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Control of stem cell fate and function by engineering physical microenvironments

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Abstract

The phenotypic expression and function of stem cells are regulated by their integrated response to variable microenvironmental cues, including growth factors and cytokines, matrix-mediated signals, and cell-cell interactions. Recently, growing evidence suggests that matrix-mediated signals include mechanical stimuli such as strain, shear stress, substrate rigidity and topography, and these stimuli have a more profound impact on stem cell phenotypes than had previously been recognized, e.g. self-renewal and differentiation through the control of gene transcription and signaling pathways. Using a variety of cell culture models enabled by micro and nanoscale technologies, we are beginning to systematically and quantitatively investigate the integrated response of cells to combinations of relevant mechanobiological stimuli. This paper reviews recent advances in engineering physical stimuli for stem cell mechanobiology and discusses how micro- and nanoscale engineered platforms can be used to control stem cell niches environment and regulate stem cell fate and function.

Keywords

biomechanical cues; stem cell; micro- and nanotechnology; surface topography

1. Introduction

Stem cells possess the capability to differentiate into cell types of many varied lineages; the variety of cell types is dictated by the type of stem cell, e.g. pluri- (embryonic or induced pluripotent cells), multi- (adult stem cells), or uni-potent (tissue-specific stem cells). This capability is regulated by the various stimuli dictated by the microenvironment including soluble factors and matrix-mediated signals, as well as from cell-cell communication[1,2]. Although it is an accepted knowledge that soluble factors, including many growth factors and cytokines significantly influence various stem cell phenotypes such as self-renewal and differentiation[3,4], many of the effects are not explained by known soluble factor-mediated signaling pathways. Recently, the cues presented by the physical microenvironment are being thought to be important regulators of stem cell behavior (Fig. 1)[5]. The mechanisms through which a living organism responds to its mechanical environment, e.g. physical force, and hemodynamic shear stress is referred to as mechanotransduction[6,7];many cell responses induced by mechanotransduction are summarized in Table I.

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Recent advancement in various techniques in tissue engineering and micro/nanotechnology has allowed researchers to unveil many aspects of biomechanical regulation, especially in the context of stem cells[8]. Most cells are in contact with extracellular matrix (ECM), a 3-dimensional scaffold to which they adhere. ECM can induce mechanically-coupled signaling pathways among its various functions to direct stem cell fate. To guide stem cells into specific lineages, researchers and bioengineers are now trying to mimic ECM properties in which stem cells reside *in vivo* by combining tissue engineering and micro/nanofabrication techniques. New developments in tissue engineering such as the design of novel scaffolds for cell culture mimicking various features present in the ECM permit the stem cell researchers to understand the biomechanical interactions of cells with their microenvironment. In addition, micro/nanofabrication technique used for complex layers of integrated circuits allows one to create specific structures mimicking the *in vivo* physiological topography integrated in *in vitro* cell culture models[9]. Cells can discern the physical cues from the substrate at nanometer scales, and their phenotypic responses are highly specific in nature. These responses may initiate the expression of specific genes or the signaling pathways related to the biomechanical regulation.

This review introduces the biomechanical regulation of cellular behaviors, and especially describes the studies showing that the effects of biomechanical properties on regulating stem cell fate. This research is of high significance due to its promise in stem cell therapy and important implications in biomechanical control of cell and developmental biology.

2. Extracellular matrix as a conveyer of biochemical and mechanical signals

Cells reside in a complex microenvironment consisting of other similar or dissimilar cell types, extracellular matrix, biochemical, and physical factors. Extracellular matrix (ECM) is diversely constituted consisting of various protein molecules, proteoglycans (carbohydrate polymers attached to other ECM proteins), carbohydrate polymers and other molecules that combine with water to create the mechanical properties, and structure of the ECM matrix[10,11]. ECM signals to cells in multiple ways: as mechanical signals owing to its physical properties[12–15], as chemical signals by virtue of special motifs present in the protein molecules present in the ECM[16], and as a presenter of biochemical paracrine signals that adhere to ECM and activate receptors present on the cells[17,18]. In order to understand the role of ECM signaling, it is necessary to decouple the various aspects of ECM involved in the observed phenotypic response, which till the recent development of micro- and nanofabrication tools, presented substantial challenges in experimental designs. Use of micropatterning tools, protein immobilization, and cell constraints imposed by controlling spatial presentation of ECM signals have presented unique insights into the role of ECM based signaling and cellular morphology, and how cell shape can control cell division, death, and even fate[18–21]. Geometry of ECM can control cell spreading which could determine the cellular decision to undergo apoptosis or cell growth[22,19]. Geometrically anisotropic substrata can stimulate cells to elongate via contact guidance of focal adhesion aligned by the topographical cues, and the degree of cell elongation is determined by both the architecture, as well as the biochemical properties of the substrata[23,24]. These data indicate that ECM modulate cellular phenotypes by acting not only as biochemical modulators of cell behavior, but also by presenting biological cues in different shapes, topography, and as direct mechanical forces[25]. Modern micro- and nanotechnology based platforms that have now been perfected for biological use present a wide range of tools to allow careful experimental designs to decouple the mechanical cues presented by ECM from physical, and biochemical nature[26–28]. These considerations are important, since it is possible that the various characteristics of ECMs influence cellular phenotypes via different signaling mechanisms[25,29].

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3. Effects of mechanical stimuli on stem cell fate and function

Stem cells can sense, transduce, and respond to stimuli such as shear stress[30], mechanical strain[31], matrix topography[32], and rigidity[33] (Fig. 2). While responses in stem cells vary, these processes typically modulate fundamental behaviors of stem cells including lineage regulation. Despite the significance of these interactions in fundamental developmental biology and the biomedical applications such as stem cell therapy, their widespread incorporation in biological techniques is limited, and understanding of the mechanism of mechanotransduction still remains poorly understood. However, the establishment of microscale technologies enables us to create the well-controlled biomechanical environment, and to investigate the effect of biomechanical regulation in stem cells.

2.1 Mechanical strain

Cells maintain a balance of force between cell and ECM through their actin cytoskeleton. External mechanical strain transduced by specific molecules expressed on cell surfaces such as integrins may induce the instantaneous imbalance and the re-organization of actin cytoskeleton. The change of this balance induced can influence cell shape, stimulate various signal transduction pathways, and induce transcription of genes resulting in a switch between cell growth and differentiation. For instance, the pulsatile nature of hemodynamic stresses leads to cyclic tensile strain prevalent in cardiac tissue and the blood vessel wall, leading to differentiation of the affected cells into smooth muscle cells[34].

Although mechanical strain usually exhibits complex patterns *in vivo*, its impacts and related mechanisms can be investigated with simplified *in vitro* models. Depending on system geometry, a combination of translational, rotational and/or multi-axial strain can be applied to cells to study the interaction between strain and cellular responses. Physiologically relevant mechanical strain could induce self-renewal and maintenance of pluripotency of embryonic stem cells (ESCs). For example, human ES cells (hESCs) cultured under biaxial cyclic strain exhibited small, tightly packed morphology with high level of Oct4 and SSEA-4, exclusively expressed in undifferentiated hESCs[35]. The cross-talk between mechanical and chemical signaling was evidenced by the reduced differentiation of hESCs cultured in mouse embryonic fibroblast (MEF)-conditioned medium, but not in the unconditioned medium[35]. It was also suggested that TGFβ/Activin/Nodal signaling might play as a key pathway to regulate strain induced inhibition of differentiation via the up-regulation of Smad2/3 phosphorylation blocking hESC differentiation[36].

Owing to their multipotency, availability for stem cell therapy applications, and low immunogenicity, adult mesenchymal stem cells (MSCs) have been widely employed for potential use in stem cell-based therapy. MSCs have the potential to differentiate into cell types residing in widely varying mechanical microenvironments, making them attractive targets for mechanical perturbation studies. For example, uniaxial strain transiently stimulated the up-regulation of smooth muscle contractile marker, promoting the differentiation into smooth muscle cell[37]. In contrast, equiaxial strain induced osteo-genes is through the activation of the extracellular signal-regulated kinase (ERK1/2) and p38 mitogen-activated protein kinase pathways[38]. With translational and rotational strain in a three dimensional bioreactor, MSCs could highly express specific ligament markers such as collagen I, III, and tenascin C[31] (Fig. 2B). Mechanical strain direction also influenced vascular cell phenotypes and strain rate in physiological ranges was found to induce phosphorylation of (Erk)1/2[39]. Furthermore, chemical signaling may have a crosstalk with mechanical strain as indicated by strain-exposed human embryoid body derived cells to exhibit high chondrogenic differentiation with the treatment of TGFβ[40]. Despite various studies about the effect of mechanical strain on stem cell behavior, the role of crosstalk

between mechanotransduction, and known signal transduction pathways initiated by soluble factors has remained unexplored. The critical point, however, is that these investigations still illustrate the vast possibilities to further our understanding of strain and their phenotypic effect on stem cells.

2.2. Substrate stiffness

Many characteristics of cell including morphology, function, and fate can depend strongly on substrate stiffness, i.e. the ability of the adherent material to undergo deformation in response to applied force by the cells. Non transformed adherent tissue cells generally must attach to a solid substrate for their survival in contrast to suspended cells e.g. red blood cells[41]. Actomyosin structures within the cell then generate traction forces against these attachments, resulting in transmission of these forces to the substrate. As a reaction, the substrate resists this deformation proportionally to its stiffness and this repulsive force influences cellular behavior. Motile cells cultured on elastic substrates have been demonstrated to align their shape along the direction of highest stiffness and move toward stiffer regions[42,43], and normal fibroblast on flexible substrate exhibit decrease in the DNA synthesis rate as well as an increase in the apoptosis rate[44]. Since each tissue in the body has a different stiffness ranging from pliable brain tissue (1kPa) to calcified bone (100kPa) (Fig. 3A)[13], cells located on different tissue may show particular properties and reactions. For example, appropriate substrate elasticity can be used to guide cell proliferation, and expansion of hematoopoietic stem cells[45], and proliferation and apoptosis[44] of various cell types.

MSCs have the potential to differentiate into cells of a wide variety, from neurons to osteocytes, guided by factors that are still unknown but are probably provided by the niche environments in the different tissue types. Among these niches, the wide variation in substrate stiffness with respect to tissue and organs can switch the lineage of its differentiation (Fig. 3B and 3C).[13] Specifically, a soft substrate (1kPa) mimicking brain tissue induces neurogenesis, stiffer substrates (10kPa) similar to muscle stimulate myogenesis, whereas MSCs on rigid matrix (30+ kPa) similar to collagenous bone cellular environment exhibit osteogenesis[13]. These observations are tension-dependent and illustrate that ECM stiffness can determine the developmental lineage of stem cells to a significant extent. Furthermore, it has been shown that interplay between matrix stiffness and adhesive ligand presentation is critically important. For instance, osteogenesis of MSCs was promoted only on the stiffest collagen I-coated gels (80 kPa), not fibronectin-coated gels whereas myogenesis was induced on moderately stiff, fibronectin-coated gels (25 kPa), with similar levels of that on the stiffest collagen I-coated gels (80 kPa)[46].

The differentiation of neural stem cells (NSCs) has also been demonstrated to be modulated by matrix elasticity. NSCs exhibited peak level of the neuronal marker, β-tubulin III on gel near the physiological stiffness (0.1~1kPa) of brain tissue in serum-free neuronal media[47]. Under mixed differentiation conditions with serum, softer substrates led NSCs to neurons in contrast to harder gels that resulted in a higher proportion of glia cells in the culture, indicating that tuning substrate stiffness modulate growth factor sensitivity. In much the same light, cell spreading also acts through an actomyosin-related mechanism that generates tension within the cytoskeleton resulting in differential response to mixed growth factor conditions[48]. Again in both scenarios, focal adhesion kinase and Rho kinase pathway have been implicated as potential regulators[48,49]. p190RhoGAP, an inhibitor for RhoA, might play an important role in modulating the cellular sensing of substrate rigidity, presumably via regulation of RhoA activity, and also in RhoA independent fashion[12,48]. Src family kinases and phospho-tyrosine signaling has also been implicated to play an important role in rigidity sensing[50]. Mechanical and biochemical factors influence cellular and tissue behavior[51], and greater knowledge about the parameters of these factors will be useful in

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designing tissue constructs towards therapeutic objectives. For example, biochemical factors available in neo natal heart, and tissue stiffness of the heart were used to create a biodegradable cardiac tissue construct[52]. However, to completely appreciate the complete mechanism for substrate rigidity sensing in cells, future studies will need to unveil the underlying coupling between these signaling observations. Improving the scaffold design to be even more biomimetic, e.g. spatial and temporal changes in stiffness, would greatly enhance our understanding of stem cell behavior in response to matrix elasticity and may lead to creation of novel therapeutic biomaterials.

2.3. Shear stress

In vivo, cells experience a dynamic shear environment, both in liquid tissues (blood, lymphatics) and solid ones. Mechanical loading and bending of bones result in strain gradients as well as local pressure gradients in the bone that can drive interstitial fluid flow resulting in complex shear stress patterns[53]. Vascular and lymphatic endothelial cells are particularly exposed to shear stress stemming from blood flow and their physiology is largely guided by the shear forces present in the blood[54]. Shear stress significantly affects cell behavior at molecular levels[55–58]. Due to a lack of technological platforms to study cellular behavior in defined shear stress conditions, however, the role of shear stress in physiological processes is underappreciated. With the advent of modern tools like microfluidics, molecular mechanisms underlying stress mediated cell-cell adhesion and cell-cell interactions are now being revealed. To generate such physiological levels of shear stress, a parallel plate flow chamber[30] or microfluidic shear devices[59,60] are extensively used and demonstrate cell type-specific responses to shear stress. For example, endothelial cells can activate mechanosensitive ion channels and modify gene regulation by laminar flow-mediated shear stress originated from blood flow[61]. In addition, shear stress aligns cell shape along the direction of the flow by dynamically reorganizing cytoskeletal filaments, focal adhesions and ECM in cell density-dependent manner[62].

Since stem cells need to home to sites of injury, they are expected to be sensitive to the shear stress they encounter in their path either post intravasation, or through interstitial fluid flows. Specifically, hESC-derived endothelial cells exhibited the up-regulation ofMMP1 that degrade ECM for helping morphological change and COX2 responsible for formation of prostanoids involved in the inflammatory response on the application of physiologic level of shear stress[30]. This up-regulation can explain the morphological elongation and amplification of inflammatory signal under shear stress. The exposure of hESC-derived endothelial cells to flow could also cause the increase in angiogenic and vasculogenic potential as well as cytoskeletal rearrangement in the direction of flow[63]. Endothelial progenitor cells under shear stress up-regulated the expression of VEGF receptors and activated these receptors without corresponding ligands. Shear stress also stimulated molecular pathways leading to histone modifications in mouse ESCs, resulting in epigenetic modifications, indicating that stress response can result in even epigenomic regulation[56]. These changes control the cardiovascular cell fate as shown by up-regulation of vascular and cardiovascular marker such as smooth muscle actin, smooth muscle protein 22-alpha, platelet-endothelial cell adhesion molecule-1, VEGF receptor 2, myocyte enhancer factor-2C (MEF2C), and alpha-sarcomeric actin[56].

In addition to ESCs, shear stress may also contribute to adult stem cell differentiation into vascular cells. For instance, shear stress (15 dyn/cm^2) for a 12-hour period significantly increased the expression of mature endothelial cell-specific markers in CH3H/10T1/2 cells such as CD31 (757-fold as compared with static control), von Willebrand factor (108-fold), and vascular endothelial-cadherin (23-fold) at both the mRNA and protein levels[64]. Angiogenic growth factor was also up-regulated, while markers associated with smooth muscle cell differentiation were down-regulated. As with mixed media conditions, the

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introduction of osteogenic differentiation factors coupled with pulsatile fluid flow for adipose-derived stem cells modulates their sensitivity, but in the opposite manner: rather than pulsatile fluid flow inducing adipose- or vascular-like responses, growth factors override them to induce osteogenic responses including increase in nitric oxide production and the up-regulation of cyclooxygenase-2 are observed[65,66]. This differentiation of MSCs is suggested to result from the increase in the phosphorylation of (Erk)1/2 by shear stress[65]. Overall though, shear stress is of particular interest in vascular cell fate because it is suggested to be involved in endothelial function, and potentially in endothelial differentiation of stem cells. Again, the molecular machinery involved in shear stress sensing and the resulting activation of signaling leading to transcription of genes is only now begun to be understood.

2.4. Substrate topography

ECM can be organized into fibers, sheets, and other features that range in sizes from nanometers to many centimeters. To mimic these features electron beam lithography[67], photolithography[68], self-assembly of colloidal monolayers[69], and other micro/ nanofabrication techniques[15] have been used to create experimental platforms to understand the role of substratum topography on cellular behaviors[70]. Stem cells can sense these features and regulate their shape, apoptosis, proliferation or differentiation. For example, ECM electron beam lithography has been used to demonstrate that geometric control of cells determines cell fates[19,71]. Similarly, micro/nanofabrication techniques have been used to create topographical features of arbitrary sizes and shapes. Recently, control of randomness in nanoscale surface agglomeration was accomplished allowing understanding of anisotropy and randomness in influencing ESCs behavior[71]. In addition, combination of roughness with micro-grated groove pattern led to control the axonal growth orientation from resulting neuronal cultures from the ESCs[15].

Like other ECM properties, topographical guidance may impact various stem cell behaviors. Nanoscale substrata have recently been developed to culture MSCs in long cultures (8 weeks) while maintaining their multipotency[72]. In the case of osteoprogenitors, the topographical cue manufactured by electron beam lithography allowed stem cells to adhere more strongly to the substrate, to reorient cytoskeleton, and to produce osteoblasts specific markers such as osteocalcin and osteopontin (Fig. 4A)[67]. Furthermore, disordered nanoscale pits constructed by colloidal lithography induced differentiation of osteoprogenitors as evidenced by the production of bone mineral *in vitro*, and transcription of osteoblasts specific genes, presumably through the topography induced inhibition of Wnt/β-catenin signaling[68]. In addition to attachment and differentiation, cell-cell communication is also regulated by topographical cues. Osteoblasts on10-μm grooved substrate exhibited a reduced expression of the gap junction protein connexin-43 compared to a flat substrate, resulting in reduced communication between adjacent cells[73].

With three-dimensional (3D) topographical cues, height and diameter feature control has also been implicated to be involved in the regulation of stem cell behavior[74]. Using nanotube structures to control surface height and feature diameter, larger diameter nanotubes (70 to 100 nm) support noticeable stem cell elongation by inducing cytoskeletal stress and selective differentiation into osteoblast-like cells when compared to smaller diameter nanotubes (30 nm) (Fig. 4B and 4C)[75]. 3D networks of electrospun nanofibers also can alter substrate height and have been shown to preferentially induce NSC differentiation into neurons rather than into astrocytes[76]. In addition, nanofiber diameter also appears to regulate NSC proliferation and cell spreading, though its influence on differentiation is not as certain[77]. In general, however, these micro/nanofabrication techniques permit us to design the substrate with defined topographical cues with arbitrary features that regulate stem cell shape, fate, migration, proliferation. As with other cues, the importances of surface

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fabrication in future stem cell research or therapy is dependent on our better understanding of the mechanism behind sensing these topographical cues.

4. Co-regulation of stem cell function by multi-variant mechanical stimuli: the move to 3D cultures

In vivo microenvironment is complex and can exert multiple cues on cells, consisting of combinatorial stimuli of biomechanical, biochemical, or biophysical nature[74]. For example, endothelial cells in the lumen are exposed to growth factors, cytokines, and shear stress as well as receiving cues from the properties of their cell-cell and cell-ECM contacts[12,78]. MSCs used for vascular grafts are also affected by mechanical strains in the vascular wall, which are anisotropic and circumferential[79]. Generally though, these sorts of cues are sensed in concert with each other in a 3D environment where dramatically different behaviors can be observed compared to the conventional 2D environment discussed at length above[80,81].

Before discussing the effects a 3D environment has on stem cells and how it couples various cues together, it is necessary to compare and contrast the material requirement and technical limitations for these systems. Synthetic polymer hydrogels are typically polymerized as a 3D network that presents a 2D culture substrate as they are often composed of cytotoxic components that upon polymerization become inert. However, cells can now be mixed in situ in liquid gels that can be polymerized by temperature, chemical or photo induced polymerization[82]. Novel techniques to encapsulate cells in gels in a highly controlled manner are emerging from microfluidics community by leveraging the control over fluid flow of polymer gels[83,84]. Cells are seeded on top of the hydrogel after polymerization and functionalization with a variety of ECM proteins or cell adhesive peptides, e.g. RGD[85,86] or collagen[87,88]. On the other hand, nanofibers closely mimic the *in vivo* 3D microenvironments, are capable of encapsulating cells, and are seen to be more compatible with widely available techniques including fluorescence microscopy but suffer from not presenting the mechanical cues to the cells in a complete 3D fashion[28,89]. In addition, culture on these scaffolds can be combined with chemical and physical perturbations with relative ease. For example, nanofiber organization with immobilization of ECM protein and growth factor can mimic the native matrix fibrils in a closer manner[69]. A combination of both nanotopography and chemical signaling have also been shown to significantly enhanced skin cell migration, in the context of wound healing, while also presenting novel phenotypes that are not observable on a smooth surface[90].

For stem cells specifically, the phenotype of many stem cell types has been shown to change greatly when cultured on 3D systems as compared to the more conventional 2D systems[1]. The most common observation is that cells fail to form *in vivo* like structures on flat and hard surfaces, while collectively assemble into tissue-like structures when grown in 3D such as tube formations by endothelial cells, mammary gland formations by breast cancer cells and closed organoid formation by epithelial cells[91–93]. Stem cells also are expected to exhibit more tissue like organization when grown in compliant 3D microenvironments[33]. We conjecture that while there may be observable differences in cell autonomous behavior when cells are cultured on physiologically relevant mechanical substrata as compared to on tissue culture dishes/cover slips, collective cell behavior differences may be more visible when these physiological mechanical cues are presented to the cells in 3D. Ultimately however, the ‘devil is in the details’ for the specific stem cell and its niche, which can vary widely in terms of their anatomical organization, extracellular composition, cell-cell interactions, and mechanical cues[94].

There are many characteristics that vary between cell niches, e.g. the elasticity, strain, and topography are all different between ESCs, MSCs, *Drosophilia* germ cells, etc. Yet the

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biochemical and biophysical reactions at play in these niches must remain balanced so that the tissue remains static unless an external trigger is presented, for example, by inflammation, tissue injury, or loss of somatic cell types. This process, called dynamic reciprocity, affects cell migration, fate, and proliferation[35], and since each niche is different, the exact balance of forces in each case is different. It is this balance that leads to tissue morphogenesis, relating form and function, but what results are quantifiable and specific phenotypic changes within cells. For example, cells sense their mechanical microenvironment through integrin molecules that are part of larger focal adhesion complexes that couple the intracellular cytoskeleton and ECM protein networks. Focal adhesions, especially in stem cells, are largely dependent on their context as their composition can change based on the dimensionality of their matrix[95]. Indeed, the focal adhesions observed in 3D environment are found to be more mature consisting of many more molecules localized together (vinculin, paxillin, focal adhesion kinase, a-actinin, $\alpha5\beta1$ integrin and phosphotyrosine)[5,80]. In addition to adhesions, cytoskeletal tension in stem cells balances and aligns their internally-generated forces exerted on the ECM with that generated in neighboring cells[96]. This phenomenon differs significantly in 3D where a highly fibrillar ECM transduces unidirectional forces along fibers rather than bidirectionally as in 2D. Ultimately a more detailed examination is required of how cells sense mechanical cues in 3D via focal adhesion components, and then respond via signaling pathway activation and transcriptional activity to affect lineage commitment.

Cells reside in a 3-dimensional world, and it is increasingly being appreciated that cellular phenotypes are significantly affected by the reduction of dimensionality in which mechanical and biochemical cues are presented to the cell. An adult stem cell niche contains the cues presented to the cells in 3 dimensions in the form of a cellular neighborhood with specific identities, growth factors tethered to the ECM, mechanical forces provided by the ECM, topography and physical parameters, e.g. ischemia. Future research and development is required to create true 3D contexts of the cells that mimic the natural niche environment of the stem cell combining all the above factors.

5. Mechanisms of mechanosensing

How cells sense and respond to the mechanical stimuli remains an unresolved question, but significant progress has been made recently in determining the signaling mechanisms involved in mechanotransduction[97,98]. Mechanical forces, as described in Section 2, manifest themselves in different ways to the cells, but recent research suggests significant redundancy in the pathways used to transmit and transduce mechanical information into biochemical signals that cells can interpret[97,99]. Mechanical forces can occur both “outside-in” and “inside-out,” meaning the cell can generate traction stresses on their environment in the former case or be exposed to an external force in the latter case. For outside-in situations, forces are typically generated by actomyosin contractions in sarcomeres for muscle or in pre-myofibrils in non-muscle cells. These forces are transmitted via a complex assembly of focal adhesion proteins and integrins, which bind to ECM molecules that can transmit these forces to adjacent cells bound to the same matrix proteins[99,100]. Within the focal adhesions, guanine exchange factors (GEF) activate Rho, Rac and other small GTPases[101]. RhoA activation results in ROCK phosphorylation, which in turn activates myosin light chain kinase (MLCK). MLCK can recruit myosin II to myofibrils and induce its activation, which is ultimately responsible for cell contractility and enables the cell to “feel” its extracellular environment[102]. In addition, mDiA, another effector of RhoA, results in the polymerization and stabilization of the actin cytoskeleton to ensure that it may develop the intracellular tension necessary for an array of cell behaviors[101,103].

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While Rho GTPases are the most well studied mechanotransduction pathway, several alternative sensing mechanisms have been identified. For instance, cells employ stretch activated ion channels that allow Ca2+ influx in stretched cells transiently[104] and results in increased activation of Calmodulin (CaM) and MLCK[105]. p190RhoGAP, a GAP protein of RhoA is also known to directly influence transcription in both RhoA dependent and independent manner[12,48]. YAP, a downstream transcriptional factor in the Hippo pathway has also been found to be involved in sensing substratum rigidity[106]. There is also evidence that talin, and vinculin, both found in the focal adhesion complex, undergo conformational changes and alter their binding affinities in response to force[107], and this represents another important class of potential mechanosensing mechanisms. In this way, signaling from cryptic binding sites could either invoke a biphasic response, as suggested with MAPK[108], or a monotonically increasing one where additional stretch induces more signaling.

While this list of potential mechanosensing pathways may appear to be both extensive and redundant in many ways, these mechanism likely only represent a partial list of the ways cells feel their surroundings. From that perspective, discovering all of cell mechanosensing mechanisms may be impractical as too much overlap in the control of common functions may limit our detection capabilities. One critical area that remains somewhat uncertain are the specific ways that upstream mechano-signaling results in gene transcription. Not to be lost in the variety of mechanisms, however, is the point that mechanobiologists must appreciate that mechanical stimuli can be transient (e.g. shear stress) or static (e.g. rigidity or topography), and each may require different signaling machinery or involvement of control modules that have still not been discovered.

6. Conclusions and outlook

Stem cell investigations have often been guided by the discovery of novel soluble cues or signaling pathways that regulate stem cells fate, many of which are well known growth factors with unexpected function in stem cells. However, the role of mechanical cues in determining stem cell fate has only now come into focus with findings from the past 5 years indicating that embryonic and adult stem cells are profoundly affected by various mechanical and biophysical cues[28]. Importantly, the role of mechanical cues on stem cell fate underlines the fact that the heterogeneous mechanical milieu in an organism affects stem cells in very significant ways[109]. This underlines the need to create physiologically relevant environments for stem cell culture, maintenance, and application to regenerative medicine where these findings can be used as design criteria for new materials. A common critique of adult stem cells based experiments conducted *in vitro* is the disconnect between the physiological niches in which stem cells reside, and the plastic or glass plates in which they are observed. There is a need to combine mechanical, biochemical, and physical cues present in adult stem cell niches in a single platform to bridge the disconnect allowing in vitro observations to have increased expected extrapolation with the physiological context of the cells. Further, our understanding of these underlying biophysical mechanism(s) require significantly more investment in molecular biology tools integrated with the novel platforms to study role of mechanical forces on the cells. Only then can the role of mechanotransduction, especially in conjunction with other known signaling pathways, be fully appreciated.

References

1. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science. 2009; 324:1673–1677. [PubMed: 19556500]
2. Moore KA, Lemischka IR. Stem cells and their niches. Science. 2006; 311:1880–1885. [PubMed: 16574858]

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3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–676. [PubMed: 16904174]
4. Seuntjens E, et al. Transforming Growth Factor type beta and Smad family signaling in stem cell function. Cytokine Growth Factor Rev. 2009; 20:449–458. [PubMed: 19892581]
5. Guilak F, et al. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell. 2009; 5:17–26. [PubMed: 19570510]
6. Wozniak MA, Chen CS. Mechanotransduction in development: a growing role for contractility. Nat Rev Mol Cell Biol. 2009; 10:34–43. [PubMed: 19197330]
7. Orr AW, Helmke BP, Blackman BR, Schwartz MA. Mechanisms of mechanotransduction. Developmental cell. 2006; 10:11–20. [PubMed: 16399074]
8. Kim DH, Wong PK, Park J, Levchenko A, Sun Y. Microengineered platforms for cell mechanobiology. Annu Rev Biomed Eng. 2009; 11:203–233. [PubMed: 19400708]
9. Kim DH, Lee H, Lee YK, Nam JM, Levchenko A. Biomimetic nanopatterns as enabling tools for analysis and control of live cells. Adv Mater. 2010; 22:4551–4566. [PubMed: 20803528]
10. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. The Journal of cell biology. 2012; 196:395–406. [PubMed: 22351925]
11. Gillies AR, Lieber RL. Structure and function of the skeletal muscle extracellular matrix. Muscle & nerve. 2011; 44:318–331. [PubMed: 21949456]
12. Kshitiz, et al. Matrix rigidity controls endothelial differentiation and morphogenesis of cardiac precursors. Science Sig. 2012
13. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006; 126:677–689. [PubMed: 16923388]
14. Dela Paz NG, Walshe TE, Leach LL, Saint-Geniez M, D'Amore PA. Role of shear-stress-induced VEGF expression in endothelial cell survival. Journal of cell science. 2012; 125:831–843. [PubMed: 22399811]
15. Bakeine GJ, et al. Design, fabrication and evaluation of nanoscale surface topography as a tool in directing differentiation and organisation of embryonic stem-cell-derived neural precursors. Microelectronic Engineering. 2009; 86:1435–1438.
16. Giancotti FG. A structural view of integrin activation and signaling. Developmental cell. 2003; 4:149–151. [PubMed: 12586058]
17. Taipale J, Keski-Oja J. Growth factors in the extracellular matrix. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 1997; 11:51–59. [PubMed: 9034166]
18. Pompe T, Salchert K, Alberti K, Zandstra P, Werner C. Immobilization of growth factors on solid supports for the modulation of stem cell fate. Nature protocols. 2010; 5:1042–1050.
19. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science. 1997; 276:1425–1428. [PubMed: 9162012]
20. Lehnert D, et al. Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. Journal of cell science. 2004; 117:41–52. [PubMed: 14657272]
21. Kilian KA, Bugarija B, Lahn BT, Mrksich M. Geometric cues for directing the differentiation of mesenchymal stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:4872–4877. [PubMed: 20194780]
22. Ingber DE. Fibronectin controls capillary endothelial cell growth by modulating cell shape. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87:3579–3583. [PubMed: 2333303]
23. Lamers E, et al. The influence of nanoscale grooved substrates on osteoblast behavior and extracellular matrix deposition. Biomaterials. 2010; 31:3307–3316. [PubMed: 20122723]
24. Park J, Kim HN, Kim DH, Levchenko A, Suh KY. Quantitative analysis of he combined effect of substrate rigidity and topographic guidance on cell morphology. IEEE transactions on nanobioscience. 2012; 11:28–36. [PubMed: 21908261]

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25. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Developmental cell. 2004; 6:483–495. [PubMed: 15068789]
26. Tan JL, et al. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:1484–1489. [PubMed: 12552122]
27. Sniadecki NJ, et al. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:14553–14558. [PubMed: 17804810]
28. Kshitiz Kim DH, Beebe DJ, Levchenko A. Micro- and nanoengineering for stem cell biology: the promise with a caution. Trends Biotechnol. 2011; 29:399–408. [PubMed: 21549437]
29. Dupont S, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011; 474:179–183. [PubMed: 21654799]
30. Metallo CM, Vodyanik MA, de Pablo JJ, Slukvin II, Palecek SP. The response of human embryonic stem cell-derived endothelial cells to shear stress. Biotechnol Bioeng. 2008; 100:830–837. [PubMed: 18306423]
31. Altman GH, et al. Cell differentiation by mechanical stress. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2002; 16:270–272. [PubMed: 11772952]
32. Yim EK, Pang SW, Leong KW. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. Exp Cell Res. 2007; 313:1820–1829. [PubMed: 17428465]
33. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol. 2009; 10:21–33. [PubMed: 19197329]
34. Jeong SI, et al. Mechano-active tissue engineering of vascular smooth muscle using pulsatile perfusion bioreactors and elastic PLCL scaffolds. Biomaterials. 2005; 26:1405–1411. [PubMed: 15482828]
35. Saha S, Ji L, de Pablo JJ, Palecek SP. Inhibition of human embryonic stem cell differentiation by mechanical strain. J Cell Physiol. 2006; 206:126–137. [PubMed: 15965964]
36. Saha S, Ji L, de Pablo JJ, Palecek SP. TGFbeta/Activin/Nodal pathway in inhibition of human embryonic stem cell differentiation by mechanical strain. Biophys J. 2008; 94:4123–4133. [PubMed: 18234825]
37. Park JS, et al. Differential effects of equiaxial and uniaxial strain on mesenchymal stem cells. Biotechnol Bioeng. 2004; 88:359–368. [PubMed: 15486942]
38. Simmons CA, et al. Cyclic strain enhances matrix mineralization by adult human mesenchymal stem cells via the extracellular signal-regulated kinase (ERK1/2) signaling pathway. J Biomech. 2003; 36:1087–1096. [PubMed: 12831733]
39. McKnight NL, Frangos JA. Strain rate mechanotransduction in aligned human vascular smooth muscle cells. Annals of biomedical engineering. 2003; 31:239–249. [PubMed: 12680722]
40. Terraciano V, et al. Differential response of adult and embryonic mesenchymal progenitor cells to mechanical compression in hydrogels. Stem Cells. 2007; 25:2730–2738. [PubMed: 17702983]
41. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science. 2005; 310:1139–1143. [PubMed: 16293750]
42. Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophys J. 2000; 79:144–152. [PubMed: 10866943]
43. Saez A, Ghibaudo M, Buguin A, Silberzan P, Ladoux B. Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. Proc Natl Acad Sci USA. 2007; 104:8281–8286. [PubMed: 17488828]
44. Wang HB, Dembo M, Wang YL. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. Am J Physiol Cell Physiol. 2000; 279:C1345–1350. [PubMed: 11029281]
45. Holst J, et al. Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. Nat Biotechnol. 2010; 28:1123–1128. [PubMed: 20890282]

NIH-PA Author Manuscript

46. Rowlands AS, George PA, Cooper-White JJ. Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation. Am J Physiol Cell Physiol. 2008; 295:C1037–1044. [PubMed: 18753317]
47. Saha K, et al. Substrate modulus directs neural stem cell behavior. Biophys J. 2008; 95:4426–4438. [PubMed: 18658232]
48. Mammoto A, et al. A mechanosensitive transcriptional mechanism that controls angiogenesis. Nature. 2009; 457:1103–1108. [PubMed: 19242469]
49. Mitrossilis D, et al. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:18243–18248. [PubMed: 19805036]
50. Giannone G, Sheetz MP. Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways. Trends Cell Biol. 2006; 16:213–223. [PubMed: 16529933]
51. Li D, et al. Integrated biochemical and mechanical signals regulate multifaceted human embryonic stem cell functions. The Journal of cell biology. 2010; 191:631–644. [PubMed: 20974810]
52. Pagliari S, et al. Cooperation of biological and mechanical signals in cardiac progenitor cell differentiation. Adv Mater. 2011; 23:514–518. [PubMed: 21254254]
53. Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. Annals of biomedical engineering. 2008; 36:1978–1991. [PubMed: 18855142]
54. Riha GM, Lin PH, Lumsden AB, Yao Q, Chen C. Roles of hemodynamic forces in vascular cell differentiation. Annals of biomedical engineering. 2005; 33:772–779. [PubMed: 16078617]
55. DiStasi MR, Ley K. Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability. Trends Immunol. 2009; 30:547–556. [PubMed: 19783480]
56. Illi B, et al. Epigenetic histone modification and cardiovascular lineage programming in mouse embryonic stem cells exposed to laminar shear stress. Circ Res. 2005; 96:501–508. [PubMed: 15705964]
57. Ten Dijke P, Egorova AD, Goumans MJ, Poelmann RE, Hierck BP. TGF-beta Signaling in Endothelial-to-Mesenchymal Transition: The Role of Shear Stress and Primary Cilia. Sci Signal. 2012; 5(pt2)
58. Zeng Y, Shen Y, Huang XL, Liu XJ, Liu XH. Roles of mechanical force and CXCR1/CXCR2 in shear-stress-induced endothelial cell migration. Eur Biophys J. 2012; 41:13–25. [PubMed: 21989491]
59. Lu H, et al. Microfluidic shear devices for quantitative analysis of cell adhesion. Analytical Chemistry. 2004; 76:5257–5264. [PubMed: 15362881]
60. Plouffe BD, et al. Peptide-mediated selective adhesion of smooth muscle and endothelial cells in microfluidic shear flow. Langmuir. 2007; 23:5050–5055. [PubMed: 17373836]
61. Stolberg S, McCloskey KE. Can shear stress direct stem cell fate? Biotechnol Prog. 2009; 25:10–19. [PubMed: 19197983]
62. Mott RE, Helmke BP. Mapping the dynamics of shear stress-induced structural changes in endothelial cells. Am J Physiol Cell Physiol. 2007; 293:C1616–1626. [PubMed: 17855768]
63. McCloskey KE, Smith DA, Jo H, Nerem RM. Embryonic stem cell-derived endothelial cells may lack complete functional maturation in vitro. J Vasc Res. 2006; 43:411–421. [PubMed: 16877873]
64. Wang H, et al. Shear stress induces endothelial differentiation from a murine embryonic mesenchymal progenitor cell line. Arterioscler Thromb Vasc Biol. 2005; 25:1817–1823. [PubMed: 15994439]
65. Kapur S, Baylink DJ, Lau KH. Fluid flow shear stress stimulates human osteoblast proliferation and differentiation through multiple interacting and competing signal transduction pathways. Bone. 2003; 32:241–251. [PubMed: 12667551]
66. Knippenberg M, et al. Adipose tissue-derived mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on osteogenic stimulation. Tissue Eng. 2005; 11:1780–1788. [PubMed: 16411823]
67. Biggs MJ, et al. Interactions with nanoscale topography: adhesion quantification and signal transduction in cells of osteogenic and multipotent lineage. J Biomed Mater Res A. 2009; 91:195–208. [PubMed: 18814275]

NIH-PA Author Manuscript

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68. Dalby MJ, McCloy D, Robertson M, Wilkinson CD, Oreffo RO. Osteoprogenitor response to defined topographies with nanoscale depths. Biomaterials. 2006; 27:1306–1315. [PubMed: 16143393]

69. Silva GA, et al. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science. 2004; 303:1352–1355. [PubMed: 14739465]

70. Gupta K, et al. Lab-on-a-chip devices as an emerging platform for stem cell biology. Lab Chip. 2010; 10:2019–2031. [PubMed: 20556297]

71. Dalby MJ, et al. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater. 2007; 6:997–1003. [PubMed: 17891143]

72. McMurray RJ, et al. Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. Nat Mater. 2011; 10:637–644. [PubMed: 21765399]

73. Kirmizidis G, Birch MA. Microfabricated grooved substrates influence cell-cell communication and osteoblast differentiation in vitro. Tissue Eng Part A. 2009; 15:1427–1436. [PubMed: 19061431]

74. Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. Cell. 2007; 130:601–610. [PubMed: 17719539]

75. Oh S, et al. Stem cell fate dictated solely by altered nanotube dimension. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:2130–2135. [PubMed: 19179282]

76. Xie J, et al. The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages. Biomaterials. 2009; 30:354–362. [PubMed: 18930315]

77. Christopherson GT, Song H, Mao HQ. The influence of fiber diameter of electrospun substrates on neural stem cell differentiation and proliferation. Biomaterials. 2009; 30:556–564. [PubMed: 18977025]

78. Bergh N, Larsson P, Ulfhammer E, Jern S. Effect of shear stress, statins and TNF-alpha on hemostatic genes in human endothelial cells. Biochem Biophys Res Commun. 2012

79. Kurpinski K, Chu J, Hashi C, Li S. Anisotropic mechanosensing by mesenchymal stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:16095–16100. [PubMed: 17060641]

80. Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. Science. 2001; 294:1708–1712. [PubMed: 11721053]

81. Cukierman E, Pankov R, Yamada KM. Cell interactions with three-dimensional matrices. Current opinion in cell biology. 2002; 14:633–639. [PubMed: 12231360]

82. Lippens E, et al. Cell survival and proliferation after encapsulation in a chemically modified pluronic(R) F127 hydrogel. J Biomater Appl. 2011

83. Aikawa T, Konno T, Takai M, Ishihara K. Spherical phospholipid polymer hydrogels for cell encapsulation prepared with a flow-focusing microfluidic channel device. Langmuir. 2012; 28:2145–2150. [PubMed: 22176809]

84. Mazzitelli S, Borgatti M, Breveglieri G, Gambari R, Nastruzzi C. Encapsulation of eukaryotic cells in alginate microparticles: cell signaling by TNF-alpha through capsular structure of cystic fibrosis cells. J Cell Commun Signal. 2011; 5:157–165. [PubMed: 21484183]

85. Chikar JA, et al. The use of a dual PEDOT and RGD-functionalized alginate hydrogel coating to provide sustained drug delivery and improved cochlear implant function. Biomaterials. 2012; 33:1982–1990. [PubMed: 22182748]

86. Chandler EM, et al. Stiffness of photocrosslinked RGD-alginate gels regulates adipose progenitor cell behavior. Biotechnol Bioeng. 2011; 108:1683–1692. [PubMed: 21328324]

87. Lawrence BJ, Madihally SV. Cell colonization in degradable 3D porous matrices. Cell Adh Migr. 2008; 2:9–16. [PubMed: 19262124]

88. Moraes C, Sun Y, Simmons CA. (Micro)managing the mechanical microenvironment. Integr Biol (Camb). 2011; 3:959–971. [PubMed: 21931883]

89. Gupta, K.; Kim, DH.; Ellison, DE.; Smith, C.; Levchenko, A. Using Lab-on-a-chip technologies for stem cell biology. In: Appasani, K.; Appasani, RK., editors. Stem cells and regenerative medicine. Vol. XXVIII. Springer Science (Humana) Press; 2011. p. 483-498.

NIH-PA Author Manuscript

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90. Patel S, et al. Bioactive nanofibers: synergistic effects of nanotopography and chemical signaling on cell guidance. Nano Lett. 2007; 7:2122–2128. [PubMed: 17567179]
91. Ewald AJ, et al. Mammary collective cell migration involves transient loss of epithelial features and individual cell migration within the epithelium. Journal of cell science. 2012
92. Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Developmental cell. 2008; 14:570–581. [PubMed: 18410732]
93. Nandakumar V, et al. Isotropic 3D nuclear morphometry of normal, fibrocystic and malignant breast epithelial cells reveals new structural alterations. PLoS One. 2012; 7:e29230. [PubMed: 22242161]
94. Engler AJ, et al. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. The Journal of cell biology. 2004; 166:877–887. [PubMed: 15364962]
95. Harunaga JS, Yamada KM. Cell-matrix adhesions in 3D. Matrix Biol. 2011; 30:363–368. [PubMed: 21723391]
96. Sjostrom T, et al. Fabrication of pillar-like titania nanostructures on titanium and their interactions with human skeletal stem cells. Acta Biomater. 2009; 5:1433–1441. [PubMed: 19208503]
97. Holle AW, Engler AJ. More than a feeling: discovering, understanding, and influencing mechanosensing pathways. Current opinion in biotechnology. 2011; 22:648–654. [PubMed: 21536426]
98. Hamill OP, Martinac B. Molecular basis of mechanotransduction in living cells. Physiological reviews. 2001; 81:685–740. [PubMed: 11274342]
99. Wang JH, Thampatty BP. An introductory review of cell mechanobiology. Biomechanics and modeling in mechanobiology. 2006; 5:1–16. [PubMed: 16489478]
100. Schwartz MA. Integrin signaling revisited. Trends Cell Biol. 2001; 11:466–470. [PubMed: 11719050]
101. Mammoto A, Mammoto T, Ingber DE. Rho signaling and mechanical control of vascular development. Current opinion in hematology. 2008; 15:228–234. [PubMed: 18391790]
102. Narumiya S, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. Cancer metastasis reviews. 2009; 28:65–76. [PubMed: 19160018]
103. Bryan BA, Li D, Wu X, Liu M. The Rho family of small GTPases: crucial regulators of skeletal myogenesis. Cellular and molecular life sciences: CMLS. 2005; 62:1547–1555. [PubMed: 15905962]
104. Sachs F. Stretch-activated ion channels: what are they? Physiology. 2010; 25:50–56. [PubMed: 20134028]
105. Jaalouk DE, Lammerding J. Mechanotransduction gone awry. Nature reviews. 2009; 10:63–73.
106. Dupont S, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011; 474:179–183. [PubMed: 21654799]
107. del Rio A, et al. Stretching single talin rod molecules activates vinculin binding. Science. 2009; 323:638–641. [PubMed: 19179532]
108. Holle AW, et al. In Situ Mechanotransduction via Vinculin Regulates Stem Cell Myogenesis. The Journal of cell biology. 2012
109. Rehfeldt F, Engler AJ, Eckhardt A, Ahmed F, Discher DE. Cell responses to the mechanochemical microenvironment--implications for regenerative medicine and drug delivery. Adv Drug Deliv Rev. 2007; 59:1329–1339. [PubMed: 17900747]
110. Dalby MJ, et al. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nature materials. 2007; 6:997–1003.
111. Kaunas R, Nguyen P, Usami S, Chien S. Cooperative effects of Rho and mechanical stretch on stress fiber organization. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:15895–15900. [PubMed: 16247009]
112. Kim BS, Nikolovski J, Bonadio J, Mooney DJ. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. Nat Biotechnol. 1999; 17:979–983. [PubMed: 10504698]

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113. Kinneberg KR, et al. Chondroitin-6-sulfate incorporation and mechanical stimulation increase MSC-collagen sponge construct stiffness. J Orthop Res. 2010; 28:1092–1099. [PubMed: 20143407]
114. Nikmanesh M, Shi ZD, Tarbell JM. Heparan sulfate proteoglycan mediates shear stress-induced endothelial gene expression in mouse embryonic stem cell-derived endothelial cells. Biotechnol Bioeng. 2012; 109:583–594. [PubMed: 21837663]

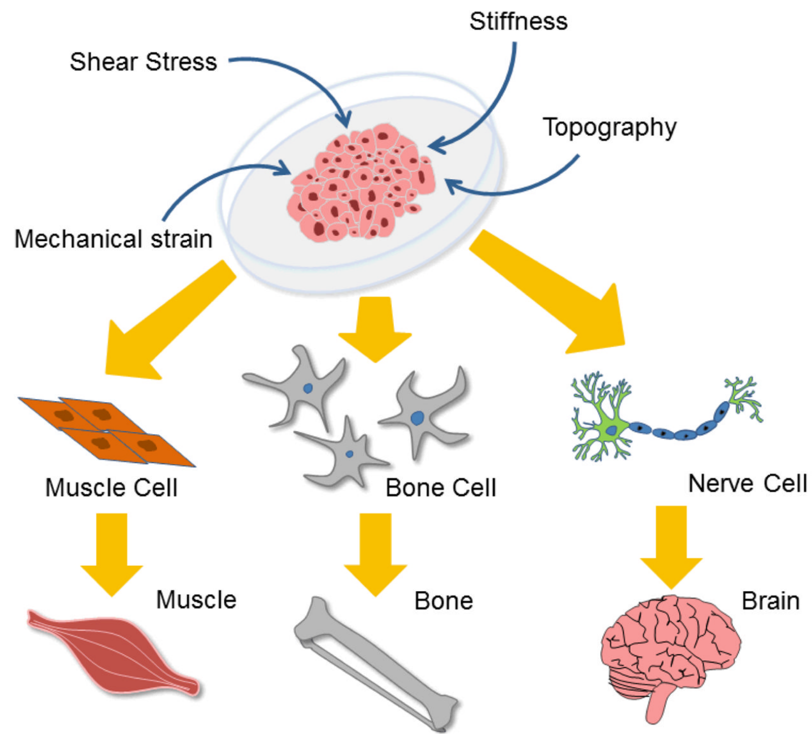

Figure 1.
Schematic diagram of biomechanical regulation of stem cell behaviors. Mechanical stimuli such as mechanical strain, substrate stiffness, shear stress and topography affect on stem cell phenotypes in a combinatorial fashion.

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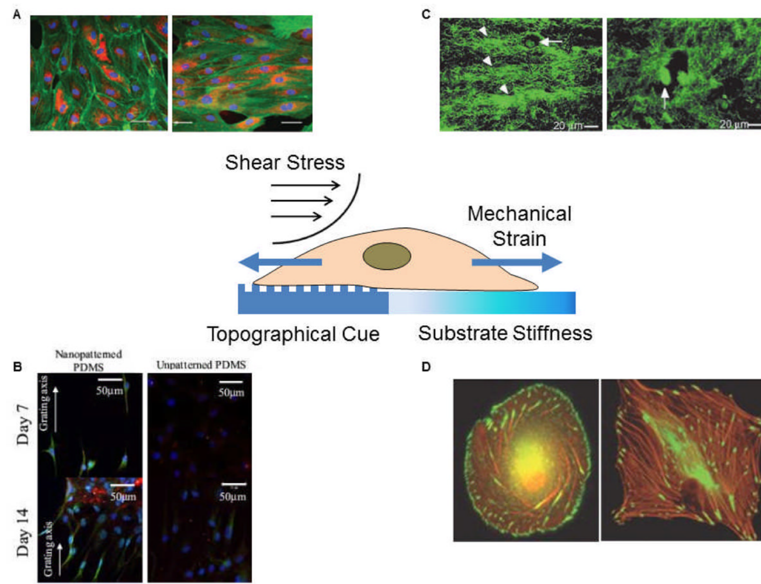

Figure 2.

Representative examples of biomechanical regulation of stem cell behaviors.(A) Actin filaments (green) and vWF (red) in the absence and presence of shear, respectively. Arrows indicate direction of flow; Scale bars = 50 μm[30]. *Reprinted with permission from John Wiley and Sons.* (B) Immunofluorescent staining of MAP2 and nestin of hMSCs cultured on nano-patterned, and unpatterned PDMS. Nestin, the neuronal differentiation marker is shown in red, MAP2 in green. The direction of the gratings on the nano-patterned PDMS is indicated with a white arrow. *Reprinted with permission from Elsevier Publishing*[32]. (C) Immunohistochemical assessment of gels either seeded with bovine bone marrow-derived progenitor cells[31]. Mechanically stimulated bovine gels (Left) compared with static controls (Right) cultured for 21 days showed stimulated cells containedordered collagen type I fiber bundles (arrowheads) in the direction of load, i.e., along the longitudinal axis of the ligaments (double-arrow); Static controls exhibited absence of fuber bundle organization. *Reprinted with permission from FASEB.* (D) The response of human fibroblasts to rigid (Left; Young's modulus (E)=100 kPa) or soft (Right; E=10 kPa) fibronectin-coated PDMS substrates[33]. Organization of paxillin-GFP-labelled focal adhesions (green) and phalloidin-labelled filamentous actin (red), as well as overall cell shape, strongly differ in cells that are plated onto the two substrates. *Reprinted with permission from Nature Publishing Company.*

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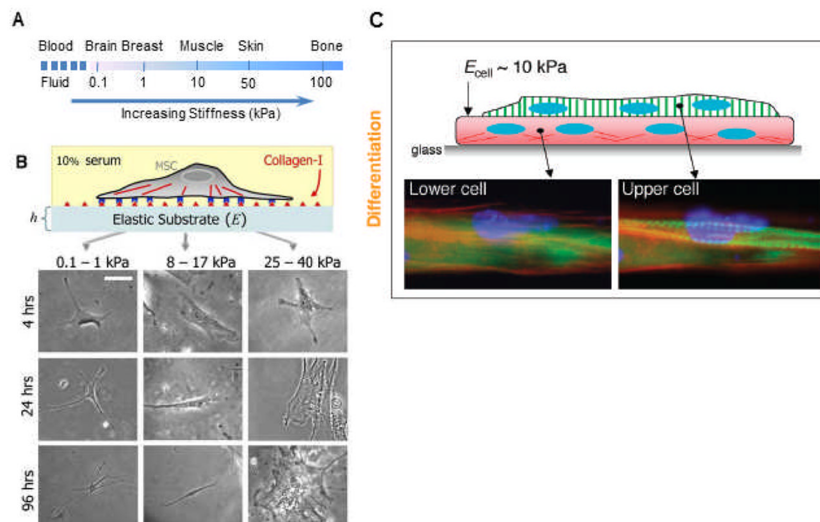

Figure 3.

Effects of substrate stiffness on stem cell phenotypes. (A) Solid tissues exhibit a range of stiffness, as measured by the elastic modulus, E. (B) The *in vitro* gel system allows for control of E through crosslinking, control of cell adhesion by covalent attachment of collagen-I, and control of thickness, h. Naive MSCs of a standard expression phenotype are initially small and round but develop increasingly branched, spindle, or polygonal shapes when grown on matrices respectively in the range typical of brain (0.1–1 kPa), muscle (8–17 kPa), or stiff crosslinked-collagen matrices (25–40 kPa). Scale bar =20 μm[13]. A, B reprinted with permission from Elsevier. (C) Substrate stiffness influences adhesion structures and dynamics, cytoskeleton assembly and cell spreading, and differentiation processes such as striation of myotubes[94]. A cell-on-cell layering demonstration; Lower layer is attached first to glass followed by the upper layer, derived from myoblasts that are added later allowing myoblasts to perceive a softer, cellular surface.

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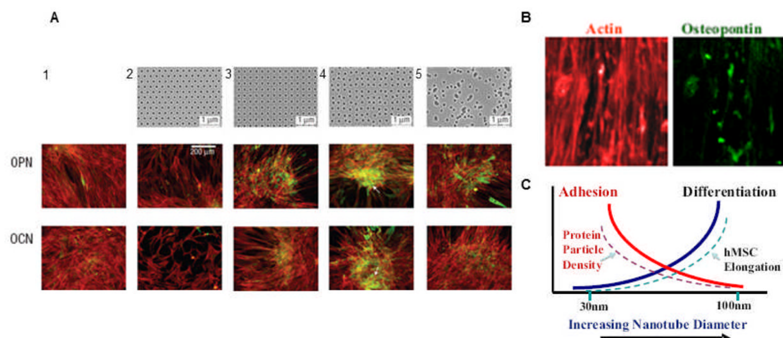

Figure 4.

Influences of substrate topographical cues on stem cell phenotypes. (A) Top row shows images of nanotopographical geometries fabricated by electron beam lithography. All have 120-nm-diameter pits (100 nm deep, absolute or average 300 nm centre–centre spacing) with hexagonal (2), square (3), displaced square (4)(50 nm from true centre) and random placements (5)[110]. Osteoprogenitors cultured on the control (1) exhibit lack of positive OPN and OCN stain; on hexagonal arrangment (2) show loss of cell adhesion; and on square (3) show decreased cell adhesion but increase in OPN and OCN stain; on displaced square geometry (4) show bone nodule formation; and on random arrangement (5) an OCN and OPN positive cell subpopulations. Actin=red, OPN/OCN=green. *Reprinted with permission from Nature Publishing Group.* (B) Immunofluorescent images of OPN (Right), as well as actin (Left) on 100-nm diameter TiO_2 nanotubes after 3 weeks of culture. (C) Schematic illustration of the overall trends of nano cue effects on hMSC fate and morphology after a 24-h culture[75]. The change in hMSC cell adhesion and growth without differentiation (solid red line) has the same trend as protein particle density (broken red line), whereas that of differentiation (solid blue line) has the same trend as hMSC elongation (broken blue line). *Reprinted with permission from National Academy of Sciences.*

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Table 1

Examples of biomechanical regulation in stem cell behaviors

Mechanical Stimuli	Cell Source	Highlights	Ref.
Mechanical Strain	Endothelial cell	The formation of stress fiber by cyclic uniaxial strain without Rho pathway	111
	Smooth muscle cell	The upregulation of elastin and collagen gene expression and the increase in the express of elastin and collagen by cyclic strain	112
	ESC	Tightly packed morphology with high level of Oct4 and SSEA-4 under biaxial strain	35
		The up-regulation of Smad2/3 phosphorylation by strain which block ESC differentiation	Saha, 2008 #31}
	MSC	The up-regulation of smooth muscle contractile marker, SM alpha-actin and SM-22alpha by uniaxial stain	37
		The activation of ERK1/2 and p38 MAPK signaling pathway by equiaxial strain	37
		The increase in the expression of the specific ligament markers such as collagen I, III and tenasin C	113
Substrate Stiffness	MSC	The direction of stem cell lineage by mimicking tissue stiffness	13
		The induction of MyoD expression, an indicator of myogenic differentiation on gel coated FN with 25kPa	94
	NSC	The increase in β-tubulin III, the neuronal marker on gel near stiffness of brain tissue	47
	CSC	Differentiation of CSC into endothelial cells via p190RhoGAP downregulation	12
Shear Stress	hESC-derived endothelial cell	The upregulation of COX2, the indicator of amplification of inflammatory signal and MMP1 that degrades matrix for morphological change by shear stress.	30
		The increase in angiogenic and vasulogenic potential as well as cytoskeletal rearrangement in the direction of flow	61,62
	Endothelial progenitor cell	The increase in the expression of VEGF receptors and the activation of VEGF receptor withoug corresponding ligands by shear stress	64
	ESC	The histone modification by shear stress	56
	MSC	The increase in the endothelial markers such as CD31, vWF and cadherin at the mRNA and protein level by shear stress	114
		The increase in nitric oxide production and the upregulation of cyclooxygenase-2 by shear stress	65
Substrate Topography	MSC	The increase in osteoblastic markers such as osteocalcin and osteopontin on disordered nanoscale pit pattern	71
	Osteoblasts	The decrease in the expression of the connexin-43, the gap junction protein by the groove pattern	73