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Forcing Stem Cells to Behave: A Biophysical Perspective of the Cellular Microenvironment

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Abstract

Physical factors in the local cellular microenvironment, including cell shape and geometry, matrix mechanics, external mechanical forces, and nanotopographical features of the extracellular matrix, can all have strong influences in regulating stem cell fate. Stem cells sense and respond to these insoluble biophysical signals through integrin-mediated adhesions and the force balance between intracellular cytoskeletal contractility and the resistant forces originated from the extracellular matrix. Importantly, these mechanotransduction processes can couple with many other potent growth factor-mediated signaling pathways to regulate stem cell fate. Different bioengineering tools and micro/nanoscale devices have been successfully developed to engineer the physical aspects of the cellular microenvironment for stem cells, and these tools and devices have proven extremely powerful to identify the extrinsic physical factors and their downstream intracellular signaling pathways that control stem cell functions.

Keywords

Stem Cell Fate; Mechanotransduction; Cellular Microenvironment; Mechanical Forces; Micro/Nanotechnology

INTRODUCTION

Stem cells are critical players during development, tissue regeneration and healthy homeostatic cell turnover, and they are an important driving force for the developing fields of functional tissue engineering and regenerative medicine owing to their self-renewal capacity and pluripotency (28). Collectively, all stem cells share the ability to self-renew and

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differentiate into specific lineages. Embryonic stem (ES) cells—which are derived from the inner cell mass of the developing blastocyst – are pluripotent, whereas stem cells derived from adult tissues generally maintain a more limited, tissue-specific, regenerative potential (28, 94). Owing to their ability to generate tissue *de novo* following disease or injury, there is a widespread hope of developing stem cell-based therapies for various degenerative diseases (71, 76). A key aspect in the enabling of these stem-cell based therapies will be the ability to manipulate stem cell interactions with their local microenvironment (a setting in vivo known as the stem cell niche) in order to regulate and direct stem cell fate (61, 90, 115).

How the in vivo stem cell niche, which filters and presents a wide range of molecular and cellular scale physical and biological signals, acts to regulate tissue regeneration based on physiological demand and pathological state remains incompletely understood (94, 105). In vivo, stem cell niches create specialized microenvironment, consisting of soluble and surface-bound signaling factors, cell-cell contacts, stem cell niche support cells, extracellular matrix (ECM), and local mechanical microenvironment (Fig. 1). While stem cell biologists have long appreciated the regulatory roles for soluble stem-cell niche signals (*e.g.*, growth factors and cytokines) in regulating stem cell fate, recent evidence demonstrates that regulation of stem cell fate by these soluble factors are strongly influenced by the co-existing insoluble adhesive, mechanical, and topological cues inherently contained and dynamically regulated in the stem cell niche (23, 30, 46, 131). These insoluble biophysical cues can be sensed and transduced into intracellular biochemical and functional responses by stem cells, a process known as mechanotransduction (16, 41, 97, 118, 128).

The sensory machinery of stem cells can sense and integrate multiple signals (both soluble and insoluble ones) simultaneously from their niche and convert them into a coherent environmental signal to regulate downstream gene expression and stem cell fate. Further, different well conserved soluble factor-mediated signal transduction pathways and the cellular mechano-sensing and –transduction processes can converge to activate the elaborate intracellular signaling network in an integrated and interacting manner to regulate stem cell fate. Taking human ES cells (hESCs) as an example, basic fibroblast growth factor (bFGF)-mediated signaling, transforming growth factor- β (TGF- β)/Activin/Nodal-mediated signaling, and canonical Wnt (wingless)/ β -catenin-mediated signaling are all central for the self-renewal of hESCs, while bone morphogenetic proteins (BMPs) induce differentiation of hESCs (33, 140–141). bFGF activates the MAPK/ERK signaling cascade in hESCs, also known to be a central mechanotransduction pathway for adaptive cellular responses to mechanical stimuli from the cellular microenvironment (63, 75). Extracellular mechanical forces have also been shown to stimulate expressions of TGF- β , Activin, and Nodal, providing an autocrine or paracrine signaling mechanism to promote maintenance of the pluripotency of hESCs (95, 112). β -catenin, which is a critical component of the canonical Wnt signaling pathway, plays an important role in cell-cell adhesions by mediating cytoskeletal attachment of E-cadherin to the actin cytoskeleton. β -catenin-mediated E-cadherin-based cell-cell adhesions have been shown to be mechanosensitive and depend on non-muscle myosin II (NMMII) activity in mouse ES cells (mESCs) (74). Further, β -catenin has been shown to be critical for the mechanical induction of Twist expression in

Drosophila, which is a transcriptional factor associated with regulation of skeletal development (36).

Moreover, recent studies show that the RhoA-GTPase/Rho-kinase (ROCK)/myosin-II signaling axis, which is the major biochemical pathway mediating the actin cytoskeleton (CSK) tension in non-muscle cells (35, 108), plays a critical role in regulating survival and cloning efficiency of single hESCs (17, 130, 133). Blocking RhoA/ROCK mediated CSK tension using drug inhibitors reduces dissociation-induced apoptosis of hESCs, suggesting that hyper-activation of CSK tension, triggered by hESC cell dissociation, is the upstream regulator and direct cause of hESC apoptosis. Importantly, RhoA/ROCK mediated CSK tension plays a critical role in the mechanotransduction process (16, 58). All together, biophysical signals in the cellular microenvironment of stem cells can have extensive potential to regulate and synergize with classical signal transduction pathways induced by soluble factors to control stem cell fate.

With recent major advances in understanding how the insoluble biophysical signals in the cellular microenvironment regulate stem cell fate, tissue engineering and regenerative medicine are becoming increasingly oriented toward biologically inspired in vitro cellular microenvironment designed to guild stem cell growth, differentiation, and functional assembly (78, 129). The premise is that to unlock the full potential of stem cells, at least some aspects of the dynamic cellular microenvironment that are associated with their renewal, differentiation, and assembly in native tissues need to be reconstructed.

A major goal of this review is therefore to offer a perspective on this new trend of designing synthetic artificial in vitro stem cell niche and their promise for stem cell research and to enable new, clinically relevant strategies for tissue regeneration. In particular, we will focus on discussing the biophysical signals in the synthetic stem cell niche and their functional effects on stem cell fate. To do so, we will take the approach by highlighting some illustrative examples of using bioengineering strategies for controllable synthetic cellular microenvironment developed through the interactions of stem cell biology, tissue engineering, and micro/nanotechnology at multiple length scales. We will first stress different biophysical factors in the cellular microenvironment that have been shown to be critical for the fate decisions of stem cells, such as extracellular matrix geometry, nanotopography, and mechanics, and describe how these biophysical factors impact cell signaling and function. We will discuss the mechanosensory machinery and mechanotransduction mechanisms stem cells can use to sense and respond to these biophysical factors and how these mechanotransduction pathways converge with classical signal transduction pathways to control stem cell fate. We will discuss different versatile and powerful bioengineering and micro/nanotechnology strategies and methods that can be used for constructing the synthetic stem cell niche. We will offer some perspectives on potential research directions and opportunities for engineering stem cell functions using well-controlled cellular microenvironments.

MECHANICAL CONTROL OF STEM CELL FATE

Functional regulation of stem cells *in vivo* normally plays out in the context of embryonic development, tissue regeneration, and the wound healing response, where extracellular mechanical forces abound and the mechanical environment surrounding the stem cells changes dynamically. Plenty of evidence exists to suggest that these biophysical signals from the local stem cell niche instruct the subsequent behaviors of stem cells. There are other extensive reviews on the topic of stem cell niche signals in regulating stem cell fate, especially for *in vivo* organismal development settings (65, 94, 105); here we provide illustrative examples using novel bioengineering and micro/nanofabrication approaches to control the local stem cell niche and where evidence suggests mechanical control of stem cell fate through synergistic regulations of stem cell shape, CSK tension, and integrin-mediated adhesion signaling.

Cell Shape and Control of Cytoskeletal Tension

Cell shape is a potent regulator of cell growth and physiology (39). Cells adapt and optimize their shape for their specific functions. For example, adipocytes are spherical in shape to maximize lipid storage while neurons have long axons to deliver signals rapidly over a long distance. In fact, many events associated with stem cell differentiation during embryonic development and tissue regeneration are designed to change cell shape, and those changes in shape can influence tissue structure and function (56, 81, 143). Thus, a natural converse question for stem cells arises whether their fate can be regulated by their intrinsic and dynamically regulated cell shape. Compelling studies to support cell shape as a key regulator of stem cell fate came from experiments using bioengineering strategies to pattern the spreading and morphology of human mesenchymal stem cells (hMSCs). hMSCs are isolated from bone marrows and can differentiate into multiple lineages of mesenchymal tissues (15, 106). By using microcontact printing to coat flat polydimethylsiloxane (PDMS) surfaces with distinct patterns of adhesive ECM islands, McBeath *et al.* reported that in response to a bipotential differentiation medium that contained inducers for both the adipogenic and osteogenic differentiations, single hMSCs confined to small ECM islands selectively underwent adipogenesis, whereas single hMSCs on large ECM islands were biased towards osteogenesis (Fig. 2 a) (86).

This osteogenic-adipogenic switch in well-spread versus poorly-spread hMSCs required generation of CSK tension through RhoA-dependent acto-myosin contractility. RhoA is a member of Rho family small GTPases involved in cellular signaling and cytoskeletal organization, and it stimulates CSK tension through its effector, ROCK, which directly phosphorylates both NMMII regulatory light chain (MLC) and MLC phosphatase to synergistically increase MLC phosphorylation and thus myosin II contractility (6, 48). Inhibition of CSK tension using either cytochalasin D (an actin depolymerization agent) or Y-27632 (a ROCK inhibitor) promoted adipogenesis, mimicking the phenotype of poorly spread hMSCs. Moreover, manipulation of the RhoA pathway could override the effects of soluble differentiation factors, such that dominant-negative RhoA induced adipogenesis even in the context of pure osteogenic medium, whereas constitutively active RhoA triggered osteogenesis in pure adipogenic medium. These findings highlight RhoA activity

as a potential convergence point for mechanical and soluble factor signaling in the control of stem cell differentiation. Importantly, McBeath *et al.* also demonstrated that expression of constitutively-active ROCK rescued osteogenic differentiation of poorly-spread MSCs, and this effect required myosin II activity, indicating that cell shape and RhoA regulate osteogenic-adipogenic switching through the development of CSK tension.

Another more recent study by Ruiz *et al.* confirmed the importance of CSK tension in regulating stem cell fate in the setting of multicellular structures (109). Ruiz *et al.* applied microscale patterning approaches to control geometries of both two-dimensional (2-D) and three-dimensional (3-D) multicellular structures of hMSCs (Fig. 2b). Ruiz *et al.* reported that in the presence of soluble factors permitting both osteogenic and adipogenic differentiations, hMSCs at the edge of the multicellular structures selectively differentiated into the osteogenic lineage, whereas those in the center became adipocytes. Using some microfabricated cellular traction force sensors (31, 126), Ruiz *et al.* further demonstrated that a gradient of traction stress across the 2-D multicellular hMSC structures could precede and mirror the patterns of multicellular differentiation, where regions of high stress were concentrated with osteogenesis of hMSCs, whereas hMSCs in regions of low stress differentiated to adipocytes. Inhibition of CSK tension using blebbistatin (a myosin II inhibitor), Y-27632, or ML-7 (an inhibitor of MLC kinase) suppressed the spatial patterns of multicellular differentiation of osteogenesis versus adipogenesis, for both the 2-D and 3-D multicellular structures of hMSCs. Interestingly, in addition to the overall cell shape, cell geometry also plays an important role in regulating stem cell fate (66, 77, 121). Kilian *et al.* demonstrated that in response to a bipotential differentiation medium that contained inducers for both the adipogenic and osteogenic differentiations, single hMSCs cultured in rectangles with increasing aspect ratio and in shapes with pentagonal symmetry but with different subcellular curvature - and with each occupying the same area - displayed different adipogenesis and osteogenesis profiles. Using cytoskeletal-disrupting pharmacological agents, Kilian *et al.* further confirmed a causal role for CSK tension in modulating the shape-based trends in lineage commitment of hMSCs. Taken together, the aforementioned studies demonstrate a causal role of cell shape and geometry in regulating stem cell fate. Importantly, there is a common theme emerging from all these studies: cell shape signal seems to converge with soluble inductive factors on the actin CSK and the RhoA/ROCK mediated CSK tension to regulate stem cell fate.

Another recent study performed by Peerani, R. *et al.* demonstrated the effect of the cellular microenvironment on hESC fate by patterning hESC colonies onto defined adhesive islands with a controlled colony diameter (Fig. 2c) (103). Peerani, R. *et al.* showed that larger colonies with high local cell density microenvironment would promote the maintenance of pluripotency in hESCs, through a niche size-dependent spatial gradient of BMP-mediated Smad1 signaling generated as a result of antagonistic interactions between hESCs and hESC-derived extra-embryonic endoderm. Thus, this colony size effect on the pluripotency maintenance of hESCs appears to be mediated by interactions between exogenously controlled parameters and autocrine and paracrine secretion of endogenously produced factors from hESCs. Even though there was no mechanotransduction mechanism revealed specifically in the study by Peerani, R. *et al.*, combining their results together with the

observations from hMSCs discussed above, it is reasonable to speculate that stem cell fate is mediated by a combination between soluble factors and insoluble biophysical signals in the local stem cell microenvironment. Thus, structural and mechanical cues associated with the cytoskeletal reorganization appear to be integrated with several developmental signaling pathways known to be critical for stem cell fate determination, and the integrated mechanochemical networks provide a mechanism for stem cells to orchestrate the many structural and mechanical changes associated with morphogenesis to direct the downstream genetic programs required to give rise to the appropriate spatiotemporal patterns of stem cell differentiation.

Nanotopography: Integrins Making the Sense

During embryonic development and tissue regeneration, stem cells do not only interact with each other but also with the 3-D porous network of the ECM, which comprises fibrillar networks of proteins such as collagen and laminin interlaced with proteoglycan. While the characteristic pore size of the ECM might provide a direct physical constraint on the stem cell size and shape, the micro- and nano-scale topography, structure and architecture of the fibrous ECM are also important biophysical signals that can regulate stem cell adhesion and cytoskeletal organization, and thus stem cell behaviors such as proliferation, migration, and differentiation (25, 44, 122, 137). Equipped with advanced sub-100 nm micro / nanofabrication techniques, materials scientists and applied physicists have successfully teamed up with stem cell biologists and tissue engineers to generate well-controlled molecular and cellular scale topography on 2-D planar substrates to investigate their independent effect on stem cell fate. Existing studies on nanotopography have suggested that instead of directly affecting CSK tension as in the case for cell shape, nanotopographical cues appears to elicit its effect on stem cells by directly modulating the molecular arrangement, dynamic organization, and signaling of the cellular adhesion machinery.

Adhesion of stem cells to the nanotopographical ECM is mediated via heterodimeric transmembrane receptors, namely, α - and β -integrins (Fig. 1). Combinations of among 18 α -chains and 8 β -chains form different heterodimers to yield a rich diversity of ECM receptors, enabling differential cell-type specific responses to variations in the ECM. Upon binding ECM, integrins can cluster to form dynamic adhesion structures called focal adhesions (FAs). On the cytoplasmic side of FAs, integrins can interact, via their cytoplasmic tails, with different adaptor and signaling proteins. Among these molecules, talin, vinculin, paxillin, and α -actinin are adaptor proteins that provide a direct physical linkage to the actin CSK. Importantly, binding of integrins to the ECM proteins can activate tyrosine kinase and phosphatase signaling to elicit downstream biochemical signals important for regulation of gene expression and stem cell fate. Thus, it is speculated that nanotopographical signals intrinsically contained in the ECM surrounding stem cells can regulate stem cell fate through their direct effect on the integrin-mediated FA signaling.

As reported by Arnold *et al.*(4) by using block-copolymer micelle nanolithography to pattern gold nanodots coated with adhesive peptides, when the spacing between these nanodots exceeded about 70 nm, cell adhesion and spreading, FA and actin stress fiber formations were significantly impaired, likely owing to the restricted clustering of integrin

molecules by the distance between the adjacent gold nanodots. In another relevant study using nanoimprint lithography (NIL) to pattern gold nanodots functionalized with binding ligand RGD (Arg-Gly-Asp), Schwartzman *et al.* reported a drastic increase in spreading efficiency of cells on arrays of different geometric arrangements of the nanodots when at least four liganded sites were spaced within 60 nm or less, with no dependence on global density (117). This interesting observation pointed to the existence of a minimal of four integrin adhesion units required for initial growth and maturation of nascent FAs on fibronectin as defined in space and stoichiometry. Together, these two studies based on well-controlled cell-ECM interactions demonstrate the molecular sensitivity and dynamic organization of FAs regulated by the local nanotopographical cue.

Given the potent influence of local nanotopography in regulating molecular organization of FAs, it is not surprising that nanotopography can significantly affect stem cell fate. Yim *et al.* showed that hMSCs cultured on the nanoscale gratings on the PDMS surface tended to align and elongate their actin CSK and nuclei along the nanogratings (145). Gene profiling and immunostaining by Yim *et al.* further showed significant up-regulation of neuronal markers such as microtubule-associated protein 2 (MAP2) for hMSCs cultured on the nanogratings, as compared to un-patterned flat controls, and the combination of nanotopography and biochemical inductive factors such as retinoic acid further enhanced the expressions of neuronal markers. Importantly, Yim *et al.* further demonstrated that nanotopography showed a stronger independent effect compared to biochemical cues (in this case, retinoic acid for neurogenic differentiation of hMSCs) alone on unpatterned control surfaces (145). In a follow-up study (144), Yim *et al.* found that on the nanogratings, expressions of most integrins except $\alpha 3$ and $\beta 5$ were considerably down-regulated, and the aligned actin CSK on the nanogratings was not as prominent or dense as on flat surface controls. Further, distributions of vinculin and focal adhesion kinase (FAK), two prominent FA proteins, were different on the nanogratings as compared to on flat surfaces. Combined together, studies from Yim *et al.* suggest that the local nanotopographical cues could affect the molecular organization and composition and signaling of FAs and such modified FA signaling might further influence CSK structure to mediate stem cell fate.

More recently, Dalby *et al.* applied the electron beam lithography (EBL) and hot embossing to pattern nanoscale pits of different symmetry and with varying degrees of disorder in the polymethylmethacrylate (PMMA) or polycaprolactone (PCL) substrates. Dalby *et al.* first reported that the nanoscale disorder in the nanopit array stimulated hMSCs to produce bone mineral *in vitro*, even in the absence of osteogenic supplements (27). Interestingly, Dalby *et al.* further showed that hMSCs plated on perfectly ordered or totally random arrays of nanopits produced much less osteoblastic differentiation. A more recent study from the same authors demonstrated another intriguing effect of nanotopography on hMSC fate regulation. McMurray *et al.* showed that the perfectly ordered arrays of nanopits, even though not efficient to promote osteogenic differentiation of hMSCs as shown in their previous work, were conducive to hMSC growth while permitting prolonged retention of their multipotency and differentiation potential (88).

The aforementioned studies strongly suggest the potential of nanoscale structured surfaces as non-invasive tools to control the local stem cell microenvironment to regulate stem cell

fate, even though the mechanisms by which stem cells can sense and respond to the nanotopographical signal is not yet clear. But as discussed earlier, molecular arrangement and dynamic organization of integrin-mediated FAs appear to be very sensitive to the local arrangement and presentation of the nanotopographical signal. Thus, it is likely that integrin-mediated FA signaling, critical for many cellular functions (42, 119) and strongly dependent on their dynamic characteristics and molecular processes (52, 102, 147), plays an important functional role in regulating the stem cell sensitivity to nanotopography.

Mechanical Forces and Matrix Mechanics: A Balanced Tensional Homeostasis

During embryonic development and tissue regeneration, cells are not only exposed to structural changes in the surrounding ECM, but also to many mechanical stresses. There are local changes in mechanical forces during development, caused by the addition or removal of cells, cell movements associated with morphogenesis, muscle contraction and relaxation, as well as during bone compression and decompression. Therefore, stem cells are constantly subjected to and adjust to external force fluctuations from their local microenvironment. Another physical signal important for regulating stem cell fate is the intrinsic elastic modulus of the ECM surrounding stem cells. Stem cells sense and response to changes of the elastic modulus of the ECM by modulating their endogenous CSK contractility, balanced by resistant forces generated by the deformation of the ECM, the magnitude of which is determined by the ECM elastic modulus. Thus, it appears that stem cells are mechano-sensitive and –responsive to mechanical forces and matrix mechanics through a modulated delicate force balance between the endogenous CSK contractility and external mechanical forces transmitted across the cell-ECM adhesions. Indeed, such tensional homeostasis in the intracellular CSK has a key role in the regulation of basic cellular functions, such as cell proliferation, apoptosis, adhesion, and migration (80, 138). Deregulation of the tensional homeostasis in cells contributes to the pathogenesis of several human diseases, such as atherosclerosis, osteoarthritis and osteoporosis, and cancer(12, 47, 57).

External forces and matrix mechanics have a key role in the regulation of stem cell fate. Yet, the detailed molecular picture of the mechanotransduction process for stem cells to sense and respond to external forces and changes in matrix mechanics have yet to be identified. The force balance transmitted across the mechanical continuum of ECM-integrin-CSK can regulate integrin-mediated adhesion signaling (such as FAK and Src signaling) to coordinate downstream integrated stem cell function. These biophysical signals are sensed at the FA sites in which integrins provide the mechanical linkage between the ECM and the actin CSK. Exposure of stem cells to mechanical strain, fluid shear stress, or plating stem cells on substrates with varying elastic moduli, will activate integrins, which promote recruitment of scaffold and signaling proteins to strengthen FAs and to transmit biochemical signals into the cell. These mechanotransduction pathways establish positive feedback loops in which integrin engagement activates actomyosin CSK contractility, which in turn reinforces FAs (16, 41). Thus, the level of CSK contractility generated inside the cell is directly proportional to the adhesion strength and the matrix elastic modulus and dictates the cellular responses of stem cells.

Effects of external forces including mechanical strain, compression, and fluid shear stress on cellular functions have long been studied for cardiovascular tissues, skeletal muscles, and adult stem cells such as hMSCs (19, 29, 98, 131). Evidence related to regulation of pluripotent stem cell fate by mechanical forces *in vitro* has only recently begun to emerge. Saha *et al.* showed that in the presence of mouse embryonic fibroblast (MEF) conditioned medium, under cyclic equibiaxial strain, hESC differentiation was reduced and self-renewal was promoted without selecting against survival of differentiated or undifferentiated cells (111). A more recent study by the same authors further showed that the TGF β /Activin/Nodal signaling pathway played a crucial role in repression of hESC differentiation under mechanical strain (112). Saha *et al.* showed that mechanical strain induced transcription of TGF β 1, Activin A, and Nodal to up-regulate Smad2/3 phosphorylation in undifferentiated hESC. Thus, the studies by Saha *et al.* demonstrated that TGF β superfamily activation of Smad2/3 was required for repression of spontaneous differentiation of hESCs under mechanical strain, which further suggested that mechanical strain might induce autocrine or paracrine signaling in hESCs through TGF β superfamily ligands (112). Inspired by the fact that *in vivo* blood vessels remodel and change their sizes by sensing the shear stress of blood flow (93), different researchers had recently showed that well controlled shear stress could be used to induce mESCs to differentiate into the endothelial cell lineage (Fig. 3a) (142) as well as hematopoietic progenitor cells (1), even though the molecular mechanisms underlying these mechanoresponsive behaviors of mESCs have not been sufficiently explored. Another recent study by Chowdhury *et al.* (21) showed that local cyclic stress through integrin-mediated adhesions by using functionalized magnetic beads induced spreading of mESCs with a concomitant down-regulation of their Oct3/4 gene expression (Fig. 3b).

The ability of stem cells to sense matrix mechanics has only been demonstrated recently. Yet, its implications for functional tissue engineering and regenerative medicine have already generated tremendous excitements. In a landmark study, Engler *et al.* demonstrated that in the absence of exogenous soluble cues, plating hMSCs on polyacrylamide gels of varying elasticity was sufficient to induce hMSCs to differentiate into different tissue types corresponding to the tissues' relative mechanical elasticity *in vivo* (Fig. 4a&b) (34). As discussed earlier, adherent cells sense matrix mechanics through a force balance between intracellular acto-myosin contractility and the resistant force of the ECM determined by its elastic deformation. Thus, the level of CSK tension generated inside stem cells is directly proportional to the elastic modulus of the substrate stem cells adhere to. Indeed, this positive correlation between the elastic modulus of the substrate and intracellular CSK contractility was reported by Engler *et al.* and others for hMSCs and many other mechanosensitive adherent cells (34, 40). Importantly, to demonstrate that this CSK contractility indeed played a causal role for regulating matrix mechanics-dependent changes in hMSC differentiation, Engler *et al.* showed that addition of blebbistatin to block intracellular CSK tension generation in hMSCs obliterated matrix mechanics-driven differentiation. Recently, Huebsch *et al.* extended the *in vitro* study of mechanobiology in MSCs to a 3-D microenvironment setting by using a 3-D hydrogel synthetic ECM formed by alginate polymers that presented integrin-binding RGD peptides (Fig. 4c) (55). Using this 3-D cell culture system with well controlled elastic modulus encapsulating mouse MSCs (mMSCs),

Huebsch *et al.* showed that osteogenesis of mMSCs occurred predominantly at 11–30 kPa, comparable to the native tissue stiffness of precalcified bone (30). Since mMSCs were encapsulated in the 3-D hydrogel, their morphology appeared to be independent on the elastic modulus of the hydrogel. Still, Huebsch *et al.* demonstrated that matrix stiffness regulated integrin binding as well as reorganization of adhesion ligands on the nanoscale, both of which were CSK contractility dependent and correlated with osteogenic commitment of mMSCs, again highlighting the importance of intracellular CSK contractility and their force balance with deforming surrounding ECM in regulating stem cell fate.

Other types of adult stem cells have also been studied recently for their mechanoresponsive behaviors to matrix mechanics in both 2-D and 3-D cellular microenvironment, including skeletal muscle stem cells (Fig. 4d) (43), hematopoietic stem cells (53), and adult neuron stem cells (8, 73, 110). The most definitive experimental evidence to demonstrate mechanosensitivity of pluripotent ESCs to matrix mechanics was shown in a recent study by Chowdhury *et al.* (20), which reported that mESCs could maintain their pluripotency on soft polyacrylamide gels (~500 Pa) even under long term culture conditions (at least 15 passages) without exogenous leukemia inhibitory factor (LIF; a soluble factor critical for maintenance of pluripotency of mESCs), in sharp contrast to mESCs seeded on the conventional rigid tissue culture plates. Importantly, traction force measurements of these mESCs demonstrated that their CSK contractility was mechanosensitive and correlated positively with the elastic modulus of the polyacrylamide gels (20), implicating involvement of CSK contractility in regulating their mechanosensitivity to changes in matrix mechanics.

Collectively, a few common observations can be drawn from the aforementioned studies of the mechanosensitivity of stem cells. All the studies have explicitly or implicitly suggested the involvement of CSK contractility in regulating the mechanosensitivity of stem cells, suggesting the importance of the force balance along the mechanical axis of the ECM-integrin-CSK linkage and their regulation by the mechanical signals in the stem cell niche. Moreover, strong evidence suggests that the differentiation potentials of stem cells toward distinct lineages can be maximized if the cells are cultured in the mechanical microenvironment mimicking their tissue elasticity *in vivo* (Fig. 4a). This observation is important for both functional tissue engineering and developmental biology as it anticipates a major role of dynamic control of matrix mechanics in controlling stem cell function and tissue development. Indeed, dynamic regulation of matrix mechanics has emerged as a critical regulator of differentiation and morphogenesis (22, 89, 146). An emerging hypothesis has further suggested a role for the long-lived CSK structures as epigenetic memories to determine responses of stem cell shape, function, and fate to changes of matrix mechanics(38).

MECHANOTRANSDUCTION PATHWAYS TO REGULATE STEM CELL FATE

Stem cells can sense and respond to local biophysical signals through the integrin-mediated FA signaling, and such signaling can be regulated by the force balance between the endogenous CSK contractility and external mechanical forces transmitted across the cell-ECM adhesions. In this section, we will discuss how this force balance across the mechanical continuum of ECM-integrin-CSK can be further transduced into the intracellular

space of stem cells to mediate signaling molecules important for fate decisions of stem cells (Fig. 5).

Integrin Signaling

1. Ras/MAPK signaling—Stem cells can sense and respond to biophysical signals through integrin-mediated FA signaling. Indeed, forces transmitted through FAs, generated either internally by CSK contractility or externally by mechanical forces, can trigger both mechanical and biochemical responses in cells. Forces at FAs activate several kinases involved in regulation of cellular functions (113, 118, 135, 139). Perhaps the most important players in this mechanotransduction system are FAK and Src family kinases such as fyn (45, 72, 101, 134). One major downstream signaling pathway following FAK/Src activation is the Ras-Raf-MEK-ERK pathway (one branch of the MAPK pathway), and the exact molecular mechanism of how integrins regulate MAPK is not yet well defined. Several possible pathways have been proposed, which include integrin-FAK-Grb2-SOS-Ras (116), integrin-fyn-Shc-Grb2-SOS-Ras (132), or through the EGF receptor (13). ERK is then translocated to nucleus to regulate gene expression by activating different transcription factors.

Ras/MAPK pathway has been shown to play a critical role in the fate decisions of stem cells. For example, MAPK signaling is required for stemness maintenance of both neural stem cells (NSCs) (14) and human epidermal stem cells (149). Interestingly, the FGF/MAPK cascade plays a functional role in promoting differentiation of mESCs, thus inhibition of MAPK signaling can support self-renewal of mESCs (107). In contrast, FGF/MAPK signaling promotes self-renewal of hESCs, indicating that hESCs may have opposite cellular responses to the biophysical signals compared to mESCs.

It is important to note that MAPK-mediated stem cell fate is dynamically required during different stages of stem cell differentiation. For instance, activated MAPK signaling is required for the early lineage specification of mESCs to adipocytes, while the MAPK pathway has to be shut down during their terminal differentiation (11). This observation further suggests that spatial and temporal dynamic modulation of the biophysical signals in the stem cell niche can be necessary for optimizing behaviors of stem cells.

2. PI3k/Akt—Another downstream pathway of Ras is the PI3k/Akt pathway, which can also be activated through integrin signaling (18). PI3k/Akt pathway is known critical for the self-renewal and differentiation of both ESCs and somatic stem cells. Paling *et al.* reported that PI3k signaling was activated by LIF and was required to maintain the self-renewal of mESCs (100), and one possible downstream target of PI3k/Akt signaling is Nanog (123). Other reports had suggested that PI3k was responsible for activation of somatic stem cells to exit from their quiescent states, such as HSCs (148) and intestinal stem cells (51). It is also known that there is also signaling cross-talk between the PI3k/Akt pathway and the Wnt signaling pathway, a central pathway that controls the fate decisions of many different stem cells(69).

3. RhoA/ROCK—RhoA is a key molecular regulator of actin CSK tension and FA formation (*i.e.* upstream regulator of integrin), by acting through its effector Rho-kinase

(ROCK). RhoA/ROCK signaling also acts as a downstream target of integrin-mediated signaling through activated FAK (32). RhoA can be activated by different growth factors and cytokines as well as the biophysical signals from the cellular microenvironment. The functional role of RhoA/ROCK mediated CSK contractility is well appreciated in the lineage commitments of hMSCs. Activating RhoA promotes osteogenesis of hMSCs by up-regulating Runx2 expression, while inhibition of RhoA leads to adipogenesis of hMSCs (5, 86). In response to activated RhoA/ROCK signaling, intact actin CSK structure is required for mechanoresponsive hMSC differentiations. RhoA/ROCK mediated CSK contractility can directly regulate certain gene expressions of transcription factors (*e.g.* PPAR- γ and Sox-9) to influence stem cell differentiation.

4. Wnt/ β -catenin—Wnt/ β -catenin signaling can regulate fate decisions of different stem cell types, including ESCs, HSCs, MSCs, and NSCs (10, 26, 84, 92). In the canonical Wnt pathway, the expression and nuclear translocation and accumulation of β -catenin is regulated through Dvl. The role of Wnt/ β -catenin signaling in regulating stem cell fates can be complicated. For example, for mESCs, Wnt signaling is necessary for maintenance of their pluripotency (7, 114), however, overexpression of β -catenin can also promote neural lineage commitment of mESCs (99). The signaling cross-talk between Wnt and integrin signaling has been identified, and two different models involving integrin-linked kinase (ILK) and FAK have been proposed. In the first model (96), ILK is suggested to stabilize and/or promote the nuclear accumulation of β -catenin, while in the other model (24), Grb2 integrated integrin signaling through FAK with Wnt signaling via Dvl and jnk, a downstream kinase of Grb2, and promoted translocation of β -catenin into nucleus.

Direct regulation of Wnt signaling by biophysical signals has been demonstrated in osteoblasts. Data has been shown that mechanical loading could regulate Wnt signaling in a time-dependent manner (60). In this study, after 15 min cyclic stretch, Wnt signaling in human osteoblasts was ultimately down-regulated despite of an initial increase of β -catenin expression.

5. TGF- β —TGF- β is a secreted protein which belongs to TGF- β superfamily. It binds to a latent TGF- β binding protein (LTBP) which is linked to ECM, therefore, TGF- β is stored in extracellular space (3). The most remarkable role of TGF- β is to inhibit cell proliferation. Given the fact that many adult stem cells need to be kept at a quiescent state, TGF- β plays important roles in this process. For example, TGF- β can inhibit expansion of NSCs and keep HSCs in their quiescent state (3, 10), and some studies have shown that TGF- β is critical for maintenance of the pluripotency of hESCs via Smad2/3 signaling (59, 127). In addition to the canonical pathway via Smad2/3, TGF- β can also activate multiple major signaling pathways including MAPK, PI3k and Rho/ROCK discussed above (85, 91).

The signaling cross-talk between integrin and TGF- β has been extensively studied. The regulation of TGF- β activation by integrin has been reviewed in detail by Sonnenberg and co-workers recently (82). Certain types of integrins can directly regulate activation of TGF- β either through cellular traction forces exerted by actin CSK or through some G-protein-coupled receptors (GPCR). In addition, integrin can indirectly control the expression of the components in the TGF- β pathway, and it has also been shown that external mechanical

forces can activate releasing of TGF- β from ECM (79, 136). TGF- β can also regulate actin CSK through the RhoA/ROCK pathway, which has been well recognized in the epithelial to mesenchymal transition (EMT) process of tumor cells (9). Taken together, forces transmitted through integrins generated either internally by CSK contractility or externally by mechanical forces can activate TGF- β signaling, which in turn regulates stem cells fate. Several studies have confirmed this important signaling cross-talk between integrin and TGF- β . For example, the pluripotency of hESCs can be improved through directly applying a cyclic mechanical strain or indirectly using stiff substrates to activate latent TGF- β from ECM or fibroblasts as feeder cells (2, 112, 136).

Mechanosensitive Ion Channels

In addition to integrin signaling, mechanosensitive ion channels can also regulate mechanoresponsiveness of stem cells (83). Interestingly, based on the tethered model, mechanosensitive ion channels can be linked with ECM and/or CSK, and the relative displacement of channels with respect to ECM or CSK is responsible for the gating of channels (49). Thus, mechanosensitive ion channels can be directly activated by external forces or intracellular CSK contractility (50, 70, 124).

The major downstream effect of the activation of mechanosensitive ion channels is the changes of the cytoplasmic Ca^{2+} concentration as well as their oscillations (70). Ca^{2+} oscillations have been observed in MSCs and are considered as both an indicator and a regulator for MSC differentiation (64, 125). This Ca^{2+} concentration oscillation has been shown to be influenced by substrates stiffness (68). Ca^{2+} oscillations have also been found in mESCs, human preadipocytes, and human cardiac progenitor cells (37, 54, 62), indicating that mechanical forces might have the potential to directly regulate the fates of these cell types through modulating calcium signals.

SUMMARY AND OUTLOOK

The molecular mechanisms by which stem cells maintain their self-renewal ability and control their differentiation are central questions that need to be addressed in order for these cells to be effectively used for functional tissue engineering and regenerative medicine. Much of our effort until now has focused on the biochemical components and soluble factors in the stem cell microenvironment that are critical for their self-renewal and differentiation. Yet, recent evidence demonstrates that stem cells are also heavily influenced by co-existing insoluble adhesive, mechanical, and topological cues contained within in the dynamic stem cell niche. Experimental evidence have clearly suggested that insoluble biophysical signals, such as cell shape and geometry, external forces and matrix mechanics, and nanotopography can elicit intracellular programs to regulate stem cell fates, likely through the integrin-mediated FA signaling and the force balance across the mechanical continuum of ECM-integrin-CSK.

The molecular mechanisms for stem cells to sense and respond to different biophysical signals are not yet clear, and likely would be cell-type specific and involve different mechanisms working in concert. It also appears that the dominant effect of different biophysical signals on stem cell functions will depend on different experimental settings,

and stem cell fate is mediated by the intricate interactions and interdependences between soluble factors and insoluble biophysical signals in their local cellular microenvironment.

Moving forward, it is important to recognize that tissue development from stem cells in vivo is a long term process where dynamic changes of both chemical and physical environment surrounding the cells abound. How we can generate in vitro stem cell microenvironments to mimic the dynamic nature and complexity of the in vivo stem cell niche is currently a significant challenge. Researchers from different disciplines have devised different bioengineering strategies and micro/nanoscale tools that can provide good controls of different aspects of the stem cell microenvironment. Some of these techniques have already been mentioned in the examples discussed earlier, which include microcontact printing, synthetic hydrogels, microfluidics, and micro/nanofabrication (Fig. 6). These tools, which span different scales from molecular to cellular to organ levels, have proven to be extremely powerful to allow stem cell biologists and tissue engineers to identify the extrinsic physical factors and their independent effects on stem cell fates. We envisage that in the future, these tools will be further polished and used in different combinations to allow researchers to generate dynamic and complex synthetic cellular microenvironments with the molecular, structural, hydrodynamic, and mechanical cues well controlled in conjunction with their spatial and temporal levels and combinations. Given the complexity of the stem cell niche signals, it is also important to utilize high throughput tools that can help screen different combinations of the environmental signals to elicit the desired stem cell behaviors. Such high throughput screening assays no doubt can benefit from more in-depth understanding of the molecular mechanisms that regulate stem cell fate.

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ACRONYMS

bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic proteins
CSK	Cytoskeleton
EBL	Electron beam lithography
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial to mesenchymal transition
ERK	Extracellular-signal regulated kinase
FA	Focal adhesion

FAK	Focal adhesion kinase
GPCR	G-protein-coupled receptors
hESCs	human embryonic stem cells
hMSCs	human mesenchymal stem cells
MAPK	Mitogen-activated protein kinase
MEF	Mouse embryonic fibroblast
NIL	Nanoimprint lithography
NMMII	Non-muscle myosin II
RGD	arginine-glycine-aspartic acid
RhoA	Ras homolog gene family, member A
ROCK	Rho-associated protein kinase
TGF-β	Transforming growth factor β
PDMS	Polydimethylsiloxane
PI3K	Phosphatidylinositol 3-kinase

MINI-GLOSSARY

Microenvironment	the soluble and insoluble surroundings of a cell, including the biochemical and mechanical properties of the ECM, soluble factors, adjacent cells, and interstitial fluid
Stem cell niche	specific stem cell microenvironment that regulates how stem cells participate in tissue generation, maintenance and repair
Mechanotransduction	the processes whereby cells convert physiological mechanical stimuli into intracellular biochemical responses
Nanotopography	Surfaces and structures with nanoscale topological features
ECM	a meshwork of proteins, polysaccharides, and glycoproteins that provides structural and adhesive support to cells and tissues
Focal adhesion	cell adhesion sites for their attachment to ECM, where intracellular actin filaments can link to ECM proteins through transmembrane proteins such as integrins
Actomyosin contractility	intracellular forces generated by the dynamic interaction of myosin motors and actin filaments

LITERATURE CITED

1. Adamo L, Naveiras O, Wenzel PL, McKinney-Freeman S, Mack PJ, et al. Biomechanical forces promote embryonic haematopoiesis. *Nature*. 2009; 459:1131–5. [PubMed: 19440194]

2. Ahamed J, Burg N, Yoshinaga K, Janczak CA, Rifkin DB, Collier BS. In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor- β 1. *Blood*. 2008; 112:3650–60. [PubMed: 18544680]
3. Aigner L, Bogdahn U. Tgf- β in neural stem cells and in tumors of the central nervous system. *Cell and Tissue Research*. 2008; 331:225–41. [PubMed: 17710437]
4. Arnold M, Cavalcanti-Adam EA, Glass R, Blümmel J, Eck W, et al. Activation of integrin function by nanopatterned adhesive interfaces. *Chem PhysChem*. 2004; 5:383–8.
5. Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation--the role of rhoa, rockii and cytoskeletal dynamics. *Journal of Cell Science*. 2009; 122:546–53. [PubMed: 19174467]
6. Aspenström P. Effectors for the rho gtpases. *Current Opinion in Cell Biology*. 1999; 11:95–102. [PubMed: 10047515]
7. Aubert J, Dunstan H, Chambers I, Smith A. Functional gene screening in embryonic stem cells implicates wnt antagonism in neural differentiation. *Nature Biotechnology*. 2002; 20:1240–5.
8. Banerjee A, Arha M, Choudhary S, Ashton RS, Bhatia SR, et al. The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells. *Biomaterials*. 2009; 30:4695–9. [PubMed: 19539367]
9. Bhowmick NA, Ghiassi M, Bakin A, Aakre M, Lundquist CA, et al. Transforming growth factor- β 1 mediates epithelial to mesenchymal transdifferentiation through a rhoa-dependent mechanism. *Molecular Biology of the Cell*. 2001; 12:27–36. [PubMed: 11160820]
10. Blank U, Karlsson G, Karlsson S. Signaling pathways governing stem-cell fate. *Blood*. 2008; 111:492–503. [PubMed: 17914027]
11. Bost F, Aouadi M, Caron L, Binetruy B. The role of mapks in adipocyte differentiation and obesity. *Biochimie*. 2005; 87:51–6. [PubMed: 15733737]
12. Butcher DT, Alliston T, Weaver VM. A tense situation: Forcing tumour progression. *Nature Reviews Cancer*. 2009; 9:108–22.
13. Cabodi S, Moro L, Bergatto E, Boeri Erba E, Di Stefano P, et al. Integrin regulation of epidermal growth factor (egf) receptor and of egf-dependent responses. *Biochemical Society Transactions*. 2004; 32:438–42. [PubMed: 15157155]
14. Campos LS, Leone DP, Relvas JB, Brakebusch C, Fassler R, et al. B1 integrins activate a mapk signalling pathway in neural stem cells that contributes to their maintenance. *Development*. 2004; 131:3433–44. [PubMed: 15226259]
15. Caplan AI. Mesenchymal stem cells. *Journal of Orthopaedic Research*. 1991; 9:641–50. [PubMed: 1870029]
16. Chen CS. Mechanotransduction - a field pulling together? *Journal of Cell Science*. 2008; 121:3285–92. [PubMed: 18843115]
17. Chen G, Hou Z, Gulbranson DR, Thomson JA. Actin-myosin contractility is responsible for the reduced viability of dissociated human embryonic stem cells. *Cell Stem Cell*. 2010; 7:240–8. [PubMed: 20682449]
18. Chen HC, Guan JL. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:10148–52. [PubMed: 7937853]
19. Chien S. Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell. *American Journal of Physiology -Heart Circulatory Physiology*. 2007; 292:H1209–24. [PubMed: 17098825]
20. Chowdhury F, Li Y, Poh Y-C, Yokohama-Tamaki T, Wang N, Tanaka TS. Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions. *Plos One*. 2010; 5:e15655. demonstrate that pluripotent stem cells are sensitive to subtle changes in external mechanical forces and matrix mechanics. [PubMed: 21179449]
21. Chowdhury F, Na S, Li D, Poh Y-C, Tanaka TS, et al. Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells. *Nature Materials*. 2010; 9:82–8. demonstrate that pluripotent stem cells are sensitive to subtle changes in external mechanical forces and matrix mechanics.

22. Chun T-H, Hotary KB, Sabeh F, Saltiel AR, Allen ED, Weiss SJ. A pericellular collagenase directs the 3-dimensional development of white adipose tissue. *Cell*. 2006; 125:577–91. [PubMed: 16678100]
23. Cohen, DM.; Chen, CS. Stembook. The Stem Cell Research Community; 2008.
24. Crampton SP, Wu B, Park EJ, Kim JH, Solomon C, et al. Integration of the β -catenin-dependent wnt pathway with integrin signaling through the adaptor molecule grb2. *Plos One*. 2009; 4:e7841. [PubMed: 19924227]
25. Curtis ASG, Wilkinson CDW. Reactions of cells to topography. *Journal of Biomaterials Science*. 1998; 9:1313–29. Polymer Edition.
26. Czyz J, Wobus AM. Embryonic stem cell differentiation: The role of extracellular factors. *Differentiation*. 2001; 68:167–74. [PubMed: 11776469]
27. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, et al. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature Materials*. 2007; 6:997–1003.
28. Daley GQ, Scadden DT. Prospects for stem cell-based therapy. *Cell*. 2008; 132:544–8. [PubMed: 18295571]
29. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiological Reviews*. 1995; 75:519–60. [PubMed: 7624393]
30. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. *Science*. 2009; 324:1673–7. [PubMed: 19556500]
31. du Roure O. Force mapping in epithelial cell migration. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:2390–5. [PubMed: 15695588]
32. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: Architectural control of mechanotransduction. *Nature Reviews Molecular Cell Biology*. 2011; 12:308–19.
33. Dvorak P, Dvorakova D, Koskova S, Vodinska M, Najvirtova M, et al. Expression and potential role of fibroblast growth factor 2 and its receptors in human embryonic stem cells. *Stem Cells*. 2005; 23:1200–11. [PubMed: 15955829]
34. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006; 126:677–89. demonstrates that matrix elasticity is sufficient to direct commitments of adult stem cells toward different lineages. [PubMed: 16923388]
35. Etienne-Manneville S, Hall A. Rho gtpases in cell biology. *Nature*. 2002; 420:629–35. [PubMed: 12478284]
36. Farge E. Mechanical induction of twist in the drosophila foregut/stomodaeal primordium. *Current biology : CB*. 2003; 13:1365–77. [PubMed: 12932320]
37. Ferreira-Martins J, Rondon-Clavo C, Tugal D, Korn JA, Rizzi R, et al. Spontaneous calcium oscillations regulate human cardiac progenitor cell growth. *Circulation Research*. 2009; 105:764–74. [PubMed: 19745162]
38. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. *Nature*. 2010; 463:485–92. [PubMed: 20110992]
39. Folkman J, Moscona A. Role of cell shape in growth control. *Nature*. 1978; 273:345–9. [PubMed: 661946]
40. Fu J, Wang Y-K, Yang MT, Desai RA, Yu X, et al. Mechanical regulation of cell function using geometrically modulated elastomeric substrates. *Nature Methods*. 2010; 7:733–66. [PubMed: 20676108]
41. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. *Nature Reviews Molecular Cell Biology*. 2009; 10:21–33.
42. Giancotti FG, Ruoslahti E. Integrin signaling. *Science*. 1999; 285:1028–33. [PubMed: 10446041]
43. Gilbert PM, Havenstrite KL, Magnusson KEG, Sacco A, Leonardi NA, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science*. 2010; 329:1078–81. demonstrates that the regenerative capability of stem cells *in vivo* can be strongly influenced by matrix mechanics of the substrates where the cells are cultured *in vitro*. [PubMed: 20647425]
44. Griffith LG, Swartz MA. Capturing complex 3d tissue physiology in vitro. *Nature Review Molecular Cell Biology*. 2006; 7:211–24.

45. Guan J-L. Focal adhesion kinase in integrin signaling. *Matrix Biology*. 1997; 16:195–200. [PubMed: 9402009]
46. Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell*. 2009; 5:17–26. [PubMed: 19570510]
47. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature Review Molecular Cell Biology*. 2009; 10:53–62.
48. Hall A. Rho gtpases and the actin cytoskeleton. *Science*. 1998; 279:509–14. [PubMed: 9438836]
49. Hamill OP, McBride DW Jr. Induced membrane hypo/hyper-mechanosensitivity: A limitation of patch-clamp recording. *Annual Review of Physiology*. 1997; 59:621–31.
50. Hayakawa K, Tatsumi H, Sokabe M. Actin stress fibers transmit and focus force to activate mechanosensitive channels. *Journal of Cell Science*. 2008; 121:496–503. [PubMed: 18230647]
51. He XC, Yin T, Grindley JC, Tian Q, Sato T, et al. Pten-deficient intestinal stem cells initiate intestinal polyposis. *Nature Genetics*. 2007; 39:189–98. [PubMed: 17237784]
52. Hoffman BD, Grashoff C, Schwartz MA. Dynamic molecular processes mediate cellular mechanotransduction. *Nature*. 2011; 475:316–23. [PubMed: 21776077]
53. Holst J, Watson S, Lord MS, Eamegdool SS, Bax DV, et al. Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. *Nature Biotechnology*. 2010; 28:1123–8.
54. Hu R, He ML, Hu H, Yuan BX, Zang WJ, et al. Characterization of calcium signaling pathways in human preadipocytes. *Journal of Cellular Physiology*. 2009; 220:765–70. [PubMed: 19475562]
55. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nature Materials*. 2010; 9:518–26. demonstrates that matrix mechanics can regulate lineage commitments of stem cells in a 3-D setting.
56. Ingber D. Extracellular matrix and cell shape: Potential control points for inhibition of angiogenesis. *Journal of Cellular Biochemistry*. 1991; 47:236–41. [PubMed: 1724246]
57. Ingber DE. Mechanobiology and diseases of mechanotransduction. *Annals of Medicine*. 2003; 35:564–77. [PubMed: 14708967]
58. Ingber DE. Cellular mechanotransduction: Putting all the pieces together again. *FASEB J*. 2006; 20:811–27. [PubMed: 16675838]
59. James D, Levine AJ, Besser D, Hemmati-Brivanlou A. Tgfb β /activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development*. 2005; 132:1273–82. [PubMed: 15703277]
60. Jansen JH, Eijken M, Jahr H, Chiba H, Verhaar JA, et al. Stretch-induced inhibition of wnt/ β -catenin signaling in mineralizing osteoblasts. *Journal of Orthopaedic Research*. 2010; 28:390–6. [PubMed: 19780202]
61. Jones DL, Wagers AJ. No place like home: Anatomy and function of the stem cell niche. *Nature Reviews Molecular Cell Biology*. 2008; 9:11–21.
62. Kapur N, Mignery GA, Banach K. Cell cycle-dependent calcium oscillations in mouse embryonic stem cells. *American Journal of Physiology-Cell Physiology*. 2007; 292:C1510–8. [PubMed: 17092997]
63. Katsumi A, Orr AW, Tzima E, Schwartz MA. Integrins in mechanotransduction. *The Journal of Biological Chemistry*. 2004; 279:12001–4. [PubMed: 14960578]
64. Kawano S, Otsu K, Shoji S, Yamagata K, Hiraoka M. Ca²⁺ oscillations regulated by na⁺-ca²⁺ exchanger and plasma membrane ca²⁺ pump induce fluctuations of membrane currents and potentials in human mesenchymal stem cells. *Cell Calcium*. 2003; 34:145–56. [PubMed: 12810056]
65. Keller G. Embryonic stem cell differentiation: Emergence of a new era in biology and medicine. *Genes & Development*. 2005; 19:1129–55. [PubMed: 15905405]
66. 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; 107:4872–7. [PubMed: 20194780]

67. Kim L, Vahey MD, Lee H-Y, Voldman J. Microfluidic arrays for logarithmically perfused embryonic stem cell culture. *Lab on a Chip*. 2006; 6:394–406. [PubMed: 16511623]
68. Kim TJ, Seong JH, Ouyang MX, Sun J, Lu SY, et al. Substrate rigidity regulates ca^{2+} oscillation via rhoa pathway in stem cells. *Journal of Cellular Physiology*. 2009; 218:285–93. [PubMed: 18844232]
69. Kimura, T.; Nakano, T. Regulatory networks in stem cells. Rajasekhar, VK.; Vemuri, MC., editors. Humana Press; 2009. p. 309-18.
70. Kobayashi T, Sokabe M. Sensing substrate rigidity by mechanosensitive ion channels with stress fibers and focal adhesions. *Current Opinion in Cell Biology*. 2010; 22:669–76. [PubMed: 20850289]
71. Laflamme MA, Murry CE. Heart regeneration. *Nature*. 2011; 473:326–35. [PubMed: 21593865]
72. Lehoux S, Esposito B, Merval R, Tedgui A. Differential regulation of vascular focal adhesion kinase by steady stretch and pulsatility. *Circulation*. 2005; 111:643–9. [PubMed: 15668343]
73. Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials*. 2009; 30:6867–78. [PubMed: 19775749]
74. Li D, Zhou J, Wang L, Shin ME, Su P, et al. Integrated biochemical and mechanical signals regulate multifaceted human embryonic stem cell functions. *Journal of Cell Biology*. 2010; 191:631–44. [PubMed: 20974810]
75. Li J, Wang G, Wang C, Zhao Y, Zhang H, et al. Mek/erk signaling contributes to the maintenance of human embryonic stem cell self-renewal. *Differentiation*. 2007; 75:299–307. [PubMed: 17286604]
76. Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. *Nature*. 2006; 441:1094–6. [PubMed: 16810245]
77. Luo W, Jones SR, Yousaf MN. Geometric control of stem cell differentiation rate on surfaces. *Langmuir*. 2008; 24:12129–33. [PubMed: 18850687]
78. Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. *Nature*. 2009; 462:433–41. [PubMed: 19940913]
79. Maeda T, Sakabe T, Sunaga A, Sakai K, Rivera Alexander L, et al. Conversion of mechanical force into $tgf-\beta$ -mediated biochemical signals. *Current Biology*. 2011; 21:933–41. [PubMed: 21600772]
80. Mammoto T, Ingber DE. Mechanical control of tissue and organ development. *Development*. 2010; 137:1407–20. [PubMed: 20388652]
81. Manasek FJ, Burnside MB, Waterman RE. Myocardial cell shape change as a mechanism of embryonic heart looping. *Developmental Biology*. 1972; 29:349–71. [PubMed: 4120601]
82. Margadant C, Sonnenberg A. Integrin- $tgf-\beta$ crosstalk in fibrosis, cancer and wound healing. *EMBO Reports*. 2010; 11:97–105. [PubMed: 20075988]
83. Martinac B. Mechanosensitive ion channels: Molecules of mechanotransduction. *Journal of Cell Science*. 2004; 117:2449–60. [PubMed: 15159450]
84. Maruyama T, Mirando AJ, Deng CX, Hsu W. The balance of wnt and fgf signaling influences mesenchymal stem cell fate during skeletal development. *Science Signal*. 2010; 3:ra40.
85. Massague J, Blain SW, Lo RS. $Tgf\beta$ signaling in growth control, cancer, and heritable disorders. *Cell*. 2000; 103:295–309. [PubMed: 11057902]
86. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and rhoa regulate stem cell lineage commitment. *Developmental Cell*. 2004; 6:483–95. [PubMed: 15068789]
87. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and rhoa regulate stem cell lineage commitment. *Developmental Cell*. 2004; 6:483–95. demonstrates that cell shape plays a critical role in lineage commitments of stem cells by regulating Rho A-mediated CSK contractility. [PubMed: 15068789]
88. McMurray RJ, Gadegaard N, Tsimbouri PM, Burgess KV, McNamara LE, et al. Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. *Nature Materials*. 2011; 10:637–44.

89. Moore KA, Polte T, Huang S, Shi B, Alsberg E, et al. Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by rho and cytoskeletal tension. *Developmental Dynamics*. 2005; 232:268–81. [PubMed: 15614768]
90. Morrison SJ, Spradling AC. Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008; 132:598–611. [PubMed: 18295578]
91. Moustakas A, Heldin C-H. Non-smad $\text{tgf-}\beta$ signals. *Journal of Cell Science*. 2005; 118:3573–84. [PubMed: 16105881]
92. Muroyama Y, Kondoh H, Takada S. Wnt proteins promote neuronal differentiation in neural stem cell culture. *Biochemical and Biophysical Research Communications*. 2004; 313:915–21. [PubMed: 14706629]
93. Murray CD. The physiological principle of minimum work: I. The vascular system and the cost of blood volume. *Proceedings of the National Academy of Sciences of the United States of America*. 1926; 12:207–14. [PubMed: 16576980]
94. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: Lessons from embryonic development. *Cell*. 2008; 132:661–80. [PubMed: 18295582]
95. O'Callaghan CJ, Williams B. Mechanical strain-induced extracellular matrix production by human vascular smooth muscle cells: Role of $\text{tgf-}\beta_1$. *Hypertension*. 2000; 36:319–24. [PubMed: 10988258]
96. Oloumi A, Syam S, Dedhar S. Modulation of wnt3a-mediated nuclear β -catenin accumulation and activation by integrin-linked kinase in mammalian cells. *Oncogene*. 2006; 25:7747–57. [PubMed: 16799642]
97. Orr AW, Helmke BP, Blackman BR, Schwartz MA. Mechanisms of mechanotransduction. *Developmental Cell*. 2006; 10:11–20. [PubMed: 16399074]
98. Osol G. Mechanotransduction by vascular smooth muscle. *Journal of Vascular Research*. 1995; 32:275–92. [PubMed: 7578796]
99. Otero JJ, Fu W, Kan L, Cuadra AE, Kessler JA. B-catenin signaling is required for neural differentiation of embryonic stem cells. *Development*. 2004; 131:3545–57. [PubMed: 15262888]
100. Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. *The Journal of Biological Chemistry*. 2004; 279:48063–70. [PubMed: 15328362]
101. Parsons JT. Focal adhesion kinase: The first ten years. *Journal of Cell Science*. 2003; 116:1409–16. [PubMed: 12640026]
102. Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: Integrating cytoskeletal dynamics and cellular tension. *Nature Reviews Molecular Cell Biology*. 2010; 11:633–43.
103. Peerani R, Rao BM, Bauwens C, Yin T, Wood GA, et al. Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *Embo Journal*. 2007; 26:4744–55. [PubMed: 17948051]
104. Peerani R, Rao BM, Bauwens C, Yin T, Wood GA, et al. Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *EMBO J*. 2007; 26:4744–55. [PubMed: 17948051]
105. Pera MF, Tam PPL. Extrinsic regulation of pluripotent stem cells. *Nature*. 2010; 465:713–20. [PubMed: 20535200]
106. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:143–7. [PubMed: 10102814]
107. Qi X, Li TG, Hao J, Hu J, Wang J, et al. Bmp4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:6027–32. [PubMed: 15075392]
108. Riento K, Ridley AJ. Rocks: Multifunctional kinases in cell behaviour. *Nature Reviews Molecular Cell Biology*. 2003; 4:446–56.
109. Ruiz SA, Chen CS. Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells*. 2008; 26:2921–7. [PubMed: 18703661]
110. Saha K, Keung AJ, Irwin EF, Li Y, Little L, et al. Substrate modulus directs neural stem cell behavior. *Biophysical Journal*. 2008; 95:4426–38. [PubMed: 18658232]

111. Saha S, Ji L, de Pablo JJ, Palecek SP. Inhibition of human embryonic stem cell differentiation by mechanical strain. *Journal of Cellular Physiology*. 2006; 206:126–37. [PubMed: 15965964]
112. Saha S, Ji L, de Pablo JJ, Palecek SP. Tgf β /activin/nodal pathway in inhibition of human embryonic stem cell differentiation by mechanical strain. *Biophysical Journal*. 2008; 94:4123–33. [PubMed: 18234825]
113. Sastry SK, Burridge K. Focal adhesions: A nexus for intracellular signaling and cytoskeletal dynamics. *Experimental Cell Research*. 2000; 261:25–36. [PubMed: 11082272]
114. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of wnt signaling by a pharmacological gsk-3-specific inhibitor. *Nature Medicine*. 2004; 10:55–63.
115. Scadden DT. The stem-cell niche as an entity of action. *Nature*. 2006; 441:1075–9. [PubMed: 16810242]
116. Schlaepfer DD, Hanks SK, Hunter T, van der Geer P. Integrin-mediated signal transduction linked to ras pathway by grb2 binding to focal adhesion kinase. *Nature*. 1994; 372:786–91. [PubMed: 7997267]
117. Schvartzman M, Palma M, Sable J, Abramson J, Hu XA, et al. Nanolithographic control of the spatial organization of cellular adhesion receptors at the single-molecule level. *Nano Letters*. 2011; 11:1306–12. [PubMed: 21319842]
118. Schwartz MA, DeSimone DW. Cell adhesion receptors in mechanotransduction. *Current Opinion in Cell Biology*. 2008; 20:551–6. [PubMed: 18583124]
119. Schwartz MA, Ginsberg MH. Networks and crosstalk: Integrin signalling spreads. *Nature Cell Biology*. 2002; 4:E65–E8.
120. Skelley AM, Kirak O, Suh H, Jaenisch R, Voldman J. Microfluidic control of cell pairing and fusion. *Nat Meth*. 2009; 6:147–52.
121. Song W, Lu HX, Kawazoe N, Chen GP. Adipogenic differentiation of individual mesenchymal stem cell on different geometric micropatterns. *Langmuir*. 2011; 27:6155–62. [PubMed: 21486006]
122. Stevens MM, George JH. Exploring and engineering the cell surface interface. *Science*. 2005; 310:1135–8. [PubMed: 16293749]
123. Storm MP, Bone HK, Beck CG, Bourillot PY, Schreiber V, et al. Regulation of nanog expression by phosphoinositide 3-kinase-dependent signaling in murine embryonic stem cells. *The Journal of Biological Chemistry*. 2007; 282:6265–73. [PubMed: 17204467]
124. Sukharev S, Corey DP. Mechanosensitive channels: Multiplicity of families and gating paradigms. *Science STKE*. 2004; 2004:re4.
125. Sun S, Liu YM, Lipsky S, Cho M. Physical manipulation of calcium oscillations facilitates osteodifferentiation of human mesenchymal stem cells. *The FASEB Journal*. 2007; 21:1472–80.
126. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. 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; 100:1484–9. [PubMed: 12552122]
127. Valdimarsdottir G, Mummery C. Functions of the tgfb superfamily in human embryonic stem cells. *APMIS*. 2005; 113:773–89. [PubMed: 16480449]
128. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nature Reviews Molecular Cell Biology*. 2006; 7:265–75.
129. Vunjak-Novakovic G, Scadden David T. Biomimetic platforms for human stem cell research. *Cell Stem Cell*. 2011; 8:252–61. [PubMed: 21362565]
130. Walker A, Su H, Conti MA, Harb N, Adelstein RS, Sato N. Non-muscle myosin ii regulates survival threshold of pluripotent stem cells. *Nature Communications*. 2010; 1:71.
131. Wang JHC, Thampatty BP. Mechanobiology of adult and stem cells. *International Review of Cell and Molecular Biology*. 2008; 271:301–46. [PubMed: 19081546]
132. Wary KK, Mariotti A, Zurzolo C, Giancotti FG. A requirement for caveolin-1 and associated kinase fyn in integrin signaling and anchorage-dependent cell growth. *Cell*. 1998; 94:625–34. [PubMed: 9741627]

133. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, et al. A rock inhibitor permits survival of dissociated human embryonic stem cells. *Nature Biotechnology*. 2007; 25:681–6.
134. Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, et al. Fak-src signalling through paxillin, erk and mlck regulates adhesion disassembly. *Nature Cell Biology*. 2004; 6:154–61.
135. Wehrle-Haller B, Imhof BA. The inner lives of focal adhesions. *Trends in Cell Biology*. 2002; 12:382–9. [PubMed: 12191915]
136. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent tgf- β_1 from the extracellular matrix. *Journal of Cell Biology*. 2007; 179:1311–23. [PubMed: 18086923]
137. Wong JY, Leach JB, Brown XQ. Balance of chemistry, topography, and mechanics at the cell-biomaterial interface: Issues and challenges for assessing the role of substrate mechanics on cell response. *Surface Science*. 2004; 570:119–33.
138. Wozniak MA, Chen CS. Mechanotransduction in development: A growing role for contractility. *Nature Reviews Molecular Cell Biology*. 2009; 10:34–43.
139. Wozniak MA, Modzelewska K, Kwong L, Keely PJ. Focal adhesion regulation of cell behavior. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2004; 1692:103–19.
140. Xiao L, Yuan X, Sharkis SJ. Activin a maintains self-renewal and regulates fibroblast growth factor, wnt, and bone morphogenic protein pathways in human embryonic stem cells. *Stem Cells*. 2006; 24:1476–86. [PubMed: 16456129]
141. Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. Basic fgf and suppression of bmp signaling sustain undifferentiated proliferation of human es cells. *Nature Methods*. 2005; 2:185–90. [PubMed: 15782187]
142. Yamamoto K, Sokabe T, Watabe T, Miyazono K, Yamashita JK, et al. Fluid shear stress induces differentiation of flk-1-positive embryonic stem cells into vascular endothelial cells in vitro. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1915–24. [PubMed: 15576436]
143. Yang Y, Relan NK, Przywara DA, Schuger L. Embryonic mesenchymal cells share the potential for smooth muscle differentiation: Myogenesis is controlled by the cell's shape. *Development*. 1999; 126:3027–33. [PubMed: 10357945]
144. Yim EK, Darling EM, Kulangara K, Guilak F, Leong KW. Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. *Biomaterials*. 2010; 31:1299–306. [PubMed: 19879643]
145. Yim EKF, Pang SW, Leong KW. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Experimental Cell Research*. 2007; 313:1820–9. [PubMed: 17428465]
146. Young JL, Engler AJ. Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. *Biomaterials*. 2011; 32:1002–9. [PubMed: 21071078]
147. Zaidel-Bar R, Geiger B. The switchable integrin adhesome. *Journal of Cell Science*. 2010; 123:1385–8. [PubMed: 20410370]
148. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, et al. Pten maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature*. 2006; 441:518–22. [PubMed: 16633340]
149. Zhu AJ, Haase I, Watt FM. Signaling via β_1 integrins and mitogen-activated protein kinase determines human epidermal stem cell fate in vitro. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96:6728–33. [PubMed: 10359780]

SUMMARY POINTS

1. Physical signals in the local cellular microenvironment can strongly influence stem cell fate.
2. Cell shape is a key regulator of stem cell fate by controlling CSK tension.
3. Nanotopographical cues can control stem cell behaviors by modulating the molecular arrangement, dynamic organization, and signaling of the cellular adhesion machinery.
4. External mechanical forces and matrix mechanics can regulate stem cell behaviors through the force balance along the mechanical continuum of the ECM-integrin-CSK linkage and their regulation by the mechanical signals in the stem cell niche.
5. The force balance across the mechanical linkage of ECM-integrin-CSK can be further transduced into the intracellular space of stem cells to mediate signaling molecules important for fate decisions of stem cells, such as those mediated by integrin signaling and mechanosensitive ion channels.

Figure 1.

Schematic showing biophysical signals in the stem cell niche and the intricate reciprocal molecular interactions between stem cells and their microenvironment to regulate stem cell fate. The extracellular microenvironment of stem cells is a hydrated protein- and proteoglycan-based gel network comprising soluble and physically bound signals as well as signals arising from cell-cell interactions. Biophysical signals in the stem cell niche include matrix rigidity and topography, flow shear stress, strain forces, and other mechanical forces exerted by adjacent support cells. Stem cells can sense these biophysical stimuli through mechanosensitive ion channels, focal adhesions, cell surface receptors, actin cytoskeleton, and cell-cell adhesions. A magnified view of the focal adhesion structure is also shown, which includes transmembrane heterodimeric integrin, paxillin (Pax), talin, focal adhesion kinase (FAK), vinculin (Vin), Zyxin, and vasodilator-stimulated phosphoprotein (VASP).

Figure 2.

Microcontact printing to manipulate the cell shape and colony size of stem cells to control their fate. (a) Brightfield micrographs of single hMSCs plated on different sized adhesive ECM islands. hMSCs were stained for alkaline phosphatase activity (ALP, blue) and lipid droplet accumulation (Lip, red) after 7 days of culture in either the growth (top row) or the bipotential differentiation (bottom row) medium. Scale bar, 50 μm . Reprinted from Ref. (86), copyright (2004), with permission from Elsevier. (b) Brightfield micrographs of different shaped multicellular hMSC colonies stained for ALP (blue) and Lip (red) after 14 days of culture in the bipotential differentiation medium. Scale bar, 250 μm . Reprinted from Ref. (109), copyright (2008), with permission from John Wiley and Sons. (c) Immunofluorescent images showing different sized hESC colonies (H9) cultured in XV media after withdrawal of all exogenous growth factors for 48 hrs. hESCs were stained for Hoechst, Oct4, and pSmad1 to indicate the effect of colony size on the pluripotency maintenance of hESCs. Reprinted from Ref. (104), copyright (2007), with permission from Nature Publishing Group.

Figure 3.

External mechanical forces regulate stem cell fate. **(a)** Fluid shear stress induces differentiation of Flk-1-positive mouse ESCs into vascular endothelial cells in vitro. Flk-1+ mouse ESCs were incubated under a static culture condition (top) or subjected to shear stress (5 dyn/cm^2) (bottom). After 24 hrs, the cells were stained for PECAM-1 (an endothelia cell marker; purple) and SM α -actin (a smooth muscle cells marker; brown). Shear stress appeared to induce PECAM-1-positive cell sheets. Adapted from Ref. (142), copyright (2005), with permission from the American Physiological Society. **(b)** Brightfield images (top row) with corresponding GFP images of Oct3/4 expression (bottom row) for single mouse ESCs. The cells were attached with RGD-coated magnetic beads (black dots in the brightfield images) and were continuously stressed for about 1 hr. Oct3/4 expression of these stressed cells was continuously monitored over time. Adapted from Ref. (21), copyright (2010), with permission from Nature Publishing Group.

Figure 4.

Matrix mechanics directs stem cell fate. **(a)** Soft tissue elasticity scale ranging from soft brain, fat, and striated muscle, to stiff cartilage and precalcified bone. In contrast, conventional tissue culture plates (TCPs) have a much stiffer elastic modulus (E 10^6 kPa). Varying matrix elasticity or rigidity can induce multipotent hMSCs to differentiate into different tissue cell types corresponding to the tissues' relative mechanical elasticity *in vivo*. The top part of **a** is adapted from Ref. (30), copyright (2009), with permission from the American Association for the Advancement of Science. The bottom part of **a** is adapted from Ref. (34), copyright (2006), with permission from Elsevier. **(b)** Matrix mechanics-dependent differentiation of hMSCs. hMSCs were stained for β 3-Tubulin, MyoD, and CBF α 1 as markers of neurogenic, myogenic, and osteogenic differentiation, respectively. Scale bar, μ m. hMSC differentiation correlates to tissue-specific mechanical properties (e.g., soft matrix leads to neural differentiation, whereas stiff one leads to osteogenic differentiation). Reprinted from Ref. (34), copyright (2006), with permission from Elsevier. **(c)** Matrix mechanics regulates murine MSC (mMSC) fate in 3-D matrix culture. Top row: *in situ* staining of encapsulated clonally derived mMSCs for ALP (blue) and Lip (red) after 1 week of culture in the presence of combined osteogenic and adipogenic chemical supplements within encapsulating matrices consisting of RGD-modified alginate with varying matrix elasticity as indicated (Scale bars, 100 μ m). Bottom row: immunofluorescence staining for osteocalcin (OCN, green) and the nuclear counterstain 4', 6-diamidino-2-phenylindole (DAPI, blue) in cryosectioned alginate matrices of varying matrix elasticity containing mMSCs (Scale bars, 20 μ m). Reprinted from Ref. (55), copyright (2010), with permission from Nature Publishing Group. **(d)** Cultured muscle stem cell (MuSC) engraftment is modulated by matrix mechanics. Representative bioluminescence images of recipient mice 1 month after transplantation with 100 GFP/Fluc MuSCs after 7-day culture on substrates of varying stiffness, as indicated. The bar graph shows the percentage of mice from each experimental condition that had a bioluminescence

value above the engraftment threshold. Adapted from Ref. (43), copyright (2010), with permission from the American Association for the Advancement of Science.

Figure 5. Signaling cross-talk between the mechano transductive processes (black arrows) and other known soluble factor-mediated signaling pathways regulating the fate decisions of stem cells (blue arrows).

Figure 6.

Micro/nanotechnology for constructing synthetic in vitro stem cell niche to regulate stem cell fate. **(a)** Scanning electron micrograph (SEM) of single hMSCs plated on top of microfabricated PDMS microposts. The bending spring constant of the PDMS micropost could switch the differentiation potential of hMSCs between osteogenic and adipogenic fates. Adapted from Ref. (40), copyright (2010), with permission from Nature Publishing Group. **(b)** SEM image showing single cells spreading on an array of nanodots fabricated using advance sub-100 nm nanoimprint lithography (NIL). These nanostructured surfaces were used to explore how the geometric organization of the binding ligand RGD (Arg-Gly-Asp) affects cell adhesion and spreading. Adapted with permission from Ref. (27). Copyright (2011) American Chemical Society. **(c)** Microfluidic arrays for logarithmically perfused mouse ESC culture. The top photograph shows a microfluidic device fabricated using soft lithography with multiple chambers for long-term culture of mouse ESCs. The bottom two brightfield images show the colonies of mouse ESCs after 4 days of perfusion at different culture flow rates. Reproduced from Ref. (67) by permission of The Royal Society of Chemistry. **(d)** Microfabricated cell traps for cell pairing and fusion, by using a three-step cell-loading protocol, as indicated. Scale bar, 50 μm . Reprinted from Ref. (120), copyright (2009), with permission from Nature Publishing Group.