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Getting Physical: Material Mechanics is an Intrinsic Cell Cue

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Summary

Advances in biomaterial science have allowed for unprecedented insight into the ability of material cues to influence stem cell function. These material approaches better recapitulate the microenvironment providing a more realistic *ex vivo* model of the cell niche. However, recent advances in our ability to measure and manipulate niche properties *in vivo* have led to novel mechanobiological studies in model organisms. Thus, in this review, we will discuss the importance of material cues within the cell niche, highlight the key mechanotransduction pathways involved, and conclude with recent evidence that material cues regulate tissue function *in vivo*.

eTOC

Mechanobiology studies utilize biomaterials to closely mimic the mechanics of *in vivo* tissues. Atcha et al. discussed key mechanical properties of tissues mimicked by biomaterials and their impacts on stem cell behaviors including self-renewal and differentiation *in vitro* and *in vivo*.

Keywords

Mechanobiology; Forces; Niche; Progenitor; Extracellular Matrix

1. Introduction

The cell niche and its corresponding microenvironment are continually evolving to produce specialized tissues with distinct and varying mechanical properties. For example, the cell niche is responsible for the formation of hard tissues, such as bone, as well as soft tissues including fat and brain. Given the vast differences in function and mechanical properties

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of these tissues *in vivo*, it is clear that cell differentiation is a highly coordinated and complex process involving distinct signals ranging from biochemical, mechanical, adhesive, and spatial cues[1]. Combinations of these signals give rise to distinct cell types. While over 200 different cell types are thought to exist in the human body, single cell transcriptomics has recently questioned the true number of distinct cell types[2]. Developmental 'trajectories'[3] suggest that many cues influence cell fate, and thus it begs the question: which cues are essential in the development of each cell type? For more than two decades since the isolation of pluripotent stem cells (PSCs), the use of complex sets of soluble cues has been explored to selectively differentiate and mature stem cells into a wide variety of cell types. In addition to soluble cues, mechanical stimuli have also been shown to play significant roles in cell determination and are still currently being investigated[1]. Biophysical stimuli pose a unique challenge to recapitulate *in vitro*, but several methods have been developed to introduce externally applied, or extrinsic, as well as local, or intrinsic, mechanical cues, as shown in Figure 1. Although extrinsic mechanical cues have also been shown to modulate stem cell behavior, less is known regarding the role of material cues[4]. In particular, the use of biomaterials has significantly advanced our understanding and appreciation of biophysical stimuli in the cell niche and, as a result, material mechanics are becoming more widely accepted as an intrinsic mechanical cue that is capable of modulating cell function. Material properties such as stiffness or topography as well as extracellular matrix composition have all been shown to provide key signals necessary to modulate stem cell differentiation[5,6]. Despite these advances, there is still much that material mechanics can teach us with respect to better understanding the cell niche and the mechanotransduction pathways that are activated as a result.

In this review, we will provide a general overview detailing the material systems used to mimic the stem cell niche and the molecular machinery expressed by cells to sense and transduce intrinsic material cues. While it is important to note that other biophysical stimuli are prevalent in physiological systems and can also regulate cell function, the scope of this review will be limited to mechanical stimuli provided by biomaterials. We will also discuss matrix cues that regulate self-renewal and control lineage specification. Moreover, the impact of *in vitro* material systems–regardless of dimensionality–may be limited if they are not also found *in vivo*. Recently emerging in the literature are a series of *in vivo* examples where extracellular matrix (ECM) mechanics were genetically manipulated to impact niche properties. In the final section, the resulting effects will be described, and the impact of physiological and pathological material cues *in vivo* will be provided for various tissues to convey emerging areas and those yet to be explored.

2. Stem Cell Niche Material Mechanics and Mechanotransduction

The stem cell niche refers to the highly specialized microenvironment surrounding a stem cell and is defined by the organization and type of neighboring cells, the milieu of soluble biological signals, and physical as well as biological signaling from the ECM. In this section, we describe the basic considerations for modeling the stem cell niche with biomaterials, e.g. mechanical properties that match native tissue stiffness, and how they impact cell behavior. We will pay particular attention to the types of materials chosen and whether a synthetic, biological, or hybrid approach is ideal for a particular application or

goal. Following this discussion, we will provide an overview of the more recent molecular pathways that have been identified in the transduction of material cues *in vitro*.

2.1. Niche Elasticity vs. Viscoelasticity: A Tissue Perspective

Over the last 15 years, ECM elasticity and tissue viscoelasticity have emerged as critical features of the niche and can regulate stem cell biology. ECM elasticity has more commonly been referred to as "stiffness," though it is more technically correct to define it as the material's elastic modulus, a measure of a material's ability to undergo non-permanent deformation. Soft tissues, including brain, fat, breast, or liver tissues have elastic moduli on the order of 100s of Pascals to several kilopascals (kPa). In the musculoskeletal system, muscle has a modulus of around 10 kPa, articular cartilage 100s of kPa, and bone has an elastic modulus of 10s of Gigapascals[7–9]. For comparison, tissue culture plastic exhibits an elastic modulus on the order of 10s of Gigapascals. The elastic modulus in tissues is thought to be governed largely by the density of ECM, often dominated by fibrillar type-1 collagen networks, and its crosslinking and architecture. In contrast to material stiffness, viscoelasticity has been less well characterized compared to its elastic response, but recent efforts utilizing material science and engineering have attempted to better understand how these more complex properties influence the stem cell niche. Viscoelastic materials exhibit some mechanical responses characteristic of elastic solids and some that are characteristic of viscous liquids. One feature of viscous liquids is that they dissipate mechanical energy – resulting in hysteresis during loading and unloading –this phenomenon is illustrated in Figure 2. On the order of seconds, living tissues and extracellular matrices exhibit a loss modulus, which represents the viscous resistance of a material to deformation. Viscous effects can become increasingly apparent at longer times, with viscoelastic materials exhibiting stress relaxation in response to a deformation (i.e., decreased resistance to deformation over time) as illustrated in Figure 2. Tissues exhibit a range of stress relaxation times, substantially relaxing stresses within seconds in some tissues like brain or breast tumors, while other tissues including muscle or skin are more prolonged ranging within many minutes to an hour[10]. Time-dependent mechanical responses arise from various sources including the unbinding of weak bonds connecting matrix fibers or proteins under stress or strain, generating spatially distinct cues, allowing viscous matrix flow, and enabling fluid movement in the matrix. Moreover, cellular remodeling of natural matrix due to matrix protease expression and activity provide an additional level of complexity by which the mechanical and biochemical nature of the microenvironment is continually adapting and changing[11].The temporal and spatial dynamics of the mechanical cues provide a unique niche environment for cell development and, in turn, raises several key questions of which include: What biomaterial approaches have been developed to study material mechanics in the modulation of cell function? Through the use of these engineered materials, we can also ask: How do cells sense and transduce their mechanical environment?

2.2. Niche Elasticity vs. Viscoelasticity: A Material Perspective

Similar to tissues, biomaterials display distinct elastic and viscoelastic characteristics. They can help mimic the *in vivo* microenvironment by providing physical support as well as biological and mechanical cues to regulate stem cell function or fate through cell-matrix and cell-cell interactions[12]. Biomaterial scaffolds help tune biochemical and mechanical

properties which, when well designed, can modulate cell signaling pathways to guide lineage specification. Biomaterials consist of natural and synthetic materials, each with its own set of unique advantages and disadvantages, as outlined in Table 1. Here, we provide a brief overview of current natural and synthetic biomaterial approaches that can be used to mimic the niche environment.

Natural materials include ECM protein-based materials, e.g., collagen, fibrin, and reconstituted basement membrane matrix, as well as polysaccharide-based materials, e.g., hyaluronan, alginate, and agarose. Natural ECM protein-based materials are structurally similar to native tissues[13], have superior cytocompatibility relative to synthetic materials, and derive their mechanical properties from entangled macromolecular chains and crosslinks[14,15]. The viscoelasticity of these materials varies significantly due to a number of parameters, such as the types and strength of bonds or the molecular weight of polymers within the material[16]. These materials contain cell-adhesive binding domains to provide a functional platform with superior biocompatibility for cells to grow[12]. For example, collagen has been utilized for cartilage regeneration in the treatment of osteochondral and corneal defects through its ability to promote chondrogenesis in mesenchymal stem cells (MSCs)[13,17]. However, these natural protein-based materials tend to be susceptible to degradation by proteases allowing for cell-mediated remodeling of the matrix, which can alter both physical and chemical properties of the ECM and can thus broadly impact cell function[11]. Conversely, polysaccharide-based natural biomaterials, such as hyaluronan or alginate, must be often functionalized with crosslinkers so that they form a gel (in the case of hyaluronan), or cell adhesion sites but can be biodegradable and formed with a large dynamic range of material stiffness[12,13]. Moreover, these materials have varying degrees of viscoelastic properties which can be modified for cell culture purposes[18]. Unfortunately, batch variability, poor mechanical tunability, and laborious purification and quality control methods hinder the use of natural materials.

Conversely, synthetic materials provide superior reproducibility and have chemically defined and highly tunable mechanical and degradation properties[12,13]. Material mechanics, for example, can be modulated by changing the degree of covalent bonding, polymer weight fraction, and introduction of degradation sites such as ester bonds for hydrolytic cleavage[14]. Fewer covalent bonds improve stress relaxation, energy dissipation, and plastic deformation. On the other hand, more covalent bonds enhance stiffness but suppresses viscoelasticity[16]. Synthetic biomaterials include hydrogels (e.g., polyethylene glycol and dextran) and silicon rubbers (e.g., polydimethyl siloxane). These polymers are the most widely used synthetic materials in stem cell culture with many approved by the FDA for clinical applications[19]. When used in cell culture, polymers can act in conjunction with growth factors such as transforming growth factor-β and insulin-like growth factor to promote growth and differentiation of human embryonic stem cells[20] and human mesenchymal stem cells[21].

Unlike their natural counterparts, synthetic materials lack cell adhesion sites or biological cues and, as a result, require modification with natural materials or peptides to compensate for these disadvantages. For example, gelatin methacryloyl (GelMA) utilizes the natural properties of gelatin with photo-crosslinking of methacrylic anhydride to tune hydrogel mechanical properties with stiffness and time, and such properties can influence stem cell

mechanotransduction[22,23]. However, one must be cautious when adding adhesion cues as some ligands, such as platelet lysate, can also increase substrate viscosity and stiffness[24]. While they utilize the strengths of both natural and synthetic materials, these sorts of semi-synthetic materials require UV light mediated stiffening, which may have detrimental effects with significant exposure though most systems do not result is exposure to substantial UV energy[25,26].

Despite the numerous advances that have been made in the field of biomaterials, key areas for improvement which could further enhance the biomimetic capabilities of these materials remain. For example, while much of the discussion thus far has focused primarily on mechanical stimuli, the niche microenvironment also contains other stimuli, such as biochemical and electrophysiological stimuli, which can regulate cell function. Synergistic effects between combinations of mechanical as well as biochemical or conductive electrical stimuli have been shown to modulate cell self-renewal or differentiation[27–29]. Additionally, material properties including pore size, interconnectivity, and ECM localization can also influence cell-intrinsic forces which govern shape and volume among other factors[28]. Therefore, material strategies capable of incorporating combinations of stimuli could provide a more physiological environment for stem cell growth. These strategies could involve controlled release of soluble or bound growth factors, addition of multiple mechanical stimuli, or the use of bioconductive polymers, which independently are known to regulate cell function[28]. However, when considering the design of any biomaterial, it is important to note that context is critical. While we focused on mechanical properties of insoluble matrix, some of the examples provided herein highlight their interplay with biological[30] and soluble cues[31], not to mention their combinatorial nature with other insoluble cues *in vivo*. While it is impossible to highlight all cues – even just the insoluble ones – and the types of forces applied *in vivo*, we discussed what we believe are the most critical and/or ones that are being shown to be increasingly effective at regulating stem cell behavior. If the reader takes only one point from this section, it should be that natural and synthetic matrices *in vitro* must typically mirror their mature, *in vivo* counterparts. This, in turn, can tune cellular mechanotransduction responses, a topic which we will be further discussed in the following section.

2.3 Cellular Implications of Niche Elasticity and Viscoelasticity

Stem cells, like many other differentiated cell types, express a variety of mechanically sensitive cell surface receptors that are essential to the transduction of mechanical signals. In the presence of mechanical stimuli, these proteins activate resulting in downstream signaling pathways that, in turn, modulate cell function. Recent advances have identified novel mechanosensitive molecules and pathways that are critical in mechanotransduction within the cell niche. In this section, the role of adhesive genes, collectively referred to as the adhesome, as well as mechanically activated ion channels, and non-canonical modes of mechanotransduction will be discussed, all of which are exemplified in Figure 3.

2.3.1 Adhesome—The adhesome refers to structural and signaling proteins that are essential to cell-matrix and cell-cell interactions, such as integrins[32]. Integrins are cell surface proteins that connect the cellular cytoskeleton to the ECM. In response

to mechanical stimuli, integrins activate through clustering and facilitate cytoskeletal remodeling, which results in signaling pathway activation to modulate stem cell function and fate[33] in a stiffness sensitive manner[34]. For example, soft hydrogels improve adipose derived stem cell adhesion and activate an integrin-vascular endothelial growth factor receptor-extracellular signal-regulated kinase signaling pathway resulting in enhanced angiogenesis[35]. Moderately stiff matrix can support myogenic commitment of MSCs via β_3 integrin clustering[36] while stiffer matrix supports osteogenesis via α_2[37] and α_5[38] integrin signaling. These data support the growing body of literature suggesting loss of these integrins, changes to their binding partners, or downstream signals can impair differentiation[30,39]. In addition to sensing material stiffness, integrins also play key roles in sensing viscoelasticity. Materials with shorter stress relaxation times resulted in enhanced β_1 integrin clustering and activation, which in turn enhanced yes-associated protein (YAP) nuclear localization, when compared to materials with longer stress relaxation times[40]. These observations suggest the possibility for a threshold required in integrin activation, whereby slow or gradual changes to material properties are not detected by integrins. When cultured on viscoelastic hydrogel, cells exert forces on the matrix which are relaxed overtime through matrix reorganization. The cyclic nature of cellular force exertion and the resulting matrix stress relaxation promotes ligand clustering and cell spreading. Moreover, the rate of stress relaxation regulates the degree of mechanics-mediated remodeling of the matrix. Therefore, materials with slower stress relaxation result in reduced adhesion ligand clustering, integrin activation, and cell spreading[40]. However, additional experiments are needed to confirm the exact sensitivity of integrins to differing material systems as each integrin complex can have varying thresholds. Nevertheless, these data suggest that integrins compose a large portion of the molecules involved in sensing stiffness and viscoelasticity, but equally important in the niche are cell-cell interactions.

Cell-cell interactions play a critical role in maintaining niche architecture, and cadherin-mediated adhesions are central to these types of interactions. Cadherins are calcium dependent transmembrane proteins that mediate cell-cell adhesions and help in the formation of adherens junctions[41]. They are expressed within the stem cell niche and are involved in regulating selfrenewal, proliferation, differentiation[42–44] and even ECM production[45]. Their modulation can impair differentiation[46], but conversely, when stem cells are patterned to control the number of cell-cell contacts, some differentiation markers are dependent on the size of MSC clusters, e.g., calcium deposition, whereas others are not, e.g., transcription factor localization[47]. By modulating cell number or cadherin expression, one can modulate PSC contractility, which can impact Rho-ROCK-myosin II activation and PSC differentiation[48]. A variety of downstream signals, such as mitogen-activated protein kinase and extracellular signal-regulated kinase 1/2, modulate stem cell proliferation and fate decisions[45] similar to integrins, suggesting signal convergence further downstream. Together, these studies of both cell-matrix and cell-cell adhesion complexes highlight their central role in modulating stem cell function and their regulation by material mechanics.

2.3.2 Ion Channels—Similar to adhesion molecules, mechanically gated ion channels can also confer mechanosensitivity to a cell, thus allowing cells to sense the stiffness of their environment[49]. While there are few mechanically gated channels, they include well-studied

ones such as the Piezo or transient receptor potential families of ion channels. Stiffness-mediated Piezo1 activation plays a role in the depletion of oligodendrocyte progenitor cells, where enhanced Piezo1 activity on stiff or aged surfaces reduced cell proliferation and differentiation[50]. Moreover, stiffer substrates or fluid shear stress enhance Piezo1 activity resulting in osteoblast differentiation[51]. While Piezo activity, traction forces, and differentiation are correlated with stiffer substrates[52], causal mechanisms for differentiation have only recently been suggested, including influencing cholesterol biosynthesis[53]. Cholesterol stiffens cell membranes and the reliance of Piezo1 on membrane tension, a potential feedback mechanism between Piezo1 and cholesterol, could be responsible for changes in channel activity[53–55]. Alternatively, Piezo1 stiffness sensing has been suggested gating mechanisms; when inhibited, embryonic stem cells (ESCs) fail to proliferate and differentiate[56]. Within neural progenitor cells, Piezo1 activity is also necessary for nuclear localization of YAP, a mechanosensitive transcriptional coactivator in Hippo signaling that regulates stem cell differentiation[52,57].

In contrast to material stiffness, the effects of viscoelasticity in the regulation of Piezo1 are relatively unknown and provide a potentially exciting avenue for future research. Like integrins, materials with shorter relaxation times were observed to enhance the expression of Piezo1 when compared to materials with longer stress relaxation times[58]. This again suggests the potential for channel inactivation in the presence of mechanical stimuli below gating thresholds in materials with longer stress relaxation times. While expression and activity of the channel was not explicitly evaluated, it is plausible that prolonged channel inactivation could be responsible for reduced expression, as was also observed through pharmacological inhibition of the channel. Interestingly, Piezo1 and β_1 integrin expression were found to be coregulated, suggesting potential crosstalk between the two molecules, which has also been observed in different cell types[58]. Despite these recent observations, the full extent of Piezo1 sensing of viscoelasticity and the crosstalk between multiple mechanosensitive molecules remain unclear. Further understanding of Piezo1-dependent signaling pathways is another piece of the mechanosensing puzzle that will further our understanding in how mechanical cues influence stem cell function. While the focus of this section has been on Piezo channels, it also important to note that other mechanosensitive ion channels have also been implicated in mechanotransduction, such as TRPV4 in mesenchymal stem cell sensing of hydrogel viscoelasticity[59].

2.3.3 Intracellular and Other Non-Canonical Modes of Mechanotransduction

—In additional to cell surface receptors, a growing body of evidence suggests that intracellular components, e.g., the cytoskeleton and nucleus, are pivotal in sensing and transducing of mechanical cues. The cytoskeleton is connected by LINC complexes, Lamin A/C, and nuclear envelope proteins to enable direct force transmission from the niche or cytoplasm to the nucleus[60]. Modulation of these components, e.g., changes in Lamin A/C induced by substate stiffness, influences mesenchymal stem cell differentiation. Lamin A/C can also act as a rheostat where its expression can oppose signals from the matrix; overexpression or knockout can result in a phenotype resembling cells cultured on stiff and soft surfaces, respectively, even when the substrate is of opposite stiffness[61]. Lamin A/C also acts as a buffer, reducing tensile forces on chromatin to protect it from damage that might

prevent differentiation; indeed, iPSCs on stiff substrates stabilize their Lamin networks by reducing turnover[62], perhaps for this very purpose. For MSCs, stiff substrates, which are more osteogenic than adipogenic, also cause them to increase nuclear envelope protein expression, which are distributed in a dome-like pattern around the nucleus[61]. Transcription factor localization also follows suite, i.e., YAP and myocardin related transcription factor A (MRTFA) which are both involved in osteogenesis in MSCs, become nuclear localized on stiffer substrates[63]. Once forces are transmitted to chromatin, it undergoes significant rearrangement, which can activate transcriptionally repressed DNA regions in differentiating stem cells. Classically, PSCs are known to exhibit softer and more compliant nuclei than their differentiated counterparts[64], and as a consequence of differentiating, they acquire expression of Lamins A/C[65]. Conversely, loss of Lamins can result in reduced nuclear viscosity, detrimental rearrangements of lamina-associated domains, and self-renewal[66]. However, in committed cells, normal force transmission can increase heterochromatin content by removing acetylation marks on histone tails[67]. These changes appear to set preferred positions for chromatin-chromatin interactions[68] and lamina associated domain location, which are cell type specific, and to establish a mechanotransductive link from stiff, differentiation-inducing matrix to chromatin.

While stiffness is clearly important, its effects can be additive with other mechanical stimuli. For example, nanogroove topographies reduced Lamin A/C expression and enhanced adipogenesis in a soft niche[69]. In contrast, cells cultured on stiff, convex surfaces had increased expression and osteogenesis[70]. Cell spreading and morphology also interplay with stiffness; for example, embryonic stem cells cultured on triangular-shaped micropatterned islands had reduced Lamin A/C expression which resulted in suppressed F-actin levels and softer nuclei, suggesting mechanical interplay between Lamins, cytoskeletal components, and nuclear mechanics as well as highlighting the ability of mechanical cues to influence cellular forces[71]. Additionally, recent observations found that physical properties of the cell dynamically change in response to multiple extracellular cues. For example, matrix stiffness was shown to modulate stem cell self-renewal through enhancing the phase separation of TAZ and NANOG, two key transcriptional molecules. More specifically, TAZ, a transcriptional coactivator, was found to form phase separated droplets with NANOG, this in turn is known to concentrate signaling molecules[72,73], thus promoting the ability of NANOG to transcribe various pluripotency genes[74]. This increase in cytoplasmic concentration of molecules results in intracellular molecular crowding which has also been shown to influence additional molecular pathways. Similar to the effects of stiffness, volumetric compression of a cell through multiple extracellular mechanical cues, including mechanical forces, matrix rigidity, and osmotic pressure, was found to stabilize the LRP6 signalosome and activate Wnt/β-catenin signaling pathways by regulating intracellular molecular crowding[75]. Activation of this pathway was observed to promote stem cell self-renewal. Therefore, while often discussed independently, it is important to note that combinatorial effects of multiple cell extrinsic and intrinsic mechanical stimuli often work synergistically to regulate cell function. Overall, the molecules and pathways discussed in this section are by no means comprehensive, but instead provide a review of recent studies and novel pathways in niche material mechanics and stem cell mechanotransduction. We will next extend this discussion to evaluate the role of matrix cues in the modulation of

stem cell self-renewal and differentiation, focusing on the more *in vitro* based PSCs, tissue specific stem cells, as well as the more broadly defined cancer stem cells.

3. Stem Cell Paradox: Gelatin Coated Tissue Culture Polystyrene vs. Soft Animal Blastocysts

Critical features that define a stem cell are its ability to symmetrically (and asymmetrically) self-renew and its ability to exit the cell cycle and commit to a particular cell lineage. However, the term “stem cell” encompasses a broad number of vastly different biological systems, for the purposes of this review we will discuss three distinct categories: pluripotent stem cells (PSCs), tissue stem cells, and cancer stem cells (CSCs). While there is growing evidence that insoluble cues are important in the modulation of biological processes and common mechanotransduction pathways have been identified, it is important to note that each category is distinct with variable physiological outcomes. Here, we discuss the role of material mechanics and ECM in regulating the self-renewal as well as the differentiation potential of PSCs and tissue stem cells. We will reserve the discussion of CSCs for the following section.

3.1 PSCs

The most common *in vitro* cell culture system remains tissue culture treated plastic, sometimes coated with an extracellular matrix protein such as Matrigel or gelatin, which provides a vastly different mechanical environment to the early embryo inner cell mass and can influence cell self-renewal. These *in vitro* systems are easy to use and widely available, but it is rigid and can adsorb serum or cell-secreted proteins in a non-specific manner[76]. In particular, polystyrene has stiffness values within the Gigapascal range (10^{12} Pa), whereas niche environments, such as those present within animal blastocysts are known to have stiffness values <100 Pa[7,8]. As a result, there is little control and physiological relevance of the physical and biochemical cues presented within this culture system. Moreover, natural tissues display viscoelastic properties which are also not present in rigid polystyrene[40]. Finally, tissues are three-dimensional, which create different physical interactions between cells or with ECM when compared to standard two-dimensional polystyrene substrates. These apparent mechanical disparities between polystyrene and tissues emphasize the need for further studies in stem cell mechanobiology; over the last 15 years, substrate stiffness has become well appreciated as a mechanical cue involved in modulating the function of PSCs which were primarily cultured on polystyrene surfaces. For example in a soft niche, mouse PSCs and neural progenitor cells may display greater capacity for self-renewal[77,78]; consistent with this idea, others report that slightly stiffer matrices may push similar cells towards neurogenesis[79]. While these studies typically use polyacrylamide or other polymer hydrogels, similar behaviors have been reported on other compliant materials. PSCs cultured on silicone rubbers have also been implicated to self-renew at specific compliance, albeit at values significantly higher than for hydrogels[80]. Contradictory findings or differences in when self-renewal occurs between material systems may suggest that the overall heterogeneity of the microenvironment as well as the species or cell source specific factors could play a role in cell response to stiffness.

Material mechanics and ECM are also known to influence PSC differentiation. Substrate stiffness, for example, was shown to enhance endothelial differentiation of PSCs when cultured on silicone-based rubbers in conjunction with biochemical stimuli[81]. Moreover, nanopillars and biochemical stimuli, were observed to promote neuronal differentiation in PSCs[82]. Both studies identified the mechanosensitive transcriptional co-activator YAP as a key molecule involved in each mechanotransduction pathway. While our discussion has focused primarily on the importance of material mechanics, it is also important to note that combinations of biophysical and biochemical cues can have synergistic effects to modulate cell function and activate a variety of mechanosensitive pathways. Signaling that underpins such stiffness-mediated observations include regulating the formation of focal adhesion complexes, the generation of traction forces, the modulation of the cytoskeleton and nucleoskeleton, and the localization of mechanosensitive molecules[39,61,83,84]. Stiffness also combines with other material cues, such as ligand composition and concentration, to regulate the switch from self-renewal to differentiation; some ligands and their integrin counterparts support stiffness-mediated lineage commitment, e.g., Collagen type I and Fibronectin[30]. While ECM stiffness is generally accepted as a material cue, more recent observations have also identified the importance of tissue viscoelasticity in the modulation of stem cell function.

Tissues also display viscoelastic behaviors which has been shown to modulate stem cell fate independent of tissue stiffness[40]. Viscoelastic alginate hydrogels with tunable stress relaxation, stiffness, and adhesion ligand density were used to evaluate the role of material mechanics in PSC maintenance and morphogenesis in a 3D culture system. Higher RGD density and fast stress relaxation enhanced PSC viability, proliferation, and lumen formation, which was regulated by actomyosin contractility and YAP nuclear translocation. In contrast, slow stress relaxation at reduced RGD densities resulted in cellular apoptosis. Interestingly, PSCs maintained pluripotency when cultured on alginate hydrogels for a longer period when compared to cells cultured on basement membrane matrices[85]. The studies mentioned thus far have evaluated the role of material mechanics *in vitro*; however, the overall conclusions made are also applicable *in vivo* as mechanical cues and biochemical factors can both synergistically influence pluripotency and differentiation. Therefore, material mechanics should be considered as an additional tool in PSC culture.

3.2 Tissue Stem Cells

Similar to PSCs, tissue derived stem cells are also commonly cultured on stiff tissue culture plastic *in vitro*; despite distinct mechanical and biochemical features prevalent in their niche environment capable of modulating cell self-renewal. As noted previously, similar optimal mechanical and biochemical conditions may lead to self-renewal in lineage committed stem cells, e.g., satellite cells[86,87]. These authors found that self-renewal was observed *in vitro* without loss of potency, i.e., the cells could still engraft into muscle in a mouse model, when substrates matched their *in vivo* niche. However, not only does matrix change significantly between tissues[7,88,89], but it also changes substantially within them as well[8]. This can create spatial patterns where cells may self-renew in some regions and differentiate in others. Recently, the advent of 3D-bioprinting has allowed for greater spatial control of cellular or material deposition and modification[90,91]. This, in turn, allows for a

more biomimetic environment; however, restrictions in the materials capable of use with this technology currently limit the range of material, biochemical, and mechanical cues used within a cell culture system. Further advances will improve the applicability of 3D-bioprinting in the future. Moreover, combinations with other cues, e.g., ligand composition and concentration[30], dimensionality[92], or temporal stiffness gradients[93,94] can also play an equally important and modulatory role in deciding between self-renewal or differentiation. When explored in high throughput systems[95,96], it becomes easier to develop systems-level insight into how these cues regulate self-renewal. Of the many tools used to study these pathways, photo-labile hydrogels that crosslink and stiffen[93,94] or degrade and soften[97] have been a critical tool. When matrix was softened, MSCs and ESCs prolonged their stem cell marker expression via cytoskeletal disruption and decreased cell spreading[77,97], as shown in Figure 4. Variations may not be limited to tissue-level changes but what is clear from these data is that they further suggest that rigid polystyrene[10,40] – despite its popularity may not be a suitable substrate, even when coated with a thin layer of adhesive protein.

Material and ECM mechanics are also known to influence tissue stem cell differentiation. For example, MSCs cultured on soft substrates exhibit transcriptional programs and morphology consistent with lineage commitment of a tissue of corresponding stiffness; cells grown on firm substrates that mimic the muscle developed spindle morphologies and expressed myogenic programs whereas cells cultured on rigid substrates, which mimic collagenous bone, were observed to have greater osteogenic potential and morphology[5]. Beyond combinations of biophysical cues, it is important to consider that matrix is dynamic and regional. Stiffness gradients enable the comprehensive evaluation of mechanics-mediated stem cell differentiation and single cell or collective durotaxis[63,98], i.e., stiffness-directed migration. Stiffness gradients not only validate tissue stiffness-specific trilineage differentiation, but they can also screen mechanosensitive molecules including YAP/TAZ, MRTFA, and Lamin A using adipose-derived stem cells[63]. Conversely temporal changes in stiffness can better differentiate progenitor cells into mature cell types, e.g., cardiomyocytes[93,94]. Our discussion to date has assumed that the hydrogel was always continuous. While most studies use continuous materials, discontinuous materials allow one to measure physical responses in situ. For example, soft (long) or stiff (short) posts can be created with hydrogels, and post deformations can provide a direct readout of force, stem cell differentiation on these substrates scale similarly to continuous materials. Interestingly asymmetric posts can direct stem cell migration or other mechanical responses similar to spatial patterns[99]. While stiffness, its associated matrix properties, and how those change in time and space are all important, it is equally important to consider alternative approaches to how matrix can regulate stem cells.

An alternative to the prevailing thinking that stem cells undergo contractility-based differentiation by substrate deformation was that the nature of protein attachment, e.g., covalent bonding of ECM proteins on the hydrogel surface, modulated differentiation independent of underlying stiffness[100]. The varying degree of collagen tethering was based on the inverse relationship between stiffness and the pore size of the polyacrylamide hydrogel, whereby stiffer gels have smaller pores. However, contradictory findings, which utilize polyacrylamide gels with varying pore sizes while possessing very similar stiffness values, maintain stem cell differentiation is controlled in part by stiffness rather than ligand

tethering[101]. For example, when short adhesive peptides, e.g., RGD, were directly attached to polyacrylamide, stem cell differentiation could still be modulated by stiffness[101,102]. Even when native protein was used, tethered protein unfolded similarly across substrates of different stiffness by atomic force microscopy, and when cells unfolded proteins, unfolding occurred similarly across stiffness values despite differences in stem cell differentiation[101]. Changes in adhesive ligand spacing[103] and patterning[104], also still show stiffness-dependent differentiation of MSCs, suggesting a growing consensus that stiffness among other physical cues modulate lineage specification.

While variations in elastic properties may provide instructive cues to cells, viscoelasticity may be equally critical to modulating self-renewal as well as cell spreading, proliferation, and differentiation[10,40,105]. Stress relaxation – a characteristic of viscoelastic materials – influences cell function, e.g., enhancing matrix production and remodeling, cell migration, and division. In addition, fast stress relaxation mechanically confined cells providing additional barriers for cell division[106]. Alginate-based materials with constant stiffness and fast stress relaxation enhance integrin expression, and genes associated with self-renewal potential in MSCs, as illustrated in Figure 4. This depends on enhanced activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway[18]. In contrast, slow stress relaxation materials caused MSCs to enter a reversible quiescent state and weakened PI3K/Akt pathways activation[18]. However, multiple pathways were activated simultaneously in this response, hinting at the need for further assessment of stress relaxation and self-renewal. Nevertheless, these results highlight the importance of stress relaxation and viscoelasticity when considering stem cell fate. Moreover, encapsulation of adipose-derived stem cells in GelMA resulted in differential cell spreading suggesting volume expansion/adaptation as a key regulator of cell mechanotransduction and differentiation[22,23]. Therefore, material mechanics in a three-dimensional environment should be considered. While several studies were used to highlight the importance of mechanical stimuli in the modulation of stem cell function, it is important to note that biophysical cues are only one component of the microenvironment and can work synergistically with biochemical cues.

Recent studies emphasize the importance of material mechanics in the modulation of tissue stem cell and PSC fate; however, one key question remains: Are material mechanics as an intrinsic cell cue more instructive compared to biochemical cues? To answer this question, it is important to note that biophysical or biochemical cues are often studied individually, which has shown that they are both capable of modulating stem cell function and promoting differentiation; although the resulting cells are less mature compared to adult cells. More recently, studies have started to use combinatorial approaches whereby the effects of multiple biophysical and/or biochemical cues are studied together[96]. The synergistic effects of multiple stimuli suggest that both cues may well be just as important in regulating cell function. Combinatorial approaches may, therefore, provide the most biomimetic strategies to regulate stem cell function.

4. Cancer Stem Cells: Does the Matrix Promote Metastasis, Survival, or Proliferation?

While PSCs clearly self-renew, another biological system undergoes similar self-renewal to maintain and grow their niche, i.e., cancer stem cells (CSCs) or “tumor initiating cells.” CSCs are cancer cells that can self-renew, initiate clonal tumors, exhibit the potential to drive clonal repopulation, and are thought to reside in the niche. While CSCs have been studied predominantly on the soluble factors and surfaces that maintain and identify them[107,108], a wealth of data now shows that other important physical properties might serve as cancer hallmarks. For example, ECM stiffness changes accompany cancer progression, and manual palpation often reveals the presence of “stiff” masses in soft tissues of the skin, breast, etc. This has been confirmed via breast tissue stiffness measurements of normal (e.g., ~100 Pa) and malignant tissue (>5000 Pa)[109] and is the result of enhanced matrix expression[110] and crosslinking[111]. CSC maintenance is selected for on soft matrices[112] where they form embryonic stem cell like colonies that can initiate tumors in mice[113]. CSC growth and differentiation occur when the niche is stiffer than normal, activating the YAP transcriptional regulator[114] and inducing epithelial-to-mesenchymal transition (EMT) through the twist related protein transcription factor[83,115]. Activation occurs through a series of complex pathways that involve β1 integrin binding and clustering, Rho-ROCK activation and actomyosin contractility, focal adhesion kinase activation, extracellular signal-regulated kinase activation, and broad changes in gene expression[116,117]. These data, however, were obtained via a pseudo-three-dimensional culture system using flexible substrates and thick reconstituted basement membrane matrix overlays. In three-dimensional models that mirror ductal tracts, increased stiffness still promotes CSC growth and differentiation but is independent of YAP and dependent on signal transducer and activator of transcription 3 and specificity protein 1 transcription factor activity[118,119]. The systems are not often dynamic but using photo-labile systems again where crosslinking can increase with time, stiffness appears to drive EMT via multiple signaling pathways[25,120]. Redundancy in dynamic ECM may act as a therapeutic resistance mechanism, but regardless, these data provide strong evidence of the key role of increased stiffness in breast cancer progression as early as the pre-invasive stages where CSCs can transition into a metastatic state[121], as shown in Figure 4. Beyond breast cancer, increased stiffening and fibrosis are also associated with pancreatic ductal adenocarcinoma and glioblastoma cell plasticity. In pancreatic ductal adenocarcinoma, CSC plasticity may require the loss of transforming growth factor-β signaling, enhanced β1 integrin signaling, and signal transducer and activator of transcription 3 activation, which together drive fibrosis, stiffness, and cancer progression[122]. For glioblastoma, prognosis may correlate with niche stiffness, and recurring isocitrate dehydrogenase 1-mutant gliomas had a stiffer, tenascin C-enriched matrix[123]. Moreover, LOX-mediated stiffening can lead to proliferation, invasion, and metastasis in a mouse model[111]. Increased stiffness is also implicated in CSC plasticity for lung cancer[124], hepatocellular carcinoma[125], and squamous cell carcinoma[126,127]. However it should be noted that each tumor type appears to have its own set-point, thus “optimal” stiffness should vary between each cancer cell type[128], e.g., mechanical set-points for breast cancer is lower than lung, which is lower than prostate and may define a particular “mechanotype” for self-renewal vs. EMT[129].

As indicated above, increased stiffness in many contexts drives malignant signaling and promotes increased growth and invasion of the CSCs into adjacent stroma. Yet, from a mechanical perspective, increased stiffness would be expected to provide substantially increased mechanical resistance to the physical process of cell migration itself. It is possible that the activation of the invasive signaling pathways provided by increased stiffness puts the cell in a state where it can overcome this increased barrier for invasion. However, another possibility lies in another seemingly contradictory finding: the tumor microenvironment in malignant tumors is not only stiffer but also more viscous than benign tumors in some cancers depending on length scale, such as breast cancer[130,131]. Enhanced matrix viscosity, sometimes associated with increased matrix mechanical plasticity, can facilitate CSC migration and not maintenance, as seen in Figure 4, while increased stiffness alone can diminish migration and enhance maintenance of CSCs[132–134]. Alternatively, ECM structure in the tumor microenvironment is often altered, and fibrillar collagen tracks leading out of the tumor can provide a path for tumor cells to leave the tumor microenvironment[135]. However, many other niche parameters, such as hypoxia[136], or even the presence of oncogenes[137,138] may modulate mechanosensing and the set-point where CSC maintenance occurs. Another paradox is that while tumors are stiffer than normal tissue, mechanical measurements have largely indicated that CSCs are softer than normal cells[139,140]. This suggests that the surrounding ECM and the tumor microenvironment plays an outsize role in the stiffening of the entire tumor, and increased softness might allow the tumor cells to unjam within the tumor and then adopt different migration modes to squeeze through matrix more easily during invasion and migration[140,141]. These seeming contradictions call for a more detailed study of the biophysics of cancer cell invasion and migration. As mentioned above, material mechanics also plays a significant role *in vivo*, we will next extend this discussion beyond cancer to explore the role of material stimuli in various *in vivo* contexts.

5. Impact of Material Cues *In Vivo*

Material cues are not only critical in guiding cell differentiation and function *in vitro*, but they also play important roles in maintaining tissue homeostasis and in the development and progression of disease *in vivo*. For example, during development in *Xenopus laevis*, mesoderm stiffening promotes epithelial-to-mesenchymal transition in neural crest cells, which triggers their collective cell migration, a key step throughout embryogenesis[142]. Similarly, *in vitro* collective cell migration has been observed in durotactic gradients with asymmetrical migration towards stiffer substrates[98] as seen with these neural crest cells. Underlying these migration patterns *in vitro* and *in vivo* appear to be long-range collective sensing, perhaps brought on in this example by mesoderm convergent extension. More generally speaking, changes in the viscoelastic properties of embryonic tissue throughout development are known to modulate cellular organization, collective motion, and shape changes[143–145]. Given that embryonic tissues exhibit a mixture of solid and fluid like behaviors, various forms of deformations and shape changes are prevalent[146]. Moreover, during embryonic morphogenesis, maturation of the heart not only involves mechanical contraction, but also tissue stiffness. Coordinated heartbeats arise in response to progressive increases in tissue stiffness, which is also thought to occur prior to electromechanical coupling of the cells, thus further emphasizing the importance of material cues in heart

formation[147,148]. In addition, during development to birth, the cardiac matrix undergoes 10-fold stiffening[148].

Material cues also play important roles in the development and progression of many diseases and therapeutic interventions. Following injury, stem cells circulate away from their niche and are recruited to the site of injury where they aid in tissue repair. However, due to the resulting inflammatory response and overproduction of extracellular matrix proteins, the native tissue is replaced with less compliant and stiffer scar tissues that pose a non-inducible microenvironment and limit the regenerative capacity for the migrated stem cells[149,150]. Biomaterial-based tissue engineering approaches have attempted to reproduce the microenvironment for improved regeneration and repair[150]. However, the stiffness or biochemical characteristics of implanted materials also pose many challenges and can trigger undesirable immune responses resulting in tissue damage, further exacerbating scar tissue formation, and fibrosis. In addition to wound healing, maladaptive myocardial remodeling, a characteristic of heart failure and often characterized by increased deposition of collagen and fibronectin, also results in tissue stiffening which, in turn, enhances immune cell infiltration, inflammation, and cellular damage, ultimately resulting in myocardial fibrosis[147,151,152]. Aging provides another example where stiffening of the extracellular matrix *in vivo* modulates pathophysiology. Age-associated increased stiffness has been attributed to collagen synthesis which is shown to increase from 3.9 ± 0.8% in 20–25 year old individuals to 5.9 ± 0.8% of the total collagen content in 67–87 year old individuals[153]. Given that collagen I has high tensile strength, its increase with age could affect cardiac mechanics. Moreover, enhanced collagen synthesis combined with increased crosslinking promotes stiffer tissues[153,154]. Similarly, fibronectin expression is also shown to increase with age, and this may also contribute to stiffening of tissues[155]. While these cells are significantly more differentiated and older than their stem cell counterparts, they perhaps provide a guide for how *in vivo* changes correlate to those observed both during development and in vitro using biomaterials, e.g., matrix changes stiffening the niche and inducing cell plasticity. Further understanding the importance and implication of material cues *in vivo* could provide novel therapeutic targets and material strategies to improve tissue repair and regeneration. Several studies have utilized *in vitro* systems to identify material characteristics as well as molecular mechanisms responsible for stiffness-mediated wound healing, fibrosis, cancer, and cardiovascular disease[25,156–158]. Further efforts will help uncover novel molecular targets or material strategies to improve healing outcomes.

6. Conclusions/Next Steps in the Field

Advances in biomaterial science have allowed for unprecedented insight into the role of material mechanics in the modulation of stem cell differentiation and self-renewal. These natural and/or synthetic materials vary in their chemical composition and mechanical properties allowing for broad characterization of cellular responses to a wide variety of material cues. For example, material cues present within the stem cell niche are known to influence differentiation and self-renewal, which further highlight their importance in cell biology. In particular, it is well appreciated that substrate stiffness can modulate the differentiation of stem cells. While not as extensively studied when compared to stiffness, tissue viscosity is also emerging as additional material cue, independent of stiffness,

that can influence stem cell self-renewal and differentiation. Future studies elucidating viscosity-mediated molecular mechanisms will provide additional insight into the ability of material mechanics to influence cell function. In this review, we focused primarily on material cues; however, it is important to note that biochemical cues are also commonly present within the niche and have synergistic effects with biophysical cues to regulate stem cell function. Therefore, combinatorial material strategies, which incorporate multiple physical and biochemical cue and as a result can represent the most physiologically relevant microenvironment, would enhance our current understanding of the role of material mechanics in the modulation of cell function. These more complex systems provide additional challenges in fabrication and molecular analyses.

Future material science strategies can focus on better recapitulating the biophysical and biochemical cues present within the niche environment. Most studies discussed in this review utilized two-dimensional material systems; however, cellular responses are known to differ in two- and three-dimensional culture systems. Three-dimensional material systems provide a more physiological environment whereby cells can better interact with and actively remodel their matrix resulting in additional biophysical cues involving the regulation of cell volume. Combinations of extrinsic and intrinsic mechanical cues, such as compression or stretch, also influence cell volume which has been shown to promote cytoplasmic condensation and transcriptional regulation[72,74]. Matrix remodeling results in the release of biochemical stimuli, such as matrix bound growth factors and cytokines, that can synergistically modulate cell function[11]. Moreover, unlike two-dimensional cell culture systems, cellular and material spatial organization is more restricted in three-dimensional environments. The advent of 3D-bioprinting looks to bridge this limitation[90,91]; however, limited materials capable of use with this technology restrict the range of material, biochemical, and mechanical cues used within a cell culture system. The development and use of more advanced materials with 3D-bioprinting technologies provide an exciting avenue of research in both material science and cell mechanobiology. Creating high throughput combinatorial material strategies will allow for pharmacological and improved mechanistic studies. Nevertheless, material mechanics is becoming more widely accepted as an intrinsic cell cue that can modulate stem cell function; future advances will provide a superior biomimetic microenvironment that will provide additional insight into the molecular mechanisms involved in stem cell mechanosensing within the niche environment.

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Inclusion and Diversity

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science and received support from a program designed to increase minority representation in science.

References

1. Jones DL, and Wagers AJ (2008). No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol 9, 11–21. 10.1038/nrm2319. [PubMed: 18097443]
2. Trapnell C (2015). Defining cell types and states with single-cell genomics. Genome Res 25, 1491–1498. 10.1101/gr.190595.115. [PubMed: 26430159]
3. Waddington CH (1957). The Strategy of the Genes. A Discussion of Some Aspects of Theoretical Biology, 1st Edition (George Allen & Unwin.).
4. Petzold J, and Gentleman E (2021). Intrinsic Mechanical Cues and Their Impact on Stem Cells and Embryogenesis. Front Cell Dev Biol 9, 761871. 10.3389/fcell.2021.761871. [PubMed: 34820380]
5. Engler AJ, Sen S, Sweeney HL, and Discher DE (2006). Matrix elasticity directs stem cell lineage specification. Cell 126, 677–689. 10.1016/j.cell.2006.06.044. [PubMed: 16923388]
6. Vazin T, and Schaffer DV (2010). Engineering strategies to emulate the stem cell niche. Trends Biotechnol 28, 117–124. 10.1016/j.tibtech.2009.11.008. [PubMed: 20042248]
7. Discher DE, Mooney DJ, and Zandstra PW (2009). Growth factors, matrices, and forces combine and control stem cells. Science 324, 1673–1677. 10.1126/science.1171643. [PubMed: 19556500]
8. Handorf AM, Zhou Y, Halanski MA, and Li WJ (2015). Tissue stiffness dictates development, homeostasis, and disease progression. Organogenesis 11, 1–15. 10.1080/15476278.2015.1019687. [PubMed: 25915734]
9. Wu PH, Aroush DR, Asnacios A, Chen WC, Dokukin ME, Doss BL, Durand-Smet P, Ekpenyong A, Guck J, Guz NV, et al. (2018). A comparison of methods to assess cell mechanical properties. Nat Methods 15, 491–498. 10.1038/s41592-018-0015-1. [PubMed: 29915189]
10. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, and Shenoy VB (2020). Effects of extracellular matrix viscoelasticity on cellular behaviour. Nature 584, 535–546. 10.1038/s41586-020-2612-2. [PubMed: 32848221]
11. Girigoswami K, Saini D, and Girigoswami A (2021). Extracellular Matrix Remodeling and Development of Cancer. Stem Cell Rev Rep 17, 739–747. 10.1007/s12015-020-10070-1. [PubMed: 33128168]
12. Hiew VV, Simat SFB, and Teoh PL (2018). The Advancement of Biomaterials in Regulating Stem Cell Fate. Stem Cell Rev Rep 14, 43–57. 10.1007/s12015-017-9764-y. [PubMed: 28884292]
13. Xu Y, Chen C, Hellwarth PB, and Bao X (2019). Biomaterials for stem cell engineering and biomanufacturing. Bioact Mater 4, 366–379. 10.1016/j.bioactmat.2019.11.002. [PubMed: 31872161]
14. Tan Y, Huang H, Ayers DC, and Song J (2018). Modulating Viscoelasticity, Stiffness, and Degradation of Synthetic Cellular Niches via Stoichiometric Tuning of Covalent versus Dynamic Noncovalent Cross-Linking. ACS Cent Sci 4, 971–981. 10.1021/acscentsci.8b00170. [PubMed: 30159394]
15. Brovold M, Almeida JI, Pla-Palacin I, Sainz-Arnal P, Sanchez-Romero N, Rivas JJ, Almeida H, Dachary PR, Serrano-Aullo T, Soker S, and Baptista PM (2018). Naturally-Derived Biomaterials for Tissue Engineering Applications. Adv Exp Med Biol 1077, 421–449. 10.1007/978-981-13-0947-2_23. [PubMed: 30357702]
16. Elosegui-Artola A (2021). The extracellular matrix viscoelasticity as a regulator of cell and tissue dynamics. Curr Opin Cell Biol 72, 10–18. 10.1016/j.ceb.2021.04.002. [PubMed: 33993058]
17. Freyria AM, Ronziere MC, Cortial D, Galois L, Hartmann D, Herbage D, and Mallein-Gerin F (2009). Comparative phenotypic analysis of articular chondrocytes cultured within type I or type II collagen scaffolds. Tissue Eng Part A 15, 1233–1245. 10.1089/ten.tea.2008.0114. [PubMed: 18950259]
18. Lin C, He Y, Feng Q, Xu K, Chen Z, Tao B, Li X, Xia Z, Jiang H, and Cai K (2021). Self-renewal or quiescence? Orchestrating the fate of mesenchymal stem cells by matrix viscoelasticity via PI3K/Akt-CDK1 pathway. Biomaterials 279, 121235. 10.1016/j.biomaterials.2021.121235. [PubMed: 34749070]
19. Mandal A, Clegg JR, Anselmo AC, and Mitragotri S (2020). Hydrogels in the clinic. Bioeng Transl Med 5, e10158. 10.1002/btm2.10158. [PubMed: 32440563]

20. Levenberg S, Huang NF, Lavik E, Rogers AB, Itskovitz-Eldor J, and Langer R (2003). Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. Proc Natl Acad Sci U S A 100, 12741–12746. 10.1073/pnas.1735463100. [PubMed: 14561891]
21. Nuttelman CR, Tripodi MC, and Anseth KS (2004). In vitro osteogenic differentiation of human mesenchymal stem cells photoencapsulated in PEG hydrogels. J Biomed Mater Res A 68, 773–782. 10.1002/jbm.a.20112. [PubMed: 14986332]
22. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, and Khademhosseini A (2015). Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials 73, 254–271. 10.1016/j.biomaterials.2015.08.045. [PubMed: 26414409]
23. Kim C, Young JL, Holle AW, Jeong K, Major LG, Jeong JH, Aman ZM, Han DW, Hwang Y, Spatz JP, and Choi YS (2020). Stem Cell Mechanosensation on Gelatin Methacryloyl (GelMA) Stiffness Gradient Hydrogels. Ann Biomed Eng 48, 893–902. 10.1007/s10439-019-02428-5. [PubMed: 31802282]
24. Kirsch M, Birnstein L, Pepelanova I, Handke W, Rach J, Seltsam A, Scheper T, and Lavrentieva A (2019). Gelatin-Methacryloyl (GelMA) Formulated with Human Platelet Lysate Supports Mesenchymal Stem Cell Proliferation and Differentiation and Enhances the Hydrogel's Mechanical Properties. Bioengineering (Basel) 6. 10.3390/bioengineering6030076.
25. Ondeck MG, Kumar A, Placone JK, Plunkett CM, Matte BF, Wong KC, Fattet L, Yang J, and Engler AJ (2019). Dynamically stiffened matrix promotes malignant transformation of mammary epithelial cells via collective mechanical signaling. Proc Natl Acad Sci U S A 116, 3502–3507. 10.1073/pnas.1814204116. [PubMed: 30755531]
26. Fairbanks BD, Schwartz MP, Bowman CN, and Anseth KS (2009). Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility. Biomaterials 30, 6702–6707. 10.1016/j.biomaterials.2009.08.055. [PubMed: 19783300]
27. Kharbikar BN, Mohindra P, and Desai TA (2022). Biomaterials to enhance stem cell transplantation. Cell Stem Cell 29, 692–721. 10.1016/j.stem.2022.04.002. [PubMed: 35483364]
28. Zhao X, Li Q, Guo Z, and Li Z (2021). Constructing a cell microenvironment with biomaterial scaffolds for stem cell therapy. Stem Cell Res Ther 12, 583. 10.1186/s13287-021-02650-w. [PubMed: 34809719]
29. Jo Y, Hwang SH, and Jang J (2021). Employing Extracellular Matrix-Based Tissue Engineering Strategies for Age-Dependent Tissue Degenerations. Int J Mol Sci 22. 10.3390/ijms22179367.
30. Rowlands AS, George PA, and Cooper-White JJ (2008). Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation. Am J Physiol Cell Physiol 295, C1037–1044. 10.1152/ajpcell.67.2008. [PubMed: 18753317]
31. Wells RG, and Discher DE (2008). Matrix elasticity, cytoskeletal tension, and TGF-beta: the insoluble and soluble meet. Sci Signal 1, pe13. 10.1126/stke.110pe13.
32. Zaidel-Bar R, Itzkovitz S, Ma'ayan A, Iyengar R, and Geiger B (2007). Functional atlas of the integrin adhesome. Nat Cell Biol 9, 858–867. 10.1038/ncb0807-858. [PubMed: 17671451]
33. Zhou P, Feng F, Songa Y, Li J, Li Q, Xu Z, Shi J, Qin L, He F, Li H, et al. (2022). Novel RGD-containing peptides exhibited improved abilities to integrin receptor binding and cultures of human induced pluripotent stem cells. Materials & Design 219, 110762.
34. Du J, Chen X, Liang X, Zhang G, Xu J, He L, Zhan Q, Feng XQ, Chien S, and Yang C (2011). Integrin activation and internalization on soft ECM as a mechanism of induction of stem cell differentiation by ECM elasticity. Proc Natl Acad Sci U S A 108, 9466–9471. 10.1073/pnas.1106467108. [PubMed: 21593411]
35. ZhimoLiu BinfanZhao, Zhang L, Qian S, Mao J, Cheng L, Mao X, Cai Z, Zhang Y, Cui W, and Sun X (2022). Modulated integrin signaling receptors of stem cells via ultra-soft hydrogel for promoting angiogenesis. Composites Part B: Engineering 234, 109747.
36. Yu H, Lui YS, Xiong S, Leong WS, Wen F, Nurkahfianto H, Rana S, Leong DT, Ng KW, and Tan LP (2013). Insights into the role of focal adhesion modulation in myogenic differentiation of human mesenchymal stem cells. Stem Cells Dev 22, 136–147. 10.1089/scd.2012.0160. [PubMed: 22765653]

37. Shih YR, Tseng KF, Lai HY, Lin CH, and Lee OK (2011). Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells. J Bone Miner Res 26, 730–738. 10.1002/jbmr.278. [PubMed: 20939067]
38. Sun M, Chi G, Xu J, Tan Y, Xu J, Lv S, Xu Z, Xia Y, Li L, and Li Y (2018). Extracellular matrix stiffness controls osteogenic differentiation of mesenchymal stem cells mediated by integrin alpha5. Stem Cell Res Ther 9, 52. 10.1186/s13287-018-0798-0. [PubMed: 29490668]
39. Holle AW, Tang X, Vijayraghavan D, Vincent LG, Fuhrmann A, Choi YS, del Alamo JC, and Engler AJ (2013). In situ mechanotransduction via vinculin regulates stem cell differentiation. Stem Cells 31, 2467–2477. 10.1002/stem.1490. [PubMed: 23897765]
40. Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC, Huebsch N, Lee HP, Lippens E, Duda GN, and Mooney DJ (2016). Hydrogels with tunable stress relaxation regulate stem cell fate and activity. Nat Mater 15, 326–334. 10.1038/nmat4489. [PubMed: 26618884]
41. Takeichi M (1995). Morphogenetic roles of classic cadherins. Curr Opin Cell Biol 7, 619–627. 10.1016/0955-0674(95)80102-2. [PubMed: 8573335]
42. Karpowicz P, Willaime-Morawek S, Balenci L, DeVeale B, Inoue T, and van der Kooy D (2009). E-Cadherin regulates neural stem cell self-renewal. J Neurosci 29, 3885–3896. 10.1523/JNEUROSCI.0037-09.2009. [PubMed: 19321785]
43. Chen T, Yuan D, Wei B, Jiang J, Kang J, Ling K, Gu Y, Li J, Xiao L, and Pei G (2010). E-cadherin-mediated cell-cell contact is critical for induced pluripotent stem cell generation. Stem Cells 28, 1315–1325. 10.1002/stem.456. [PubMed: 20521328]
44. Zhao M, Tao F, Venkatraman A, Li Z, Smith SE, Unruh J, Chen S, Ward C, Qian P, Perry JM, et al. (2019). N-Cadherin-Expressing Bone and Marrow Stromal Progenitor Cells Maintain Reserve Hematopoietic Stem Cells. Cell Rep 26, 652–669 e656. 10.1016/j.celrep.2018.12.093. [PubMed: 30650358]
45. Passanha FR, Geuens T, and LaPointe VLS (2022). Cadherin-11 Influences Differentiation in Human Mesenchymal Stem Cells by Regulating the Extracellular Matrix Via the TGFbeta1 Pathway. Stem Cells 40, 669–677. 10.1093/stmcls/sxac026. [PubMed: 35416252]
46. Oh J, Kim CS, Kim M, Jo W, Sung YH, and Park J (2021). Type VI collagen and its cleavage product, endotrophin, cooperatively regulate the adipogenic and lipolytic capacity of adipocytes. Metabolism 114, 154430. 10.1016/j.metabol.2020.154430. [PubMed: 33188786]
47. Mao AS, Shin JW, and Mooney DJ (2016). Effects of substrate stiffness and cell-cell contact on mesenchymal stem cell differentiation. Biomaterials 98, 184–191. 10.1016/j.biomaterials.2016.05.004. [PubMed: 27203745]
48. Toh YC, Xing J, and Yu H (2015). Modulation of integrin and E-cadherin-mediated adhesions to spatially control heterogeneity in human pluripotent stem cell differentiation. Biomaterials 50, 87–97. 10.1016/j.biomaterials.2015.01.019. [PubMed: 25736499]
49. Hamza A, Amit J, Elizabeth LE, Medha MP, Michael DC, and Wendy FL (2021). Ion channel mediated mechanotransduction in immune cells. Curr Opin Solid State Mater Sci 25. 10.1016/j.cossms.2021.100951.
50. Segel M, Neumann B, Hill MFE, Weber IP, Viscomi C, Zhao C, Young A, Agley CC, Thompson AJ, Gonzalez GA, et al. (2019). Niche stiffness underlies the ageing of central nervous system progenitor cells. Nature 573, 130–134. 10.1038/s41586-019-1484-9. [PubMed: 31413369]
51. Zhou T, Gao B, Fan Y, Liu Y, Feng S, Cong Q, Zhang X, Zhou Y, Yadav PS, Lin J, et al. (2020). Piezo1/2 mediate mechanotransduction essential for bone formation through concerted activation of NFAT-YAP1-ss-catenin. Elife 9. 10.7554/eLife.52779.
52. Pathak MM, Nourse JL, Tran T, Hwe J, Arulmoli J, Le DT, Bernardis E, Flanagan LA, and Tombola F (2014). Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. Proc Natl Acad Sci U S A 111, 16148–16153. 10.1073/pnas.1409802111. [PubMed: 25349416]
53. Nourse JL, Leung VM, Abuwarda H, Evans EL, Izquierdo-Ortiz E, Ly AT, Truong N, Smith S, Bhavsar H, Bertaccini G, et al. (2022). Piezo1 regulates cholesterol biosynthesis to influence neural stem cell fate during brain development. J Gen Physiol 154. 10.1085/jgp.202213084.
54. Lewis AH, and Grandl J (2015). Mechanical sensitivity of Piezo1 ion channels can be tuned by cellular membrane tension. Elife 4. 10.7554/eLife.12088.

55. Syeda R, Florendo MN, Cox CD, Kefauver JM, Santos JS, Martinac B, and Patapoutian A (2016). Piezo1 Channels Are Inherently Mechanosensitive. Cell Rep 17, 1739–1746. 10.1016/j.celrep.2016.10.033. [PubMed: 27829145]
56. Ma N, Chen D, Lee JH, Kuri P, Hernandez EB, Kocan J, Mahmood H, Tichy ED, Rompolas P, and Mourkioti F (2022). Piezo1 regulates the regenerative capacity of skeletal muscles via orchestration of stem cell morphological states. Sci Adv 8, eabn0485. 10.1126/sciadv.abn0485.
57. Barry ER, and Camargo FD (2013). The Hippo superhighway: signaling crossroads converging on the Hippo/Yap pathway in stem cells and development. Curr Opin Cell Biol 25, 247–253. 10.1016/j.ceb.2012.12.006. [PubMed: 23312716]
58. Wu Z, Yang Z, Sha D, Ma Y, Kim BYS, Jiang W, Yuan Y, and Liu C (2022). Injectable, viscoelastic hydrogel precisely regulates developmental tissue regeneration. Chemical Engineering Journal 434, 133860. 10.1016/j.cej.2021.133860.
59. Lee HP, Stowers R, and Chaudhuri O (2019). Volume expansion and TRPV4 activation regulate stem cell fate in three-dimensional microenvironments. Nat Commun 10, 529. 10.1038/s41467-019-08465-x. [PubMed: 30705265]
60. Heo SJ, Driscoll TP, Thorpe SD, Nerurkar NL, Baker BM, Yang MT, Chen CS, Lee DA, and Mauck RL (2016). Differentiation alters stem cell nuclear architecture, mechanics, and mechano-sensitivity. Elife 5. 10.7554/eLife.18207.
61. Swift J, Ivanovska IL, Buxboim A, Harada T, Dingal PC, Pinter J, Pajerowski JD, Spinler KR, Shin JW, Tewari M, et al. (2013). Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 341, 1240104. 10.1126/science.1240104. [PubMed: 23990565]
62. Cho S, Vashisth M, Abbas A, Majkut S, Vogel K, Xia Y, Ivanovska IL, Irianto J, Tewari M, Zhu K, et al. (2019). Mechanosensing by the Lamina Protects against Nuclear Rupture, DNA Damage, and Cell-Cycle Arrest. Dev Cell 49, 920–935 e925. 10.1016/j.devcel.2019.04.020. [PubMed: 31105008]
63. Hadden WJ, Young JL, Holle AW, McFetridge ML, Kim DY, Wijesinghe P, Taylor-Weiner H, Wen JH, Lee AR, Bieback K, et al. (2017). Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels. Proc Natl Acad Sci U S A 114, 5647–5652. 10.1073/pnas.1618239114. [PubMed: 28507138]
64. Pajerowski JD, Dahl KN, Zhong FL, Sammak PJ, and Discher DE (2007). Physical plasticity of the nucleus in stem cell differentiation. Proc Natl Acad Sci U S A 104, 15619–15624. 10.1073/pnas.0702576104. [PubMed: 17893336]
65. Constantinescu D, Gray HL, Sammak PJ, Schatten GP, and Csoka AB (2006). Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation. Stem Cells 24, 177–185. 10.1634/stemcells.2004-0159. [PubMed: 16179429]
66. Amar K, Wei F, Chen J, and Wang N (2021). Effects of forces on chromatin. APL Bioeng 5, 041503. 10.1063/5.0065302. [PubMed: 34661040]
67. Damodaran K, Venkatachalapathy S, Alisafaei F, Radhakrishnan AV, Sharma Jokhun D, Shenoy VB, and Shivashankar GV (2018). Compressive force induces reversible chromatin condensation and cell geometry-dependent transcriptional response. Mol Biol Cell 29, 3039–3051. 10.1091/mbc.E18-04-0256. [PubMed: 30256731]
68. Finn EH, and Misteli T (2022). Nuclear position modulates long-range chromatin interactions. PLoS Genet 18, e1010451. 10.1371/journal.pgen.1010451. [PubMed: 36206323]
69. Kulangara K, Yang J, Chellappan M, Yang Y, and Leong KW (2014). Nanotopography alters nuclear protein expression, proliferation and differentiation of human mesenchymal stem/stromal cells. PLoS One 9, e114698. 10.1371/journal.pone.0114698. [PubMed: 25521962]
70. Werner M, Blanquer SB, Haimi SP, Korus G, Dunlop JW, Duda GN, Grijpma DW, and Petersen A (2017). Surface Curvature Differentially Regulates Stem Cell Migration and Differentiation via Altered Attachment Morphology and Nuclear Deformation. Adv Sci (Weinh) 4, 1600347. 10.1002/advs.201600347. [PubMed: 28251054]
71. Talwar S, Jain N, and Shivashankar GV (2014). The regulation of gene expression during onset of differentiation by nuclear mechanical heterogeneity. Biomaterials 35, 2411–2419. 10.1016/j.biomaterials.2013.12.010. [PubMed: 24388387]

72. Boija A, Klein IA, Sabari BR, Dall'Agnese A, Coffey EL, Zamudio AV, Li CH, Shrinivas K, Manteiga JC, Hannett NM, et al. (2018). Transcription Factors Activate Genes through the Phase-Separation Capacity of Their Activation Domains. Cell 175, 1842–1855 e1816. 10.1016/j.cell.2018.10.042. [PubMed: 30449618]
73. Seif E, Kang JJ, Sasseville C, Senkovich O, Kaltashov A, Boulier EL, Kapur I, Kim CA, and Francis NJ (2020). Phase separation by the polyhomeotic sterile alpha motif compartmentalizes Polycomb Group proteins and enhances their activity. Nat Commun 11, 5609. 10.1038/s41467-020-19435-z. [PubMed: 33154383]
74. Liu X, Ye Y, Zhu L, Xiao X, Zhou B, Gu Y, Si H, Liang H, Liu M, Li J, et al. (2023). Niche stiffness sustains cancer stemness via TAZ and NANOG phase separation. Nat Commun 14, 238. 10.1038/s41467-023-35856-y. [PubMed: 36646707]
75. Li Y, Chen M, Hu J, Sheng R, Lin Q, He X, and Guo M (2021). Volumetric Compression Induces Intracellular Crowding to Control Intestinal Organoid Growth via Wnt/beta-Catenin Signaling. Cell Stem Cell 28, 170–172. 10.1016/j.stem.2020.12.003. [PubMed: 33417868]
76. Vining KH, and Mooney DJ (2017). Mechanical forces direct stem cell behaviour in development and regeneration. Nat Rev Mol Cell Biol 18, 728–742. 10.1038/nrm.2017.108. [PubMed: 29115301]
77. Chowdhury F, Li Y, Poh YC, Yokohama-Tamaki T, Wang N, and Tanaka TS (2010). Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions. PLoS One 5, e15655. 10.1371/journal.pone.0015655. [PubMed: 21179449]
78. Leipzig ND, and Shoichet MS (2009). The effect of substrate stiffness on adult neural stem cell behavior. Biomaterials 30, 6867–6878. 10.1016/j.biomaterials.2009.09.002. [PubMed: 19775749]
79. Keung AJ, Asuri P, Kumar S, and Schaffer DV (2012). Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. Integr Biol (Camb) 4, 1049–1058. 10.1039/c2ib20083j. [PubMed: 22854634]
80. Evans ND, Minelli C, Gentleman E, LaPointe V, Patankar SN, Kallivretaki M, Chen X, Roberts CJ, and Stevens MM (2009). Substrate stiffness affects early differentiation events in embryonic stem cells. Eur Cell Mater 18, 1–13; discussion 13–14.10.22203/ecm.v018a01. [PubMed: 19768669]
81. Smith Q, Chan XY, Carmo AM, Trempel M, Saunders M, and Gerecht S (2017). Compliant substratum guides endothelial commitment from human pluripotent stem cells. Sci Adv 3, e1602883. 10.1126/sciadv.1602883. [PubMed: 28580421]
82. Song L, Wang K, Li Y, and Yang Y (2016). Nanotopography promoted neuronal differentiation of human induced pluripotent stem cells. Colloids Surf B Biointerfaces 148, 49–58. 10.1016/j.colsurfb.2016.08.041. [PubMed: 27591570]
83. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, et al. (2011). Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183. 10.1038/nature10137. [PubMed: 21654799]
84. Pelham RJ Jr., and Wang Y (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A 94, 13661–13665. 10.1073/pnas.94.25.13661. [PubMed: 9391082]
85. Indana D, Agarwal P, Bhutani N, and Chaudhuri O (2021). Viscoelasticity and Adhesion Signaling in Biomaterials Control Human Pluripotent Stem Cell Morphogenesis in 3D Culture. Adv Mater 33, e2101966. 10.1002/adma.202101966. [PubMed: 34499389]
86. Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, and Blau HM (2010). Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329, 1078–1081. 10.1126/science.1191035. [PubMed: 20647425]
87. Moyle LA, Cheng RY, Liu H, Davoudi S, Ferreira SA, Nissar AA, Sun Y, Gentleman E, Simmons CA, and Gilbert PM (2020). Three-dimensional niche stiffness synergizes with Wnt7a to modulate the extent of satellite cell symmetric self-renewal divisions. Mol Biol Cell 31, 1703–1713. 10.1091/mbc.E20-01-0078. [PubMed: 32491970]
88. Reilly GC, and Engler AJ (2010). Intrinsic extracellular matrix properties regulate stem cell differentiation. J Biomech 43, 55–62. 10.1016/j.jbiomech.2009.09.009. [PubMed: 19800626]
89. Discher DE, Janmey P, and Wang YL (2005). Tissue cells feel and respond to the stiffness of their substrate. Science 310, 1139–1143. 10.1126/science.1116995. [PubMed: 16293750]

90. Wang LL, Highley CB, Yeh YC, Galarraga JH, Uman S, and Burdick JA (2018). Three-dimensional extrusion bioprinting of single- and double-network hydrogels containing dynamic covalent crosslinks. J Biomed Mater Res A 106, 865–875. 10.1002/jbm.a.36323. [PubMed: 29314616]
91. Brassard JA, Nikolaev M, Hubscher T, Hofer M, and Lutolf MP (2021). Recapitulating macro-scale tissue self-organization through organoid bioprinting. Nat Mater 20, 22–29. 10.1038/s41563-020-00803-5. [PubMed: 32958879]
92. Gerecht S, Burdick JA, Ferreira LS, Townsend SA, Langer R, and Vunjak-Novakovic G (2007). Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc Natl Acad Sci U S A 104, 11298–11303. 10.1073/pnas.0703723104. [PubMed: 17581871]
93. Young JL, and Engler AJ (2011). Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. Biomaterials 32, 1002–1009. 10.1016/j.biomaterials.2010.10.020. [PubMed: 21071078]
94. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordonez-Moran P, Clevers H, and Lutolf MP (2016). Designer matrices for intestinal stem cell and organoid culture. Nature 539, 560–564. 10.1038/nature20168. [PubMed: 27851739]
95. Ranga A, Gobaa S, Okawa Y, Mosiewicz K, Negro A, and Lutolf MP (2014). 3D niche microarrays for systems-level analyses of cell fate. Nat Commun 5, 4324. 10.1038/ncomms5324. [PubMed: 25027775]
96. Rape AD, Zibinsky M, Murthy N, and Kumar S (2015). A synthetic hydrogel for the high-throughput study of cell-ECM interactions. Nat Commun 6, 8129. 10.1038/ncomms9129. [PubMed: 26350361]
97. Yang C, DelRio FW, Ma H, Killaars AR, Basta LP, Kyburz KA, and Anseth KS (2016). Spatially patterned matrix elasticity directs stem cell fate. Proc Natl Acad Sci U S A 113, E4439–4445. 10.1073/pnas.1609731113. [PubMed: 27436901]
98. Sunyer R, Conte V, Escribano J, Elosegui-Artola A, Labernadie A, Valon L, Navajas D, Garcia-Aznar JM, Munoz JJ, Roca-Cusachs P, and Trepat X (2016). Collective cell durotaxis emerges from long-range intercellular force transmission. Science 353, 1157–1161. 10.1126/science.aaf7119. [PubMed: 27609894]
99. Yang MT, Fu J, Wang YK, Desai RA, and Chen CS (2011). Assaying stem cell mechanobiology on microfabricated elastomeric substrates with geometrically modulated rigidity. Nat Protoc 6, 187–213. 10.1038/nprot.2010.189. [PubMed: 21293460]
100. Trappmann B, Gautrot JE, Connelly JT, Strange DG, Li Y, Oyen ML, Cohen Stuart MA, Boehm H, Li B, Vogel V, et al. (2012). Extracellular-matrix tethering regulates stem-cell fate. Nat Mater 11, 642–649. 10.1038/nmat3339. [PubMed: 22635042]
101. Wen JH, Vincent LG, Fuhrmann A, Choi YS, Hribar KC, Taylor-Weiner H, Chen S, and Engler AJ (2014). Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 13, 979–987. 10.1038/nmat4051. [PubMed: 25108614]
102. Wen JH, Choi O, Taylor-Weiner H, Fuhrmann A, Karpiak JV, Almutairi A, and Engler AJ (2015). Haptotaxis is cell type specific and limited by substrate adhesiveness. Cell Mol Bioeng 8, 530–542. 10.1007/s12195-015-0398-3. [PubMed: 26640598]
103. Frith JE, Mills RJ, and Cooper-White JJ (2012). Lateral spacing of adhesion peptides influences human mesenchymal stem cell behaviour. J Cell Sci 125, 317–327. 10.1242/jcs.087916. [PubMed: 22250203]
104. Tee SY, Fu J, Chen CS, and Janmey PA (2011). Cell shape and substrate rigidity both regulate cell stiffness. Biophys J 100, L25–27. 10.1016/j.bpj.2010.12.3744. [PubMed: 21354386]
105. Chaudhuri O, Gu L, Darnell M, Klumpers D, Bencherif SA, Weaver JC, Huebsch N, and Mooney DJ (2015). Substrate stress relaxation regulates cell spreading. Nat. Commun 6, 6364. 10.1038/ncomms7365.
106. Nam S, Gupta VK, Lee HP, Lee JY, Wisdom KM, Varma S, Flaum EM, Davis C, West RB, and Chaudhuri O (2019). Cell cycle progression in confining microenvironments is regulated by a growth-responsive TRPV4-PI3K/Akt-p27(Kip1) signaling axis. Sci Adv 5, eaaw6171. 10.1126/sciadv.aaw6171.

107. Korkaya H, Liu S, and Wicha MS (2011). Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots. Clin Cancer Res 17, 6125–6129. 10.1158/1078-0432.CCR-10-2743. [PubMed: 21685479]
108. Zhao W, Li Y, and Zhang X (2017). Stemness-Related Markers in Cancer. Cancer Transl Med 3, 87–95. 10.4103/ctm.ctm_69_16. [PubMed: 29276782]
109. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, et al. (2005). Tensional homeostasis and the malignant phenotype. Cancer Cell 8, 241–254. 10.1016/j.ccr.2005.08.010. [PubMed: 16169468]
110. Williams CM, Engler AJ, Slone RD, Galante LL, and Schwarzbauer JE (2008). Fibronectin expression modulates mammary epithelial cell proliferation during acinar differentiation. Cancer Res 68, 3185–3192. 10.1158/0008-5472.CAN-07-2673. [PubMed: 18451144]
111. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, et al. (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139, 891–906. 10.1016/j.cell.2009.10.027. [PubMed: 19931152]
112. Chang Y, Zhang J, Huo X, Qu X, Xia C, Huang K, Xie F, Wang N, Wei X, and Jia Q (2022). Substrate rigidity dictates colorectal tumorigenic cell stemness and metastasis via CRAD-dependent mechanotransduction. Cell Rep 38, 110390. 10.1016/j.celrep.2022.110390. [PubMed: 35172140]
113. Tan Y, Tajik A, Chen J, Jia Q, Chowdhury F, Wang L, Chen J, Zhang S, Hong Y, Yi H, et al. (2014). Matrix softness regulates plasticity of tumour-repopulating cells via H3K9 demethylation and Sox2 expression. Nat Commun 5, 4619. 10.1038/ncomms5619. [PubMed: 25099074]
114. Meng Z, Qiu Y, Lin KC, Kumar A, Placone JK, Fang C, Wang KC, Lu S, Pan M, Hong AW, et al. (2018). RAP2 mediates mechanoresponses of the Hippo pathway. Nature 560, 655–660. 10.1038/s41586-018-0444-0. [PubMed: 30135582]
115. Wei SC, Fattet L, Tsai JH, Guo Y, Pai VH, Majeski HE, Chen AC, Sah RL, Taylor SS, Engler AJ, and Yang J (2015). Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. Nat Cell Biol 17, 678–688. 10.1038/ncb3157. [PubMed: 25893917]
116. Sawai H, Okada Y, Funahashi H, Matsuo Y, Takahashi H, Takeyama H, and Manabe T (2005). Activation of focal adhesion kinase enhances the adhesion and invasion of pancreatic cancer cells via extracellular signal-regulated kinase-1/2 signaling pathway activation. Mol Cancer 4, 37. 10.1186/1476-4598-4-37. [PubMed: 16209712]
117. Cooper J, and Giancotti FG (2019). Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance. Cancer Cell 35, 347–367. 10.1016/j.ccell.2019.01.007. [PubMed: 30889378]
118. Lee JY, Chang JK, Dominguez AA, Lee HP, Nam S, Chang J, Varma S, Qi LS, West RB, and Chaudhuri O (2019). YAP-independent mechanotransduction drives breast cancer progression. Nat Commun 10, 1848. 10.1038/s41467-019-09755-0. [PubMed: 31015465]
119. Stowers RS, Shcherbina A, Israeli J, Gruber JJ, Chang J, Nam S, Rabiee A, Teruel MN, Snyder MP, Kundaje A, and Chaudhuri O (2019). Matrix stiffness induces a tumorigenic phenotype in mammary epithelium through changes in chromatin accessibility. Nat Biomed Eng. 10.1038/s41551-019-0420-5.
120. Stowers RS, Allen SC, Sanchez K, Davis CL, Ebelt ND, Van Den Berg C, and Suggs LJ (2017). Extracellular Matrix Stiffening Induces a Malignant Phenotypic Transition in Breast Epithelial Cells. Cell Mol Bioeng 10, 114–123. 10.1007/s12195-016-0468-1. [PubMed: 31719853]
121. Chaudhuri O, Koshy ST, Branco da Cunha C, Shin JW, Verbeke CS, Allison KH, and Mooney DJ (2014). Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium. Nat Mater 13, 970–978. 10.1038/nmat4009. [PubMed: 24930031]
122. Laklai H, Miroshnikova YA, Pickup MW, Collisson EA, Kim GE, Barrett AS, Hill RC, Lakins JN, Schlaepfer DD, Mouw JK, et al. (2016). Genotype tunes pancreatic ductal adenocarcinoma tissue tension to induce matricellular fibrosis and tumor progression. Nat Med 22, 497–505. 10.1038/nm.4082. [PubMed: 27089513]

123. Miroshnikova YA, Mouw JK, Barnes JM, Pickup MW, Lakins JN, Kim Y, Lobo K, Persson AI, Reis GF, McKnight TR, et al. (2016). Tissue mechanics promote IDH1-dependent HIF1alpha-tenascin C feedback to regulate glioblastoma aggression. Nat Cell Biol 18, 1336–1345. 10.1038/ncb3429. [PubMed: 27820599]

124. Peng DH, Ungewiss C, Tong P, Byers LA, Wang J, Canales JR, Villalobos PA, Uraoka N, Mino B, Behrens C, et al. (2017). ZEB1 induces LOXL2-mediated collagen stabilization and deposition in the extracellular matrix to drive lung cancer invasion and metastasis. Oncogene 36, 1925–1938. 10.1038/onc.2016.358. [PubMed: 27694892]

125. Chang TT, Thakar D, and Weaver VM (2017). Force-dependent breaching of the basement membrane. Matrix Biol 57–58, 178–189. 10.1016/j.matbio.2016.12.005. [PubMed: 28025167]

126. Mittapalli VR, Madl J, Loffek S, Kiritsi D, Kern JS, Romer W, Nystrom A, and Bruckner-Tuderman L (2016). Injury-Driven Stiffening of the Dermis Expedites Skin Carcinoma Progression. Cancer Res 76, 940–951. 10.1158/0008-5472.CAN-15-1348. [PubMed: 26676755]

127. Li S, Bai H, Chen X, Gong S, Xiao J, Li D, Li L, Jiang Y, Li T, Qin X, et al. (2020). Soft Substrate Promotes Osteosarcoma Cell Self-Renewal, Differentiation, and Drug Resistance Through miR-29b and Its Target Protein Spin 1. ACS Biomater Sci Eng 6, 5588–5598. 10.1021/acsbiomaterials.0c00816. [PubMed: 33320589]

128. Jabbari E, Sarvestani SK, Daneshian L, and Moeinzadeh S (2015). Optimum 3D Matrix Stiffness for Maintenance of Cancer Stem Cells Is Dependent on Tissue Origin of Cancer Cells. PLoS One 10, e0132377. 10.1371/journal.pone.0132377. [PubMed: 26168187]

129. Yeoman B, Shatkin G, Beri P, Banisadr A, Katira P, and Engler AJ (2021). Adhesion strength and contractility enable metastatic cells to become adurotactic. Cell Rep 34, 108816. 10.1016/j.celrep.2021.108816. [PubMed: 33691109]

130. Sinkus R, Siegmann K, Xydeas T, Tanter M, Claussen C, and Fink M (2007). MR elastography of breast lesions: understanding the solid/liquid duality can improve the specificity of contrast-enhanced MR mammography. Magn Reson Med 58, 1135–1144. 10.1002/mrm.21404. [PubMed: 17969009]

131. Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, Oertle P, Hyotyla JT, Aebi U, Bentires-Alj M, Lim RY, and Schoenenberger CA (2012). The nanomechanical signature of breast cancer. Nat Nanotechnol 7, 757–765. 10.1038/nnano.2012.167. [PubMed: 23085644]

132. Wisdom KM, Adebowale K, Chang J, Lee JY, Nam S, Desai R, Rossen NS, Rafat M, West R, Hodgson L, and Chaudhuri O (2018). Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments. Nature Communications 9:4144.

133. Chang J, Pang EM, Adebowale K, Wisdom KM, and Chaudhuri O (2020). Increased Stiffness Inhibits Invadopodia Formation and Cell Migration in 3D. Biophys J 119, 726–736. 10.1016/j.bpj.2020.07.003. [PubMed: 32697977]

134. Adebowale K, Gong Z, Hou JC, Wisdom KM, Garbett D, Lee HP, Nam S, Meyer T, Odde DJ, Shenoy VB, and Chaudhuri O (2021). Enhanced substrate stress relaxation promotes filopodia-mediated cell migration. Nat Mater 20, 1290–1299. 10.1038/s41563-021-00981-w. [PubMed: 33875851]

135. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, and Keely PJ (2006). Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med 4, 38. 10.1186/1741-7015-4-38. [PubMed: 17190588]

136. Pang MF, Siedlik MJ, Han S, Stallings-Mann M, Radisky DC, and Nelson CM (2016). Tissue Stiffness and Hypoxia Modulate the Integrin-Linked Kinase ILK to Control Breast Cancer Stem-like Cells. Cancer Res 76, 5277–5287. 10.1158/0008-5472.CAN-16-0579. [PubMed: 27503933]

137. Tito Panciera AC, Daniele Di Biagio, Giusy Battilana, Alessandro Gandin,, Stefano Giulitti, M. F, Silvio Bicciato, Valeria Panzetta, Sabato Fusco,, Luca Azzolin, A. T, Angelo Paolo Dei Tos, Matteo Fassan, Vincenzo Vindigni,, Franco Bassetto, A. R, Giovanna Brusatin, Michelangelo Cordenonsi and, and Piccolo S (2020). Reprogramming normal cells into tumour precursors requires ECM stiffness and oncogenemediated changes of cell mechanical properties. Nature Materials. 10.1038/s41563-020-0615-x.

138. Plunkett C, Kumar A, Yrastorza J, Hou YH, Placone JK, Grennan G, and Engler AJ (2020). H-Ras Transformation of Mammary Epithelial Cells Induces ERK-Mediated Spreading on

Low Stiffness Matrix. Adv Healthc Mater 9, e1901366. 10.1002/adhm.201901366. [PubMed: 31951109]

139. Alibert C, Goud B, and Manneville JB (2017). Are cancer cells really softer than normal cells? Biol Cell 109, 167–189. 10.1111/boc.201600078. [PubMed: 28244605]
140. Fuhs T, Wetzel F, Fritsch AW, Li XZ, Stange R, Pawlizak S, Kiessling TR, Morawetz E, Grosser S, Sauer F, et al. (2022). Rigid tumours contain soft cancer cells. Nat Phys. 10.1038/s41567-022-01755-0.
141. Holle AW, Govindan Kutty Devi N, Clar K, Fan A, Saif T, Kemkemer R, and Spatz JP (2019). Cancer Cells Invade Confined Microchannels via a Self-Directed Mesenchymal-to-Amoeboid Transition. Nano Lett 19, 2280–2290. 10.1021/acs.nanolett.8b04720. [PubMed: 30775927]
142. Barriga EH, Franze K, Charras G, and Mayor R (2018). Tissue stiffening coordinates morphogenesis by triggering collective cell migration in vivo. Nature 554, 523–527. 10.1038/nature25742. [PubMed: 29443958]
143. Morita H, Grigolon S, Bock M, Krens SF, Salbreux G, and Heisenberg CP (2017). The Physical Basis of Coordinated Tissue Spreading in Zebrafish Gastrulation. Dev Cell 40, 354–366 e354. 10.1016/j.devcel.2017.01.010. [PubMed: 28216382]
144. Petridou NI, and Heisenberg CP (2019). Tissue rheology in embryonic organization. EMBO J 38, e102497. 10.15252/embj.2019102497. [PubMed: 31512749]
145. Shellard A, and Mayor R (2021). Durotaxis: The Hard Path from In Vitro to In Vivo. Dev Cell 56, 227–239. 10.1016/j.devcel.2020.11.019. [PubMed: 33290722]
146. Valet M, Siggia ED, and Brivanlou AH (2022). Mechanical regulation of early vertebrate embryogenesis. Nat Rev Mol Cell Biol 23, 169–184. 10.1038/s41580-021-00424-z. [PubMed: 34754086]
147. Gaetani R, Zizzi EA, Deriu MA, Morbiducci U, Pesce M, and Messina E (2020). When Stiffness Matters: Mechanosensing in Heart Development and Disease. Front Cell Dev Biol 8, 334. 10.3389/fcell.2020.00334. [PubMed: 32671058]
148. Majkut S, Idema T, Swift J, Krieger C, Liu A, and Discher DE (2013). Heart-specific stiffening in early embryos parallels matrix and myosin expression to optimize beating. Curr Biol 23, 2434–2439. 10.1016/j.cub.2013.10.057. [PubMed: 24268417]
149. Li N, Liu N, Zhou J, Tang L, Ding B, Duan Y, and Jin Y (2013). Inflammatory environment induces gingival tissue-specific mesenchymal stem cells to differentiate towards a pro-fibrotic phenotype. Biol Cell 105, 261–275. 10.1111/boc.201200064. [PubMed: 23521530]
150. Lin X, Shi Y, Cao Y, and Liu W (2016). Recent progress in stem cell differentiation directed by material and mechanical cues. Biomed Mater 11, 014109. 10.1088/1748-6041/11/1/014109. [PubMed: 26836059]
151. Berry MF, Engler AJ, Woo YJ, Pirolli TJ, Bish LT, Jayasankar V, Morine KJ, Gardner TJ, Discher DE, and Sweeney HL (2006). Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. Am J Physiol Heart Circ Physiol 290, H2196–2203. 10.1152/ajpheart.01017.2005. [PubMed: 16473959]
152. Frangogiannis NG (2017). The extracellular matrix in myocardial injury, repair, and remodeling. J Clin Invest 127, 1600–1612. 10.1172/JCI87491. [PubMed: 28459429]
153. Gazoti Debessa CR, Mesiano Maifrino LB, and Rodrigues de Souza R (2001). Age related changes of the collagen network of the human heart. Mech Ageing Dev 122, 1049–1058. 10.1016/s0047-6374(01)00238-x. [PubMed: 11389923]
154. Lopez B, Gonzalez A, Hermida N, Valencia F, de Teresa E, and Diez J (2010). Role of lysyl oxidase in myocardial fibrosis: from basic science to clinical aspects. Am J Physiol Heart Circ Physiol 299, H1–9. 10.1152/ajpheart.00335.2010. [PubMed: 20472764]
155. Ferrari S, and Pesce M (2021). Stiffness and Aging in Cardiovascular Diseases: The Dangerous Relationship between Force and Senescence. Int J Mol Sci 22. 10.3390/ijms22073404.
156. Atcha H, Jairaman A, Holt JR, Meli VS, Nagalla RR, Veerasubramanian PK, Brumm KT, Lim HE, Othy S, Cahalan MD, et al. (2021). Mechanically activated ion channel Piezo1 modulates macrophage polarization and stiffness sensing. Nat Commun 12, 3256. 10.1038/s41467-021-23482-5. [PubMed: 34059671]

157. Pensalfini M, and Tepole AB (2023). Mechano-biological and bio-mechanical pathways in cutaneous wound healing. PLoS Comput Biol 19, e1010902. 10.1371/journal.pcbi.1010902. [PubMed: 36893170]

158. Wang X, Senapati S, Akinbote A, Gnanasambandam B, Park PS, and Senyo SE (2020). Microenvironment stiffness requires decellularized cardiac extracellular matrix to promote heart regeneration in the neonatal mouse heart. Acta Biomater 113, 380–392. 10.1016/j.actbio.2020.06.032. [PubMed: 32590172]

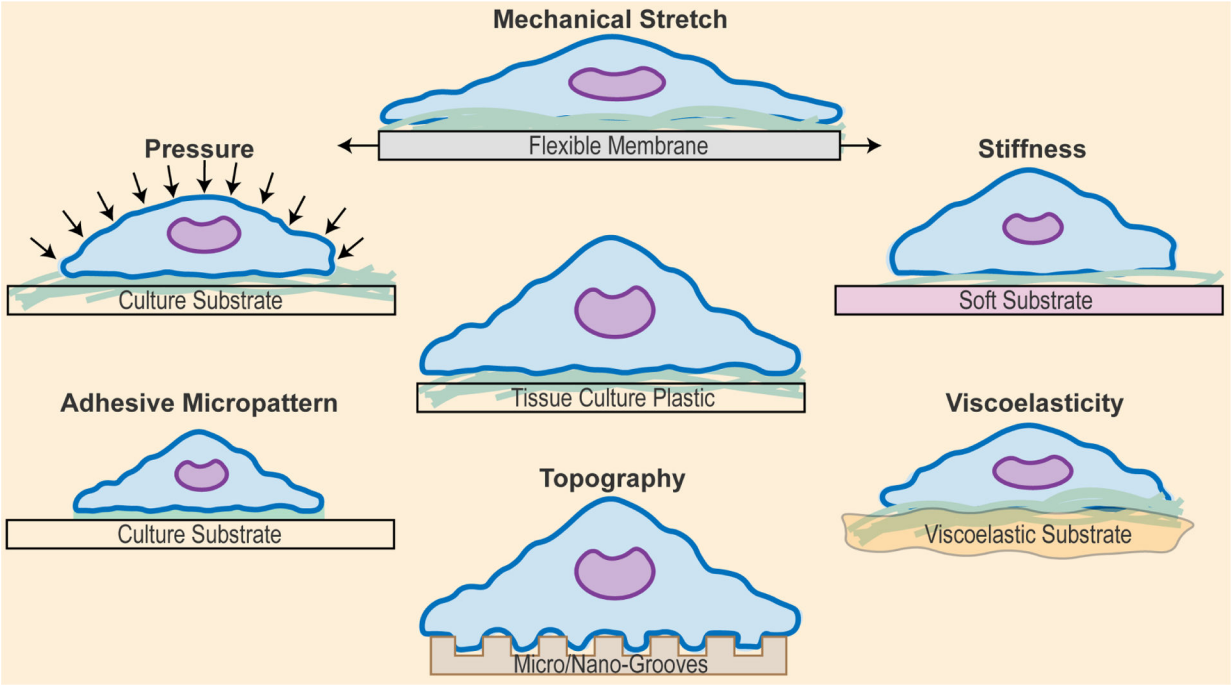

Figure 1: Mechanical stimuli used *in vitro*.

Several methods have been developed to better recapitulate the mechanical microenvironment when compared to the standard tissue culture plastic cell culture system. These include introduction of physical stimuli such as pressure or stretch as well as material derived cues such as culture substrate stiffness, viscoelasticity, topography, and adhesive geometry.

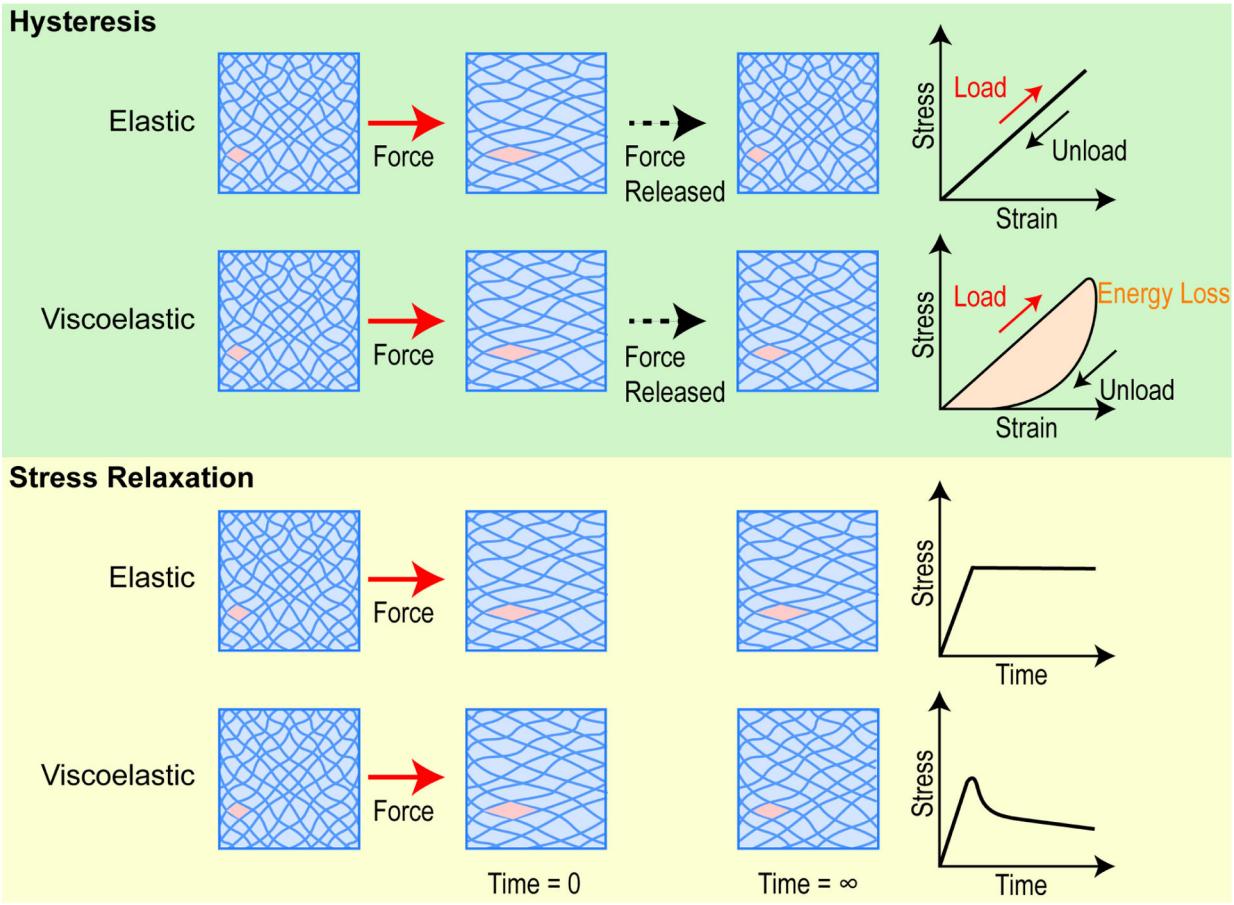

Figure 2: Elastic vs. Viscoelastic properties in materials.

(Top) When a transient force is applied an elastic material deforms and upon release of the force, the material conforms back to its original shape, as is shown by equivalent stress-strain profiles when subjected to a load or unload. Similarly, viscoelastic materials also deform when subjected to a force; however, they are unable to conform back to their original shape resulting in a net loss of energy, as shown by unequal stress-strain profiles when subjected to a load or unload. (Bottom) When a constant force is applied, elastic materials maintain a constant load and deformation, as indicated by stress vs. time (creep) and strain vs. time (stress relaxation) profiles. In contrast, viscoelastic materials dissipate a load and undergo stress relaxation over time. Highlighted sections of pictured material emphasize changes in structure under each condition.

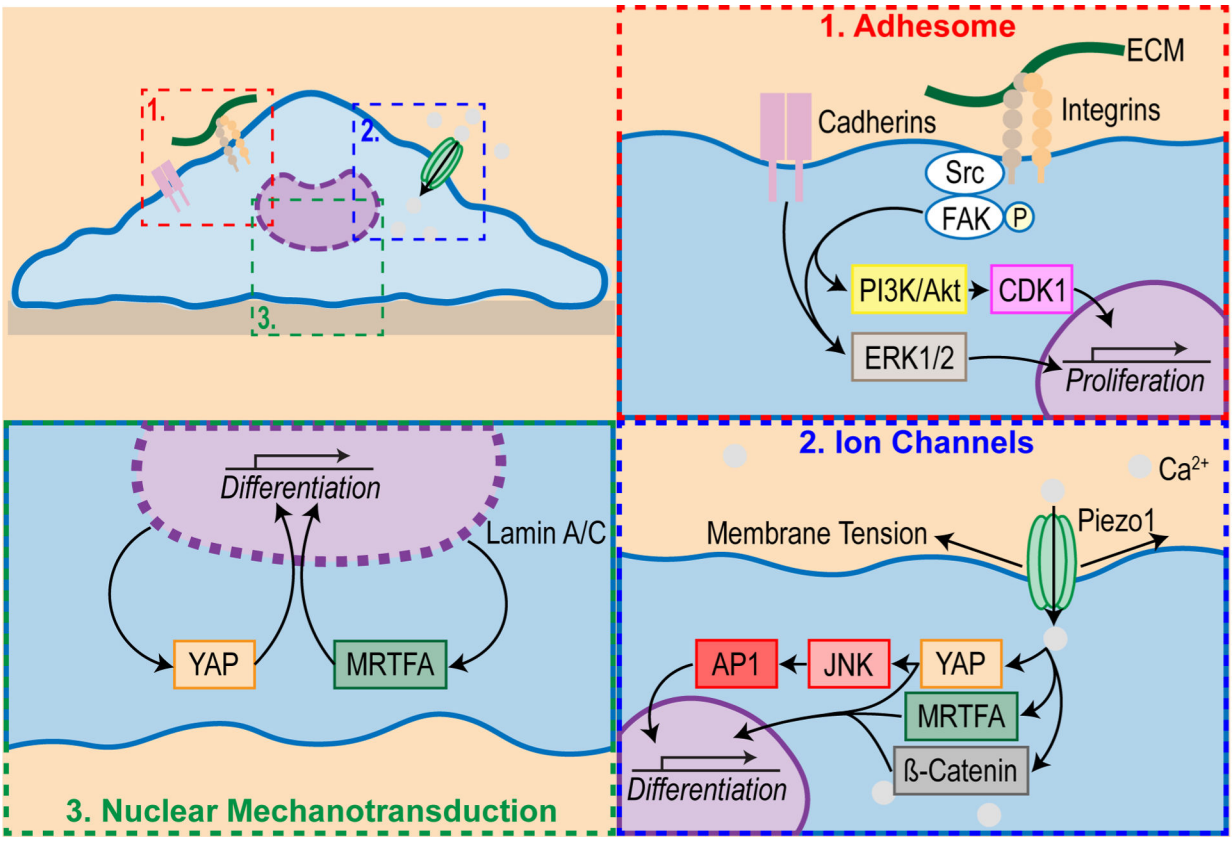

Figure 3: Examples of mechanotransduction pathways in stem cells.

The adhesome, a collection of genes involved in adhesion and cell-cell interaction, is known to activate extracellular signal-regulated kinase (ERK) / mitogen activated protein kinase (MAPK) or PI3K/Akt/CDK1 signaling pathways resulting in enhanced cell proliferation. In contrast, the mechanically gated ion channel Piezo1, activates YAP, MRTFA, and β-Catenin all of which complex and translocate into the nucleus. In addition, YAP further activates JNK/AP-1 signaling pathways. Both mechanisms result in cellular differentiation. Finally, Lamin A/C expression controls the nuclear localization of YAP and MRTFA which regulates differentiation.

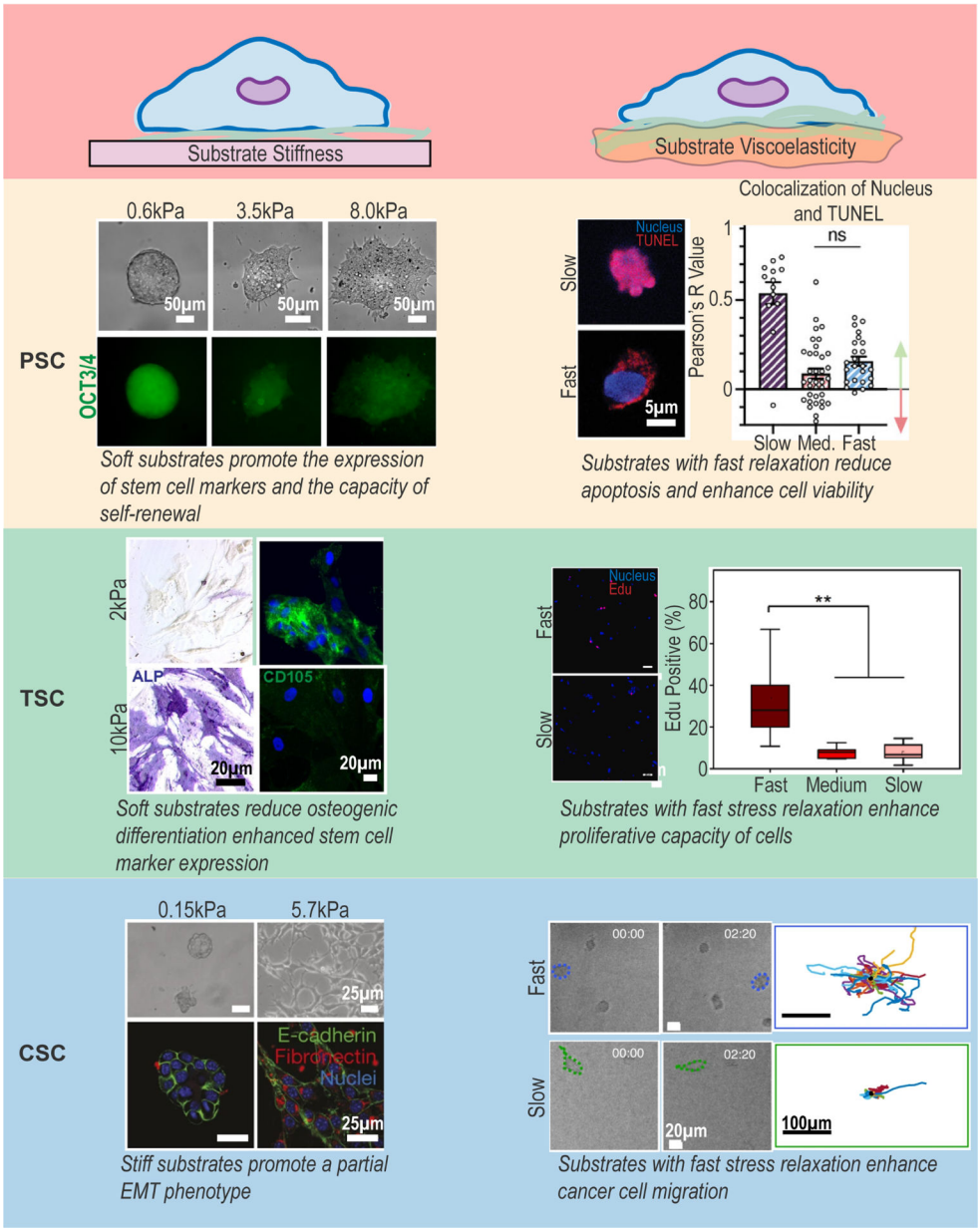

Figure 4: Substrate stiffness and viscoelasticity modulate stem cell and cancer stem cell self-renewal.

Soft surfaces were shown to promote the expression of the stem cell marker Oct3-4, figure adapted from[77]. Similarly, soft substrates were also observed to promote the expression of CD105, a stem cell marker, in human MSCs, while stiff surfaces enhanced osteogenic differentiation as seen by increased ALP staining, figure adapted from[97]. In cancer stem cells, stiff surfaces are observed to promote a partial EMT phenotype and a more spread cell morphology, figure adapted from[115]. In contrast, viscoelastic substrates with fast stress relaxation reduce cell apoptosis and promote proliferation in PSCs, as shown by reduced TUNEL staining, figure adapted from[85]. Fast stress relaxation was also observed to increase the proliferative capacity of MSCs as seen by enhanced Edu staining, figure adapted from[18]; whereas, these substrates promote a more migratory phenotype in breast cancer cells, as

shown by overall displacement over a time lapse video and displacement maps for individual cells, figure adapted from[134].

Table 1:
Summary of natural, synthetic, and semi-synthetic materials.

Each material has distinct advantages and disadvantages which should be considered when choosing a material for a specific application. Typical stiffness ranges and examples of *in vivo* applications for each material category are also listed.

	Natural	**Synthetic**	**Semi-synthetic**
Examples	Collagen, Fibrin, Elastin, Hyaluronan, Alginate, Agarose	Polyacrylamide, PGA, PLLA, PEG, PLGA	GelMA, MeHA
Advantages	• Superior biocompatibility • Native protein structure • High viscoelasticity • Can be remodeled	• Tunable mechanical properties • Possibility of tunable degradation rate	• Native protein structure • Tunable mechanical properties • Can be remodeled
Disadvantages	• Batch variability • Degradation • Poor mechanical tuning	• Poor biocompatibility • Lack of adhesion sites • Lack of biological cues • Unwanted degradation byproducts	• UV light detrimental to cell health
Stiffness Range	~Pa – kPa	~Pa – MPa	~Pa-kPa
***In Vivo* Applications**	• Cartilage tissue engineering • Neural tissue engineering • Bone tissue engineering • Muscle tissue engineering	• Bone tissue engineering	• Cartilage tissue engineering • Spinal cord repair • Cardiovascular engineering