and chromatin [51–54]. The “linker of nucleoskeleton and cytoskeleton” (LINC) complex is a protein complex that might contribute to mechanical transduction of signals to the nucleus [55, 56]. Specifically, nesprins – a family of nuclear membrane proteins – connect to actin filaments in the cytoplasm through their N-terminal domains, while their C-terminal domains are anchored to SUN proteins, providing a direct physical link between the cytoskeleton and nucleoskeleton by spanning the inner nuclear membrane and in turn binding to the lamina.

Tissue analyses have shown that lamin-A,C levels scale with the stiffness of a tissue, with stiffer tissues such as bone having higher lamin-A,C than softer tissues such as fat (Figure 2A) [52]. While the expression of lamin-A,C increased 30-fold across tissues, the constitutively expressed B-type lamins varied by less than 3-fold. Although ‘the truth is in the tissues’, culture studies are likely to hint at a possible mechanism. For at least several types of cells in culture, the lamin-A,C level was also affected by cellular tension in a

process regulated by mechano-sensitive phosphorylation (Figure 3B, E) [57]. Studies of MSCs cultured on plastic had shown that lamin-A,C knockdown favors adipogenesis [58] and lamin-A,C overexpression favors osteogenesis [59]. By using PA gels with stiffness tuned to mimic either soft fat tissue or stiff osteoid, lamin-A,C was further shown to be a mechanosensitive enhancer of matrix elasticity directed lineage specification *in vitro* (Figure 2A) [52].

As with myosin-II isoform switching in hematopoiesis, the composition of the nuclear lamina not only changes with differentiation to blood lineages but knockdown and overexpression also demonstrate a functional influence [53]. Although direct roles of matrix mechanics on nuclear lamin turnover of haematopoietic cells remain unstudied, a stiffer lamina impedes migration and – in principle – exit from the marrow. Furthermore, lamin-A,C changes less than B-type lamins across different hematopoietic lineages [53]. Since marrow matrix might be too soft to impact lamin-A,C, relatively unique marrow factors likely regulate B-type lamins in this essential compartment, which generates millions of new blood cells per second in humans. The interplay between matrix, cytoskeletal, and nuclear mechanics during hematopoietic lineage specification certainly requires further investigation, but studies thus far with hematopoietic lineages provide initial insight into how adhesion, matrix elasticity, and external shear forces can couple to cytoskeletal and nuclear mechanics in processes central to stem cell self-renewal, fate decisions, and even migration.

Mechanical regulation of transcription factors

An understanding of how mechanical signals are transmitted to the nucleus to activate different gene expression programs is slowly emerging. Nucleo-cytoplasmic shuttling of transcription factors appears to be an essential step in several mechanosensitive pathways implicated in differentiation across different cell types (Figure 3). For example, SRF (serum response factor) signaling is known to play a key role in myogenic differentiation in diverse muscle cells (e.g. [60]) and depends on RhoA and actin polymerization via interactions with cofactors including MRTF (myocardin-related transcription factor) A and B (also known as MAL). MRTFs A and B bind to monomeric G-actin in the cytoplasm but are released upon actin polymerization and transferred into the nucleus to bind and activate SRF [61, 62]. Matrix constraints can be a sufficient cue for transcription factors to change their nuclear-cytoplasmic localization and thereby regulate gene expression. Restricted ECM – cell contacts have been shown to trigger human epidermal stem cells to initiate terminal differentiation as mediated by the actin cytoskeleton and SRF pathway regulation [63]. Cytoskeletal components including non-muscle myosin-IIA (MYH9) regulate SRF as does substrate stiffness, lamin-A,C and emerin levels [52, 57, 63, 64].

Retinoic acid, a metabolite of vitamin A, is widely known to regulate development, differentiation, proliferation and apoptosis [65, 66]. Retinoic acid receptors (RAR), RAR-alpha, RAR-beta and RAR-gamma encoded respectively by the *RARA*, *RARB* and *RARG* genes bind to retinoid X receptors in the nucleus and regulate transcription of many genes, including LMNA. Lamin-A,C expression is regulated by RARs (Figure 3C, G), but the nuclear-to-cytoplasmic ratio of RARG also increases with matrix elasticity and lamin-A,C

overexpression while decreasing with lamin-A,C knockdown. Pharmacological perturbations of the RA pathway in MSCs show that RARG antagonists increase lamin-A,C and enhance osteogenesis on rigid substrates whereas RARG antagonists decrease lamin-A,C and favor adipogenesis on soft substrates [52].

YAP and TAZ transcriptional co-activators are classically part of the Hippo pathway that regulates organ growth and development but might also have a role in mechanotransduction [67] [68]. Two kinases MST and LAT canonically control the activity of the transcription factors YAP/TAZ [69]. MST phosphorylates and activates LATS kinase, and LATS phosphorylation of YAP/TAZ inhibits its activity and traps the transcription factor in the cytoplasm. If the LATS kinase is inhibited then YAP and TAZ enter the nucleus and bind TEAD transcription factors that drive the transcriptional regulation of proliferation (Figure 3C, G) [70, 71].

YAP/TAZ subcellular localization depends on matrix stiffness and actin cytoskeleton in a mechanism distinct from the Hippo pathway and independent of MST/LATS, perhaps involving other unknown kinases [72]. Nuclear localization of YAP/TAZ in MSCs increases on stiff matrix similar to osteoid (40 kPa) (Figure 3G), and requires Rho GTPase activity and actomyosin contractility (Figure 3F). A high nucleus:cytoplasmic ratio of YAP/TAZ correlates with increased osteogenic potential in MSCs in culture (Figure 3G) [73], although tissue profiling of YAP does not reveal a clear trend [52]. YAP/TAZ nuclear accumulation that is driven by a prolonged passive mechanical stimulation might increasingly restrict MSC fate choices as a “mechanical memory” [74]. Inhibition of nuclear localization of YAP in hPSCs (human pluripotent stem cells) through compliant substrates that mimic brain stiffness (0.7 kPa) or by inhibition of F-actin polymerization has been found to promote highly efficient differentiation of hPSCs into neurons even in the absence of soluble stimuli (Figure 3C) [75].

A next step in dimensionality and greater complexity

In vivo cells are often surrounded by other cells or matrix, and generally these 3D environments constrain cell shape and dynamics, restrict access to soluble factors such as oxygen, and impact how secreted matrix is deposited, among other effects. Given the complexities of the locations in which stem cells reside *in vivo*, it remains difficult to recapitulate these environments *in vitro* and to pinpoint the precise mechanical cues that influence cell fate. Among the new cell culture approaches being developed, one hydrogel system could be patterned in 3D and remodeled *in situ* with a focused laser to control cell location and to spatially define incorporation of RGDS peptides for cell tethering [76]. MSCs encapsulated in such systems mimic several features of *in vivo* chondrogenesis: cells remodel the matrix through down-regulation and degradation of fibronectin, with subsequent up-regulation of glycosaminoglycans (GAGs) and type-II collagen.

MSCs have also been encapsulated within a hydrogel-based matrix where stiffness and ligand density can be manually controlled [77]. Osteogenesis occurred in high stiffness matrices (11–30 kPa), while adipogenesis occurred in the softest substrates, which is consistent with findings on simpler 2D hydrogels. In addition, cells rearranged their integrin

adhesions with the number of bonds per cell depending non-monotonically on matrix elasticity and peaking at ~ 20 kPa. However, another finding argues that hydrogels with breakable cross-links differ from covalently cross-linked hydrogels [78]: MSCs encased in non-degradable, covalently cross-linked hyaluronic acid (HA) hydrogels with RGD functionalization favored adipogenesis, even in nominally stiff gels. Through adaptation of a 3D version of traction force microscopy [79], rounded cells were seen to generate forces similar to those observed in cells with pharmacologically inhibited contractility. However, the introduction of proteolytically cleavable cross-links made the gels susceptible to cell-mediated degradation, which allowed the cells to break symmetry, pull and spread within matrix, and undergo osteogenesis. Access to soluble factors and impact on secreted matrix deposition remain to be understood in such systems. However, when symmetry is forcibly broken by using thin, non-degradable HA gels that are layered on top of MSCs pre-cultured on a soft or stiff gel, the cells appear to exhibit cytoskeletal characteristics and polarization similar of cells cultured on a stiffer gel [80].

Soluble factors are major drivers of differentiation. Lineage-specific cocktails are often added or even mixed in deconstruction studies of substrate and ECM effects. Sometimes the cocktails include growth factors such as TGF-beta that bind to ECM and are released depending on matrix stiffness and cell tension [81]. Glucocorticoids such as dexamethasone are also commonly used *in vitro* in differential doses to impact differentiation, but whether soft or stiff matrix modulates such important transcription factors as the glucocorticoid receptors has not been examined *in vitro* or *in vivo*. Perhaps the insights gained into matrix- and lamina- regulated RAR shuttling will prove relevant. Regardless, the relative contribution of each soluble and 'insoluble' factor and the extent to which the pathways are coupled in series or in parallel needs to be explored in detail with modern methods such as proteomics, transcriptomics, and certainly epigenetics.

Concluding remarks

Although insights into the molecules that contribute to the many types of stem cells and niches has accumulated over the last several decades, measurements of niche mechanics and the forces that can influence stem cells have emerged only within the last decade. Bone marrow stem cells that make blood and bone among other lineages have been the most deeply studied with considerable evidence from tunable hydrogels, showing that matrix elasticity and perhaps viscosity can affect cell fates. Pathways involve actomyosin contractile forces, which cells use to probe the surrounding matrix, and the nucleoskeletal protein lamin-A, which adjusts nuclear mechanics to the microenvironment and enhances differentiation programs. In these contexts, mechanical regulation of gene expression at least sometimes occurs via mechanosensitive translocation of key transcription factors. Some of these pathways have also been described for cells growing on substrates that are patterned, although physical relationships to niches in tissues are not always clear. Three-dimensional hydrogels with controlled properties and patterns are also emerging, but the added complexity always needs to be carefully considered for its effects on cell confinement, permeation of factors, and the two dimensional locality of some tissue formation processes.

The physical properties of intact and fresh tissue indeed need to be studied in greater detail for normal and diseased states in order to deepen our understanding of stem cell microenvironments. Such information is already foundational to developing and employing optimal cell culture models. Functional studies and characterizations of the epigenetic changes in stem cells in response to the mechanical cues of their environment have gained important mechanistic insight into how cells choose their fate. Further measurements of tissues (and proposed culture models) will enable many more types of molecular biology perturbations, super-resolution microscopy analyses of key structures, and detailed evaluation of the cells and their nuclei during differentiation. ‘Kinetics reveal mechanism’ in terms of the order in which processes occur and so increasing attention to timing will be essential to understand and control the maturation of the many types of pluripotent and multi-potent stem cell systems that are emerging.

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Glossary

Elasticity	a property of a material that causes it to be restored to its original state after deformation. The terms elasticity, stiffness and rigidity are often used interchangeably. Stiffness is a measure of how resistant an object is to deformation.
Monotonic function	a function that within a given interval, either only increases or decreases with the argument
Strain	the deformation of an object in response to stress. When an object is deformed, distances between points within the object change. Strain describes the change in distance between points relative to the separation of the points prior to deformation. It is a tensor (multicomponent measurement) as it is a function of the position within the object
Stress	a physical quantity describing the forces acting through a given cross-sectional area in an object subjected to strain. Stress is also a tensor.
Storage and loss moduli	in viscoelastic materials they are measures of the stored energy (elastic component) and the energy dissipated as heat (viscous component)
Traction forces	the forces that cells exert on their surroundings.
Viscoelasticity	a property of a material that displays both viscous and elastic characteristics when deformed.

Viscosity	a measure of the resistance of a fluid to gradual deformation by ‘shearing’ stresses (i.e. when layers within a fluid are moved laterally with respect to each other).
Young’s modulus	the ratio of stress to strain for deformation in one-dimension a measure of elasticity.

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Highlights

- The stem cell niche is defined in part by mechanical factors
- High resolution physical methods probe *in vivo* niches and *in vitro* models
- Extracellular matrix mechanics affect actomyosin contractility within cells
- Forces propagate into the nucleus and remodel the lamina

BOX 1**Common techniques for measuring mechanical properties of ECM, cells and nucleus****Rheological methods**

The material properties of natural or synthetic gels can be characterized using rheological methods. Measurements can be made of the complex modulus $G^* = G' + iG''$ under shear stress, where the storage modulus G' describes the elastic component and the loss modulus G'' describes the viscous contribution. Gels formed from different cytoskeletal and extracellular proteins exhibit strain stiffening for small to intermediate strains, measured with a cone and plate rheometer [3] (Figure IA). The deformations of tissues, cells, or nuclei can be measured on micron scales as they are drawn into a micropipette under negative pressure. Optical microscopy is used to image the deformations over time and often the proteins of interest are fluorescently labelled. The nucleus shown in Figure IB is GFP tagged Lamin A protein expressed by a cell. This method was used to show that nuclei stiffen during differentiation [84], embryonic heart tissues stiffen during development [85], and lamina composition determines the viscoelastic response of nuclei [52–54].

Atomic force microscopy (AFM)

AFM is a widely used instrument to measure a variety of forces between a sample and a nano-sized probe [86]. The working principal behind the method is to raster scan a surface with a small probe at the end of a flexible cantilever. Interactions with the sample cause the cantilever to bend and its deflection is detected by measuring the position of a laser beam reflected from the back of the lever (Figure IC). AFM can be used for force spectroscopy or “force mode.” With this application, the tip approaches the sample surface vertically, and is then retracted. When the tip indents the sample, a force indentation curve is recorded that can be used to obtain the properties of the material under compression. When the probe is retracted, material properties that are under stretching can be measured, or proteins that are unfolding under tension can be examined. Using the Hertz model on contact mechanics of elastic solids and its modifications for different geometries, one can extract Young’s modulus E from force-indentation curves (Figure ID). Another application of AFM is imaging structures at high resolution such as the organization and assembly of matrix proteins. The AFM image in Figure IE shows the topography of nano fibrils in a thin molecular cross-linked collagen film. Moreover, AFM can be used for “force mapping” (Figure IF). With this method, force curves are recorded at an array of points across the sample. Elasticity maps of ECM, cells, or tissues on stiff or flexible substrates can be generated. The image in Figure IF shows E of an MSC on a flexible, molecularly thin collagen film.

Figure I.
Common techniques for measuring mechanical properties of ECM, cells and nucleus.

Figure 1. Matrix material properties are important both on cellular and nanometer scales

A) Schematic representation of the spatial organization of bone marrow cells at their interface with mineralized bone. **B)** Transmission electron microscopy (TEM) image of part of an osteoblast (nucleus, NU, and ER are visible inside), and its extracellular environment with two distinct layers: the fibrillar osteoid (OS) and the mineralized bone (Min. Bone). Adapted from F. Bronner & M. C. Farach-Carson [4]. The scale bar is 1 micron.

Figure 2. From outside in and out again – the relationship between matrix stiffness, cell contractility, nuclear composition and differentiation

A) Matrix stiffness increases cell contractility through regulation of non-muscle myosin-II levels. Nuclear stiffness, set by the level of lamin-A,C protein, scales with tissue microelasticity, E , and in cultured MSCs is higher on stiffer matrix and increases with cellular tension. MSCs in culture and expanded on plastic deposit their own matrix with unknown stiffness and have high lamin-A,C levels. Lamin-A,C knockdown (KD) on soft matrix enhances adipogenesis; lamin-A over-expression (OE) combined with stiffer matrix enhances osteogenesis. **B)** Myosin-II proteins polarize in HSCs on stiff matrix, leading to asymmetric division and differentiation. In contrast, soft matrix suppresses myosin polarization and cells divide symmetrically and proliferate.

Figure 3. Stem cell mechanobiology

A) Mesenchymal stem cells (MSCs) cultured on soft substrates exhibit a low contractility phenotype: low spread area, poorly developed focal adhesions (FAs) and stress fibers, and lower levels of lamin-A,C in the nuclear lamina. **B)** Lamin-A,C level is regulated by tension-dependent kinase activity. Low tension allows lamin to be phosphorylated, leading to increased solubility and turn-over. The filamentous lamina is thus maintained in a state appropriate to the mechanical environment of the nucleus. **C)** On soft substrates, transcription factor (TF) RARG remains in the cytoplasm, reducing transcription of the

lamin-A,C gene (LMNA) and favoring adipogenesis, an effect enhanced by agonists to the retinoic acid (RA) pathway. TF YAP/TAZ also remains cytoplasmic, favoring adipose and neuronal lineages over osteogenesis. **D)** By contrast, MSCs that have been cultured on stiff substrates have a high contractility phenotype: high spread area, well developed FAs and stress fibers, high levels of TF serum response factor (SRF), and a nuclear lamina rich in lamin-A,C. **E)** High tension on the nuclear lamina inhibits phosphorylation and turn-over of lamin-A,C. **F)** The contractility of the acto-myosin cytoskeleton is regulated in response to substrate stiffness by the Rho/ROCK signalling cascade. **G)** On stiff substrates, TF RARG translocates to the nucleus, increasing transcription of LMNA and driving osteogenesis, an effect enhanced by the addition of antagonists to the RA pathway. YAP/TAZ levels in the nucleus are also increased, favoring osteogenesis over adipogenic or chondrogenic cell fates.

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