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Review Article

Extracellular matrix elasticity and topography: Material-based cues that affect cell function via conserved mechanisms

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Abstract: Chemical, mechanical, and topographic extracellular matrix (ECM) cues have been extensively studied for their influence on cell behavior. These ECM cues alter cell adhesion, cell shape, and cell migration and activate signal transduction pathways to influence gene expression, proliferation, and differentiation. ECM elasticity and topography, in particular, have emerged as material properties of intense focus based on strong evidence these physical cues can partially dictate stem cell differentiation. Cells generate forces to pull on their adhesive contacts, and these tractional forces appear to be a common element of cells' responses to both elasticity and topography. This review focuses on recently published work that links ECM topography and mechanics and their influence on differentiation and other cell behaviors. We also highlight signaling pathways typically implicated in mechanotransduction that are (or may be) shared by cells subjected to topographic cues. Finally, we conclude with a brief discussion of the potential implications of these commonalities for cell based therapies and biomaterial design. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 103A: 1246–1258, 2015.

Key Words: topography, matrix mechanics, cell fate, mechanotransduction, differentiation

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INTRODUCTION

Integrin-mediated adhesion to the extracellular matrix (ECM) is critical for cell differentiation, function, and tissue organization.[1,2] When these receptors recognize and bind to ECM proteins (e.g., laminins, collagens, fibronectin), they cluster together and associate with numerous intracellular proteins to form a focal complex. As this focal complex grows and matures into a focal adhesion (FA), it provides a direct physical bridge and a biochemical nexus to transduce mechanochemical cues from the ECM to the cell (and vice-a-versa) and thereby alter cell migration, proliferation and differentiation.[3]

The biochemical composition of the ECM largely determines the specificity of integrin binding and subsequent cell responses. The simplest adhesion motif to which most cells can bind is an amino acid sequence arginine, glycine, and aspartic acid (RGD). The RGD sequence was first identified in fibronectin,[4] but is also present in vitronectin, osteopontin, and laminin,[5,6] and has been ubiquitously applied throughout the biomaterials literature to functionalize materials and facilitate cell adhesion. Other peptide sequences capable of mediating or influencing cell adhesion have also been identified in other ECM proteins and have been used to promote cell adhesion to materials.[5,7,8] Despite their widespread adoption, whether to use short peptides or full-length ECM proteins remains an ongoing debate in the field of biomaterials.[9,10] Moreover, cells manipulate the initial adhesion surface either through secretion of new ECM components,[11] or through manipulation of the "native" ECM[12] or serum proteins.[13] This manipulation may involve traction forces that expose otherwise cryptic peptide sequences (indicating the adhesion environment is very dynamic).[14]

In addition to its chemical composition, the ECM's physical properties are also important regulators of cell behavior. The most often characterized and reported physical influence is the ECM's elasticity (or rigidity), best defined as the material's ability to undergo non-permanent deformation. Tissues in the body span a wide range of elastic moduli [Fig. 1(A)], and it has been suggested that different tissue mechanical properties may be instructive and actively

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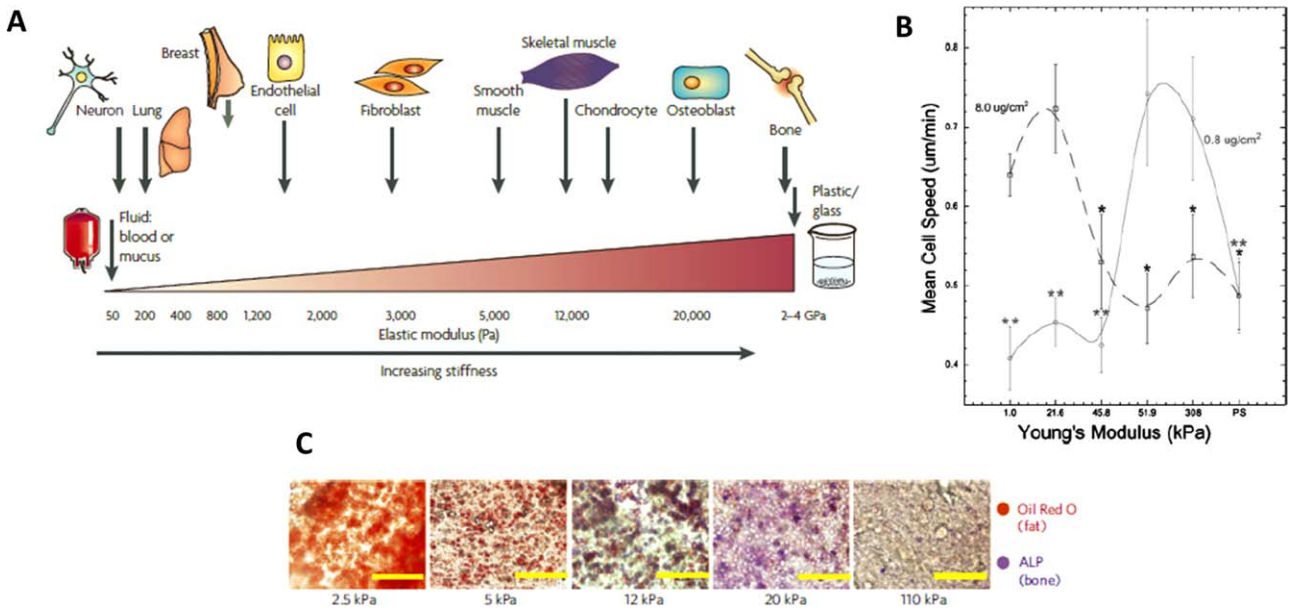

FIGURE 1. ECM elasticity and its influence on cell behavior. A: Schematic illustrating the varied mechanical properties of different *in vivo* tissues.[133] (Adapted with permission from Macmillan Publishers Ltd: *Nat Rev Cancer* 9:2, copyright 2009.) B: Migration of human aortic smooth muscle cells depends on substrate elasticity in a biphasic manner. The dashed line represents a high ECM ligand density (8.0 μg/cm^2 fibronectin) whereas the solid line represents a low ECM ligand density (0.8 μg/cm^2 fibronectin).[16] (Reprinted with permission from John Wiley and Sons, Inc.: *J Cell Physiol* 204:1, copyright 2005.) C: Differentiation of MSCs in 3D matrices *in vivo* also depends on ECM elasticity, with maximal osteogenic differentiation observed for cells entrapped within hydrogels of intermediate rigidity (scale bar = 100 μm).[33] (Adapted with permission from Macmillan Publishers Ltd: *Nat Mater* 9:6, copyright 2010.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

influence cell phenotypes in a tissue-specific manner. In fact, natural ECMs are viscoelastic, with properties of both viscous liquids and elastic solids; however, the viscous characteristics of the ECM and their influence on cell functions remain relatively underexplored. "Soft" materials are easily deformed at low stresses, whereas "hard" materials require greater stresses to produce the same amount of deformation (strain).[15] The ECM also provides topographic stimuli, primarily in the form of fibrous proteins with micro- and nanoscale features. In this review article, we will present the mounting evidence that ECM elasticity and topography act as instructive cues to influence cell phenotype, focusing mostly on cells' responses *in vitro*. In addition, we will also consider the similarities cells use to sense these physical cues, and the possibility that the mechanisms they use in response are conserved.

Cellular responses to matrix elasticity

A variety of material platforms and methods have been used to explore the influence of ECM elasticity on cell function. Most widely used include polymers such as poly(dimethylsiloxane) (PDMS), poly(urethane acrylate) (PUA), and hydrogels made from polyacrylamide (PAA) or poly(ethylene glycol) (PEG).[16–21] To alter elasticity in these materials, the amount of polymer, cross-linker, and in some cases the amount of photoinitiator, can be varied to produce substrates of desired elastic properties. The molecular weights of these polymers also affect the mechanical properties of the resulting substrates.

Cell adhesion and spreading were amongst the first cell functions shown to be influenced by ECM elasticity in a seminal article by Pelham and Wang.[22] Subsequent studies demonstrated that smooth muscle cell (SMC) spreading increased quantitatively with substrate elasticity.[16,23] The magnitude of spreading depended strongly on ECM ligand density (fibronectin) for SMCs cultured on soft substrates (PAA), but was invariant to these changes on rigid polystyrene controls, suggesting that matrix elasticity may override ligand density after some threshold is surpassed.[16] Similar findings were reported for endothelial cells grown on substrates of varied elasticity.[24] By contrast, the spreading area of a preosteoblastic cell line (MC3T3-E1) has been shown to be insensitive to changes in matrix elasticity for two different ligand densities (type I collagen).[25] These results suggest that cell spreading's dependence on substrate elasticity varies with both cell type and ligand identity.

ECM elasticity has also been shown to influence two-dimensional (2D) cell migration. Pelham and Wang[22] first demonstrated that 3T3 fibroblasts become less motile as substrate rigidity increased. A subsequent study showed that 3T3 fibroblasts migrate in a directional fashion from softer substrates to stiffer substrates, but not vice-a-versa, indicating a dependence on the mechanical properties of the substrate in the absence of any soluble chemical stimuli.[26] This phenomenon was dubbed durotaxis (or sometimes mechanotaxis).[26–28] A study exploiting this concept demonstrated that the direction of SMC migration could be controlled via patterned gradients in ECM elasticity.[29] Prior work from our laboratory demonstrated that SMC migration speeds depend on ECM elasticity in a nonlinear (i.e., biphasic) manner [Fig. 1(B)].[16] In that study, the value of the

optimal substrate stiffness at which cell migration speed was maximized was found to depend on the density of immobilized ECM ligand (fibronectin), suggesting a strong coupling between ECM chemistry and mechanics to tightly regulate cell migration. Higher density of adhesive ligand shifted the optimal ECM elasticity to lower values, while lower density required higher elastic moduli to achieve maximal migration speeds.[16]

While the influence of ECM elasticity on cell adhesion, spreading, and motility generated significant interest amongst many researchers in the bioengineering and mechanobiology fields in the early-to-mid 2000s, it was arguably a 2006 study by Engler et al.[30] that catapulted the importance of ECM elasticity into the scientific mainstream consciousness. In that seminal study, mesenchymal stem cell (MSC) differentiation was shown to depend on matrix elasticity and ECM identity. MSCs cultured on compliant matrices mimicking the elasticity of brain exhibited characteristics of neuronal cells, while those cultured on stiff substrates consistent with a premineralized osteoid matrix expressed markers of osteoblasts.[30] Substrates with intermediate stiffness supported a skeletal muscle-like phenotype. A slightly earlier study by the same authors investigated the effects of matrix elasticity on the differentiation of multinucleated skeletal muscle myotubes,[23] and subsequent studies by others showed that this phenomenon extended to other stem cell populations as well.[31]

How ECM elasticity affects cells in three-dimensional (3D) materials that more accurately mimic the native microenvironment of many cell types in the human body has been a more difficult question to address, due in large part to the coupling of ECM mechanics, chemistry, and microstructure in most hydrogel platforms. In natural protein-based hydrogels (e.g., collagen, fibrin, and matrigel), increasing protein concentration affects elastic modulus but also alters the number of binding sites available for cell adhesion and can disrupt the diffusive transport of soluble morphogens.[32] A 2010 article by Huebsch et al.[33] tackled this question using RGD-modified alginate gels, demonstrating that the osteogenic differentiation of MSCs was best supported by gels of intermediate elasticity in 3D [Fig. 1(C)]. The various formulations of alginate exploited in that study permitted equal levels of diffusive transport, and also inhibited the ability of the MSCs to spread. The authors made the argument that these material characteristics enabled decoupling of ECM elasticity from spreading and diffusive transport. Unlike the 2D case where MSC differentiation toward an osteogenic lineage is positively correlated with increasing elastic modulus,[30] the relationship between cell fate and ECM elasticity in 3D is distinct. Nevertheless, these data support an instructive role for ECM elasticity, a nearly dogmatic paradigm reviewed elsewhere.[34,35]

Cellular responses to matrix topography

Paralleling the increased focus on ECM elasticity in the recent literature, the past 10–15 years have witnessed a very large number of studies investigating the effects of physical topographical features (e.g., lines, gratings, holes, pillars, etc.) and/or chemical topographical features (e.g., "tracks" or "islands" of printed or adsorbed ECM proteins). This section of our review will focus mostly on nanotopography, as it is already well established that chemical and physical microtopographies influence cell shape and morphology, and methods to control shape have been widely used in the literature for the past two decades. A full discussion of micropatterning and other methods used to pattern ECM ligands and thereby control cell shape is beyond the scope of this review and can be found elsewhere.[36–39] However, we will discuss a few important microtopography studies in the context of control of cell migration and fate below as the biologic mechanisms appear to be conserved with those used by cells to sense ECM elasticity.

Producing surfaces with defined physical topographical features can be achieved by a number of techniques, including nanoimprint lithography,[40] capillary force lithography,[41] ultraviolet assisted lithography,[42] embossing, photolithography, and micromachining (Fig. 2).[43] These methods are typically used for polymeric substrates and are discussed in greater detail in the references cited for each above. Other methods have been used to impart topography or enhance roughness on ceramic, semiconductive, and metallic substrate surfaces; these include deep reactive ion-etching, acid etching, photolithography, sandblasting, and mechanical machining.[43] These methods can produce micro- or nanoscale features. Other methods such as self-assembled monolayers and microcontact printing have been extensively used to pattern proteins of defined areas on a substrate surface.[44,45] In some cases, substrates containing both physical and chemical topographic features have been used to provide distinct control of surface features and adhesion islands.[46]

Numerous studies linking nanoscale physical topographies with cell adhesion and morphology have appeared in the literature in the past decade.[47–53] The rationale to explore this linkage is that native ECM contains nanoscale physical topographies, and thus features of similar size on engineered substrates may better mimic the native ECM.[54] Early examples from the literature used substrates with various nanoscale features to investigate the adhesive characteristics of fibroblasts and endothelial cells.[55–58] In a more recent study, human MSC (hMSC) adhesion was examined on roughened titanium surfaces, and found to be enhanced on those with 150 and 450 nm features compared to 20 nm features.[59] However, similar nanoscale features on roughened titanium reportedly had no differential effects on osteoblast cell adhesion.[60] Such discrepancies underscore the idea that different cell types respond differently to topography,[47] and leave some doubt as to whether or not cell adhesions are impacted by physical nanotopography.

By contrast, there is an abundance of evidence that nanotopography can influence cell shape/morphology. Perhaps the most obvious manifestation of this observation can be seen with cells cultured on nanogrooves (often called nanoridges or nanogratings), which have large axial dimensions (~mm) and nanoscale lateral dimensions, typically with periodic patterns of variable ridge height and width.

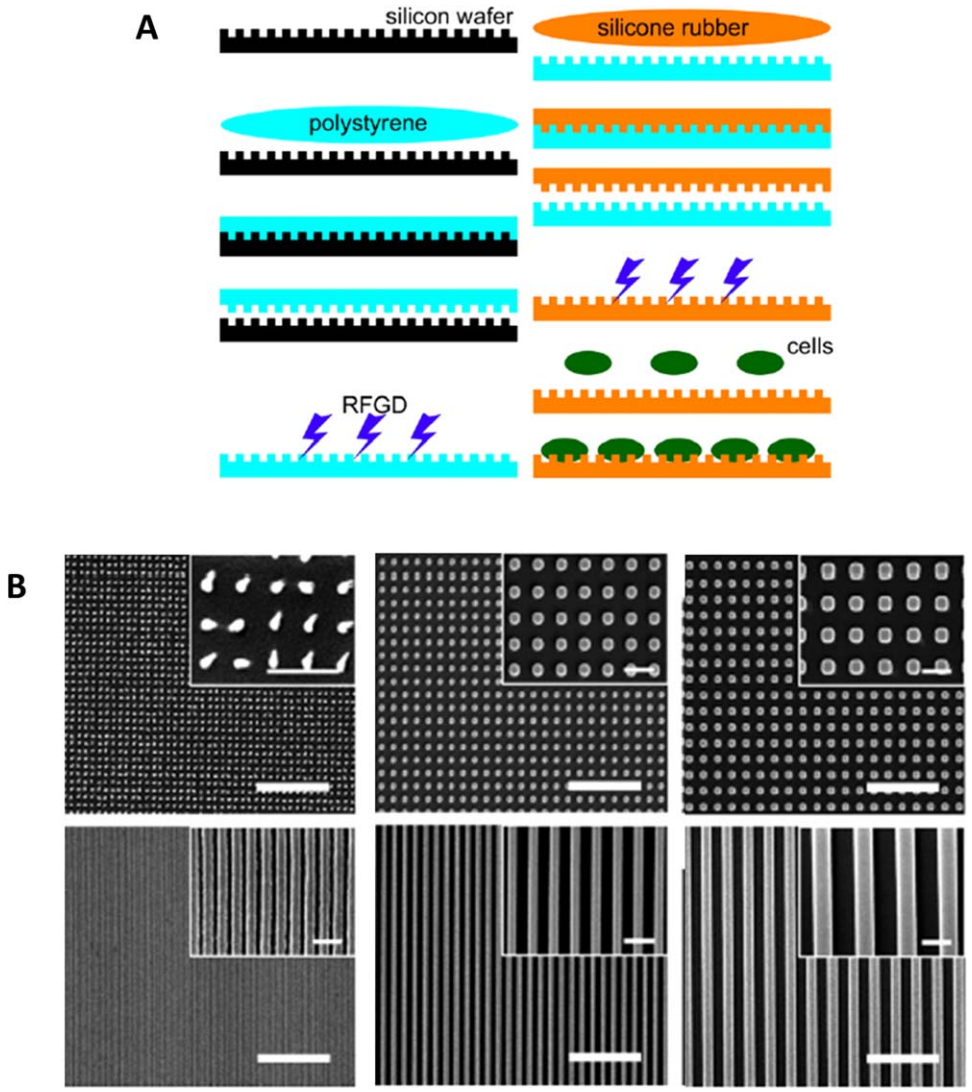

FIGURE 2. Lithographic method to fabricate nanopatterned substrates for cell culture. A: Illustration depicting method to produce nanotopographic surfaces via a multistep lithographic process that involves first creating a polystyrene mold from a silicon master, and then transferring the topography to a secondary substrate [e.g., poly(dimethylsiloxane) (PDMS)]. Cells can then be seeded on these surfaces for experimentation.[134] (Adapted with permission from Elsevier: *Biomaterials* 31:30, copyright 2010.) B: Scanning electron micrographs of nanopatterned poly(urethane acrylate) (PUA) substrates fabricated by UV-assisted capillary force lithography. Sizes range from 150 to 600 nm. Scale bar 5 and 1 μm (inset).[78] (Adapted with permission from the American Chemical Society: *Biomacromolecules* 11:7, copyright 2010.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Cells of many different origins readily align parallel to these grooved substrates.[21,49,51,53,55,61–64] At least for rat osteoblastic cells, a critical size threshold (75 nm width and 33 nm depth) has been reported to achieve this parallel alignment; nanogrooves of smaller lateral dimension failed to induce alignment of the cells.[63] A prior study suggested that groove depth plays a central role in cells' sensitivity to nanotopographic ridges.[65] However, whether such physical nanotopographic cues can be more important that chemical cues remains unknown. In the context of microtopography, a prior study created both physical and chemical features to investigate preosteoblast alignment using a polymeric base surface coated with titanium and gold with micron sized gratings. Microcontact printing was utilized to imprint fibronectin lanes either parallel or perpendicular to the underlying physical surface. Despite a perpendicular adhesive protein cue, cells in this case preferentially aligned with the underlying physical topography (qualitatively and quantitatively shown in Fig. 3).[46]

There is also increasing evidence that micro- and nanotopographies influence cell migration.[48,66–69] One study demonstrated that nanogratings can alter the polarization of smooth muscle cells in a wound healing migration assay, with orientation of the microtubule organizing center toward the wound on unpatterned surfaces and along the axis of cell alignment in cells cultured on patterns.[53] Another study used micropatterned chemical topography to compare the responses of multiple cell types in 3D matrices, on 2D surfaces, and on "1D" lines (1–10 μm width) coated with various ECM proteins (fibrinogen, vitronectin, and

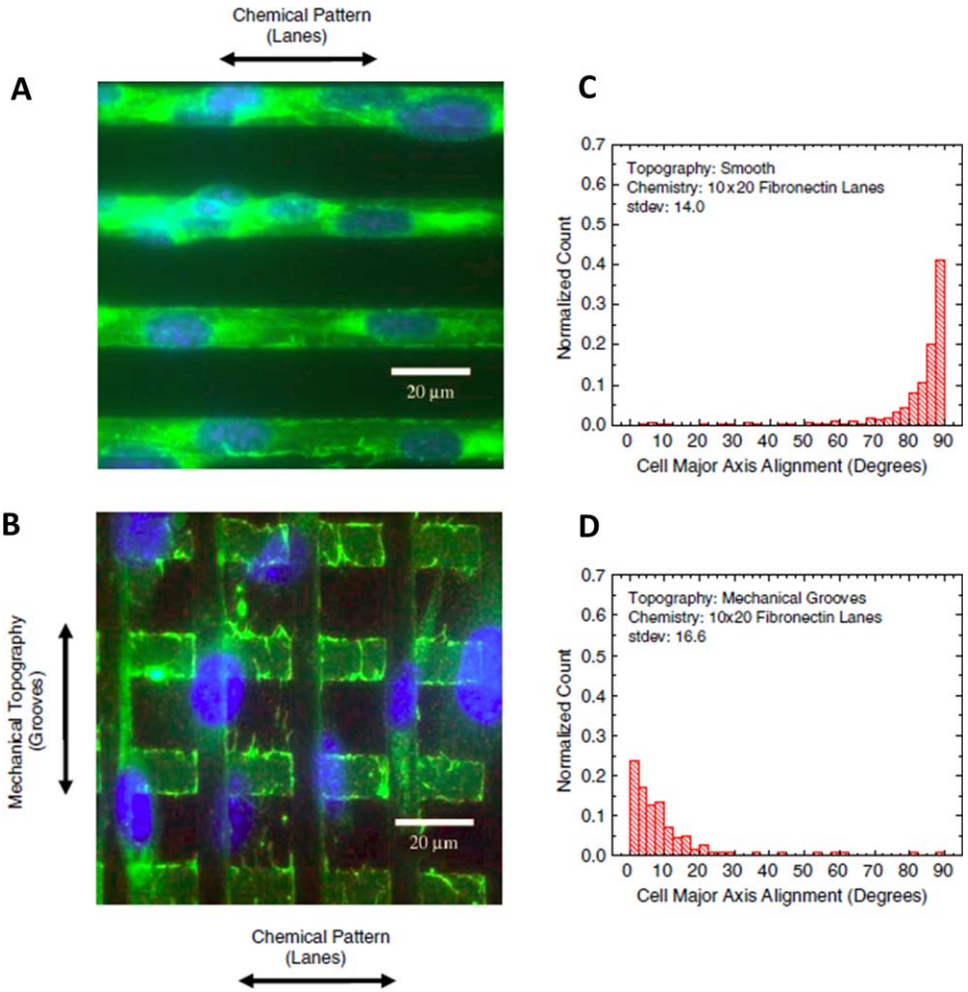

FIGURE 3. Physical topography can override chemical topography. Immunofluorescence images of mouse calvarial preosteoblasts on (A) substrates patterned with chemical topography (fibronectin lanes) or (B) substrates patterned with both chemical and physical topography perpendicular to one another. Analyses of cell orientation on the patterned surfaces in (A) and (B) via histograms of alignment in (C) and (D), respectively, suggest that physical topography more strongly influences cell alignment than chemical topography.[46] (Adapted with permission from Elsevier: *Biomaterials* 27:11, copyright 2006.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fibronectin).[70] Fibroblast adhesion and spreading on the 1D lines were similar to their behavior in 3D. Knockdown of the small GTPase Rac in cells cultured in 2D produced an elongated cell morphology similar to that observed on the 1D substrates. However, the migration speeds of the Rac knockdown cells did not increase, and vinculin staining of these cells revealed that their adhesions were still distinct from those observed for the 1D and 3D cases.

Physical nanotopography may also influence cell proliferation, but the results are somewhat mixed. For example, one study demonstrated that human osteoblast proliferation on nanorough Ti films was the same as that on smooth surfaces,[60] while another report that used similar nanorough Ti substrates reported that hMSC proliferation was influenced by nanotopographic feature size.[59] In the latter of these two studies, substrates with features on the order of picometers (which the authors referred to as subnano) failed to support MSC proliferation to the same degree as those with nano- and microscale roughness.[59] Polymeric surfaces with nanoridges and holes induced a greater proliferation rate in canine MSCs after five days.[71] Proliferation of hMSCs grown on polyurethane nanogratings was not affected by topography.[64] Our own work on nanotopographic poly(methyl methacrylate) (PMMA) has shown that hMSC proliferation is not altered at early time points, consistent with results from other studies,[72] but is enhanced at day 14 compared to smooth controls.[49] Thus, proliferation might be enhanced by physical nanotopography but is dependent upon cell type, surface chemistry, and surface feature.

The influence of physical nanotopography on differentiation has also been extensively investigated over the past 10–15 years. A very wide range of material platforms and wide range of topographies have been explored. One of the earliest and most highly cited articles reported enhanced alkaline phosphatase (ALP) activity and extracellular calcium deposition for rat osteoblasts cultured on nanophase ceramics.[73] Experiments documenting MSC response to

nanotopographies appeared a few years later.[74,75] In one study with MSCs, arrangements of 120-nm diameter, 100-nm deep nanopits in PMMA that were asymmetric and more disordered (i.e., deviated from perfectly square or hexagonal arrays) were found to enhance the expression of osteogenic genes and proteins, even in the absence of soluble osteogenic supplements.[76] The same group of investigators later demonstrated that regular square arrays of these nanopits embossed in polycaprolactone promote MSC stemness.[77] MSCs grown on gelatin-coated PUA nanogratings also reportedly upregulate osteogenic gene expression compared to cells on control surfaces,[78] as do titanium oxide nanotubes.[79] An ambitious study recently described an approach to fabricate a library of 2176 distinct, randomly designed surface topographies on poly(DL-lactice acid) and used high-content imaging to identify formerly unknown surface nanotopographies capable of inducing MSC proliferation or ALP expression (as a surrogate for osteogenic differentiation).[80] Such an approach offers the potential to screen a wide array of topographies in much the same way that surface chemistries have been explored for their effects on cell fate.[81] Similarly, others have recently shown that spatial patterning of different nanotopographies on the same surface can be used to spatially control the switch between adipogenesis and osteogenesis in MSCs.[82]

Few studies involving MSCs, however, have gone beyond gene expression assays to characterize mineral formation, a functional metric of osteogenesis. One study that did (using committed osteoblasts) found increases in some osteogenic specific markers on nanotopography relative to smooth controls, and the presence and alignment of CaP mineral deposits on substrates with grooves 50 nm wide and 17 nm deep.[63] However, no images or quantification of CaP were shown, so it is not clear how nanotopography enhanced mineral deposition relative to smooth surfaces. A study involving MSCs on nanogratings of polyurethane reported enhanced osteogenic gene expression, and improved calcium deposition on 400 nm surfaces relative to smooth controls on days 7 and 14.[64] However, the enhancement due to topography disappeared by day 21. We recently reported a similar enhancement of calcium deposition at day 14 that disappeared by day 21.[49] These findings indicate that topography (*in vitro*) may be influential for osteogenic differentiation, but long-term investigations *in vivo* are needed to fully characterize the impact of topography on bone formation. At least one study suggests that nanogrooves on titanium have no long-term benefit in terms of bone-to-implant contact in a rabbit tibial defect model.[83]

Clearly significant attention has been focused on the links between topography and various osteoprogenitor cell types (e.g., MSCs, osteoblasts). However, there is evidence that topography influences many other cell types as well. For example, several recent studies have examined the role of nanotopography in the maintenance of human embryonic stem cells (hESCs).[84–87] However, much like the case for MSCs, the influence of nanotopography on hESCs is not yet clear due to some discrepant results. For example, one study found that hESCs better retained their expression of Oct3/4 (a transcription factor and characteristic marker of undifferentiated ESCs) when cultured on smooth surfaces than nanoroughened ones.[84] However, another study showed that hESC expression of Oct4 was better maintained by culturing the cells on polystyrene nanopillar arrays with either regular hexagonal or honeycomb lattice arrangements relative to those cultured on smooth surfaces.[85] Another recent study supported the former idea, that nanoscale topography can reduce Oct4 expression and drive differentiation of ESCs.[86]

Cardiac myocytes are another cell type shown to be responsive to ECM nanotopography. In one study in particular, PEG hydrogels were patterned with nanotopography via a UV-assisted lithography method, and covalently functionalized with fibronectin [Fig. 4(A)].[62] Neonatal rat ventricular myocytes cultured on these nanotopographic substrates not only aligned parallel to the topography [Fig. 4(B,C)], but impressively displayed anisotropic action potential propagation reminiscent of native myocardium to a greater degree than cells cultured on unpatterned substrates. The cells on nanotopography also had elevated connexin-43 expression. The authors also showed evidence that the cells penetrated into the nanogratings [Fig. 4(D,E)], and attributed the enhanced myocyte function in part to the increased adhesion between cells and the patterned substrates. When beads were embedded in the patterned PEG hydrogels and used as fiduciary markers to characterize cell-generated traction forces, the authors demonstrated that the contractile forces were highly aligned with the topography. As the feature size became smaller and the substrates approached a non-patterned environment, the beneficial effects of topography disappeared.[62]

Adhesive ligand presentation

While the preponderance of data strongly suggests that ECM elasticity and topography regulate cells in 2D and perhaps 3D cultures, recent studies suggest that these material properties may exert their effects indirectly by altering ligand presentation. Trappmann et al.[17] showed that changing PAA gel formulations to change ECM elasticity simultaneously altered the presentation of collagen tethered to the gels via sulfo-SANPAH. Due to the porous nature of PAA gels, the authors argued that collagen tethering to the gels changed as gel elasticity was varied, and attributed subsequent changes in MSC fate to changes in ligand tethering rather than ECM elasticity. Reinforcing this argument, the authors showed that PDMS gels of varied elasticity did not alter the differentiation status of MSCs.[17] Another recent study used an innovative Förster resonance energy transfer (FRET) technique to show that MSCs grown on PAA substrates of varied stiffness covalently tethered with fibronectin used tractional forces to unfold plasma fibronectin to a greater extent on stiffer substrates after 24 h [Fig. 5(A-C)]. Unfolding of fibronectin, however, was not observed on PDMS surfaces (though the stiffest PDMS surfaces were $\sim 7\times$ stiffer than the stiffest PAA surfaces), a finding again attributed to differences in material architecture (porosity) of PAA versus PDMS. The degree of unfolding and magnitude of strain of single fibronectin fibers influenced MSC

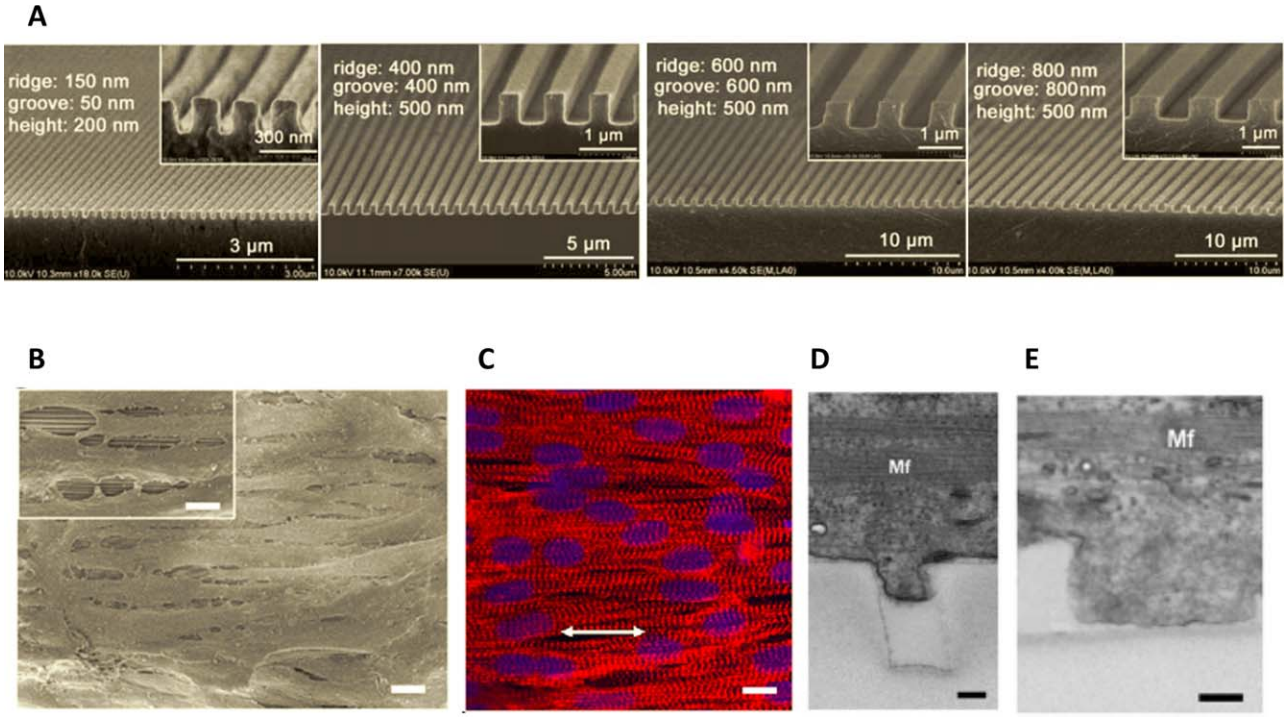

FIGURE 4. Nanotopography influences alignment of cardiac myocytes. A: Scanning electron micrographs of poly (ethylene glycol) (PEG) gels with nanotopography ranging from 50 to 800 nm in size.[62] B: SEM image of neonatal rat ventricular cardiac myocytes grown on fibronectin-coated nanopatterned PEG gel substrates show cells aligned with the underlying nanotopography. Inset shows transverse intercellular connections (scale = 5 μm). C: Immunofluorescent image of sarcomeric α-actinin (red) and nuclei (blue) observed in cardiac myocytes grown on nanopatterned PEG gel substrates (scale = 10 μm). D and E: Scanning electron micrographs illustrate that cells penetrate into nanometer grooves; "Mf" depicts myofilaments (scale = 200 nm). (Adapted with permission from the National Academy of Sciences: *PNAS* 107:2, copyright 2010.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

differentiation [Fig. 5(D-F)]. Enhanced osteogenic differentiation (assessed by ALP staining) was observed in pure osteogenic differentiation media or in mixtures of adipogenic and osteogenic differentiation media when greater strain of fibronectin occurred [Fig. 5(G-I)]. The mechanism for differentiation was attributed to differences in integrin-mediated adhesion that result from cell-mediated stretching of the fibronectin, with preferential binding of $\alpha_5\beta_1$ integrin to the stretched fibers favoring osteogenesis and binding of $\alpha_v\beta_3$ to the relaxed fibers inhibiting it.[13]

Studies in which adhesive ligands are spatially patterned in a controlled manner also underscore the significance of ligand spacing.[88–93] Using an innovative technique based on diblock copolymer micellar nanoparticles,[94,95] Spatz and coworkers[89] have devised methods to spatially pattern adhesive peptides with nanoscale precision and used these methods to investigate how cells respond to different patterns in terms of cell spreading and FA dynamics. Whether or not spatial control of adhesive ligands is able to influence more complicated cell fate decisions remains unknown. Nevertheless, changes in ECM elasticity and nanotopography may manifest in different ligand spacings on length scales relevant for individual cells, and these spacings may be the root cause of different cellular responses. Moreover, cells can use tractional forces to spatially rearrange their adhesive ligands.[33,96]

Non-specific protein adsorption may also play a critical role, particularly in the responses of cells to topographic cues. Prior studies have shown that the ability of MSCs and other progenitor cells to undergo osteogenesis *in vitro* depends on the identity of the adhesive environment.[97–100] It is plausible that substrates with nanotopographic features of different sizes may differentially adsorb ECM proteins from serum, and thereby bind different integrin receptors, activate different signaling pathways, and subsequently induce distinct cells responses. An additional aspect of relevance is the influence of material properties on the conformation of adsorbed proteins.[101] Recent articles in the biomaterials literature note that adsorbed albumin can permit adhesion of platelets and macrophages, despite the protein's lack of known cell adhesive binding sites.[102–104] In addition, fibrinogen reportedly undergoes less conformational change when adsorbed onto films of poly(lactic-glycolic acid) with nanotopography, leading to decreased platelet attachment compared to smooth surfaces.[105,106] While an extensive discussion of protein adsorption is beyond the scope of this review article (instead see Refs. 101,107,108), it is clear that different surface chemistries may differentially affect protein adsorption and downstream cell responses.[109] Consideration of this topic is notably lacking in the context of studies on ECM topography, and may significantly affect interpretation of experimental data.

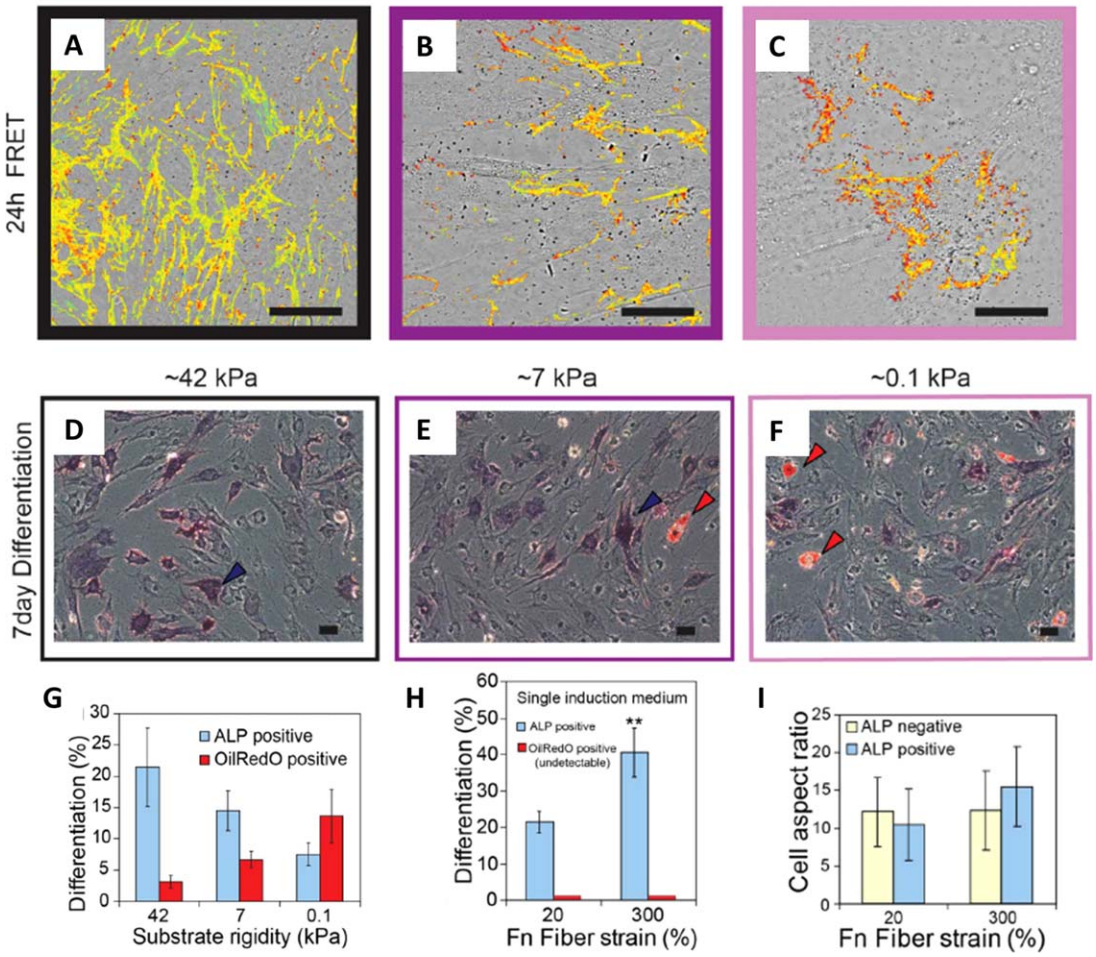

FIGURE 5. Cell-generated forces unfold fibronectin in a manner that depends on ECM elasticity. A-C: Ratiometric FRET-fibronectin images of MSC-assembled fibronectin on fibronectin-functionalized polyacrylamide gels of varying stiffness after 24 h in mixed media show that cells unfold fibronectin fibrils to a greater degree on more rigid substrates (red indicates folded fibronectin, blue indicates completely unfolded fibronectin, and yellow indicating partial unfolding). Scale bars = 50 μm. D-F: Brightfield micrographs of MSCs cultured on fibronectin-functionalized polyacrylamide gels of varying stiffness after 7 day differentiation in mixed (osteogenic and adipogenic) induction medium supplemented with trace amounts of FRET-fibronectin stained for alkaline phosphatase (ALP; blue arrows) and Oil Red O (red arrows). These images suggest the osteogenic differentiation of MSCs cultured on more rigid substrates correlates with cell-mediated fibronectin extension. Scale bars = 50 μm. G: Differentiation percentage of MSCs (mean ± SD, as determined by Oil Red O and ALP staining) after 7 days in mixed media on varied stiffness gels. H: Differentiation percentage of MSCs (mean ± SD, as determined by Oil Red O and ALP staining) after 7 days in single induction media on single strained fibronectin fibers confirmed that osteogenesis correlates with fibronectin strain, and not with cell shape (I).[13] (Adapted with permission from Macmillan Publishers Ltd: *Sci Rep* 3, copyright 2013.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Cells use conserved mechanisms to respond to ECM elasticity and topography

Early attempts to delineate the molecular mechanisms by which cells sense ECM elasticity noted the large, well-defined FA structures in cells on stiff substrates in 2D, in contrast to the small, ill-defined adhesions in cells on softer substrates.[16,22,25,110] Similarly, integrin expression[111,112] and FA morphologies[49,59,63,111–114] have been reported to be altered on topographies of various sizes and shapes. In one study, a critical FA size threshold was identified by culturing fibroblasts on "nanoislands" of fibronectin.[115] Stable integrin-fibronectin clusters did not form below an area threshold of 0.11 μm^2 when cells were confined to adhesive patterns 10 μm in diameter, which enabled the study of integrin-fibronectin cluster formation in cells with the same spread area. Importantly, this threshold limit of 0.11 μm^2 could be dynamically altered by pathways controlling adhesive force, cytoskeletal tension, and structural linkages that transmit forces between cells and the ECM.

Differences in FA size, strength, and composition often reflect changes in actin contractility and thereby implicate RhoA, a small GTPase whose activation enhances nonmuscle myosin IIa-dependent actin contractility by stimulating the formation of stress fibers and FAs.[116] A particularly important study by McBeath et al.[117] about a decade ago underscored the critical role for RhoA and its downstream effects on actomyosin contractility on the control of cell fate by cell spreading. In that study, the authors used fibronectin stamped on PDMS as adhesive islands of controlled area to reveal that MSCs differentiated along an osteogenic lineage

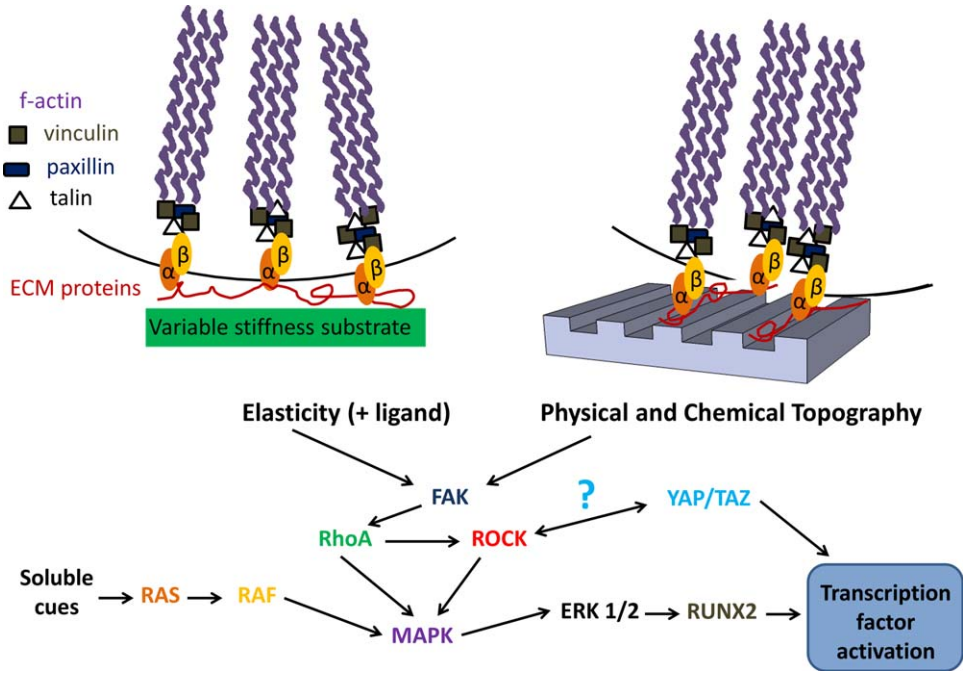

FIGURE 6. Common intracellular signaling events triggered by changes in matrix elasticity and substrate topography. Evidence in the literature suggests that cells share common mechanisms to respond to both physical and chemical topography and matrix elasticity, in some cases leading to changes in gene transcription. Key molecular players include integrins, focal adhesion-associated proteins (FAK and others), RhoA/ROCK, MAPK, and YAP/TAZ. Actomyosin-driven tractional forces, which enable cells to mechanically probe their physical microenvironment, also appear to play a critical and conserved role in cells' responses to ECM elasticity and topography. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

when allowed to spread; when spreading was restricted, they differentiated along an adipogenic lineage. Furthermore, the authors showed that RhoA/ROCK-mediated contractile forces were mechanistically at the heart of this lineage regulation by cell shape.[117] Kilian et al.[118] extended these concepts by exploring the influence of cell shape independent of cell surface area. MSCs were exposed to mixed osteogenic and adipogenic differentiation media and grown on fibronectin stamped islands of varied size and shape (but equal spread cell area). Shapes that caused cell elongation (e.g., star shapes) led to MSC differentiation along an osteoblastic lineage, while pharmacological disruption of cytoskeletal tension forced cells along an adipogenic lineage.[118]

The RhoA/ROCK-mediated signaling pathway and its effects on cell-generated forces also appear critical for ECM-dependent control of cell fate in 3D.[119] Using a dynamic hyaluronic acid hydrogel platform, Khetan et al.[120] demonstrated that MSC differentiation to an osteogenic fate in 3D requires RhoA/ROCK-mediated tractional forces, independent of changes in elastic modulus or cell shape. Specifically, they showed that MSCs capable of spreading and generating relatively high levels of traction force on their adhesive contacts undergo osteogenesis; however, when the gel substrates were effectively locked into place on the fly through a secondary cross-linking strategy, traction forces were suppressed, gel degradation was impeded, and the cells differentiated into an adipogenic fate, despite being spread.[120]

Focal adhesion kinase (FAK), another key regulator of mechanotransduction generally regarded as upstream of RhoA activation, is also influenced by changes in substrate elasticity and nanotopography.[111,121] With respect to the former, total FAK levels reportedly increased with increasing matrix elasticity in MSCs,[30] while phosphorylated (active) FAK increased in ECs[24] and preosteoblasts.[25] Nanotopographic substrates in the form of 14–45 nm nanopits[111] or 250 nm nanogratings[121] also increased FAK activity. Differential activation of FAK in turn triggers downstream signaling to the mitogen-activated protein kinase (MAPK) cascade, which conveys information about the extracellular environment to the cell nucleus and plays an important role in normal and pathologic development.[122] Evidence suggests that MAPK activity depends on ECM nanotopography[114] and matrix stiffness and is involved in the regulation of stiffness-mediated differentiation of osteogenic progenitors.[19] A subsequent study showed that changes in substrate elasticity alter the RhoA-Rho-kinase (ROCK) pathway upstream of changes in the MAPK cascade.[122] This pathway in turn influenced the transcription factor RUNX2 to control osteoblast differentiation and matrix mineralization (Fig. 6). Collectively, these findings suggest that activation of a FAK/RhoA/ROCK/MAPK signaling axis via changes in ECM elasticity and topography may play a central role in the ECM's ability to control cell fate decisions.

There is compelling evidence that these mechanosensitive signaling pathways can regulate transcriptional activity via both direct and indirect means.[123] However, how

physical cues like ECM elasticity and topography drive changes in cell fate remain incompletely understood, and new players continue to emerge on the scene. Recent evidence indicates Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), two members of the Hippo pathway implicated in organ growth control, may play essential roles linking changes in ECM cues with control of cell fate.[124–129] When phosphorylated, YAP and TAZ remain in the cytosol and are targeted for proteosomal degradation; when dephosphorylated, YAP and TAZ can translocate to the nucleus where they can regulate transcriptional activity.[126] A 2011 study by Dupont et al.[125] used fibronectin-conjugated PAA hydrogels with tunable elastic moduli to demonstrate that YAP and TAZ are differentially activated by ECM elasticity, with higher activities (elevated nuclear translocation, in this case) observed on rigid substrates. Similarly, YAP/TAZ preferentially accumulated in the nuclei of MSCs cultured on micropatterned fibronectin adhesive islands that permitted cell spreading, while remaining predominantly cytoplasmic in cells that were restricted from spreading. Importantly, osteogenic differentiation of MSCs induced by rigid substrates or cell spreading was inhibited when YAP and TAZ levels were depleted via RNA interference, leading instead to adipogenic differentiation.[125] In 3D collagen matrices, the interplay between ECM rigidity, cell shape, and matrix proteolysis is more complex, but the ability of MSCs to generate tension on their environment and activate YAP/TAZ to control MSC fate is still conserved.[128] A recent study by Sun et al.[127] showed that YAP and TAZ also play key roles in the ECM rigidity-dependent differentiation of human induced pluripotent stem cells into functional motor neurons. YAP and TAZ were recently investigated in the cellular response to ECM nanotopography,[130] but to our knowledge this is the only study linking them. Given the conserved importance of cytoskeletal tension in the ability of a cell to probe its physical/mechanical environment, one would expect additional studies linking these inputs and signals to appear in the near future.

IMPLICATIONS AND CONCLUSIONS

It is clear from the findings discussed here that matrix elasticity and topography can influence cell behavior, particularly *in vitro*. The implications of these results for biomaterial design are powerful, with the possibility that tailoring material elasticity and topography can be used as a complement to, or instead of, soluble cues to control cell phenotypes and tissue morphogenesis in clinical settings. However, many questions remain if these parameters are to be used to consistently and robustly control cell fate both *in vitro* and *in vivo*. One such question is the issue of duration: how long do ECM topography and elasticity exert control over cell function? A recent study suggests that cells have mechanical memory, and prior culture on rigid polystyrene substrates can bias their response to ECM elasticity.[129] It is possible that ECM physical cues may initiate epigenetic changes, but this possibility has yet to be investigated in depth. Another obvious question is the influence of these ECM cues on cell fate *in vivo*. Most studies cited here involve culture of cells *in vitro*, with the vast majority in 2D; whether elasticity and topography are able to drive cell fate in 3D and *in vivo* remain open questions, although there is provocative evidence that these cues are important in pathophysiological environments *in vivo*.[131,132]

There is also a compelling need for complete functional analysis of cell behavior as a function of varied material characteristics, rather than the more limited gene and protein expression studies used as surrogates for differentiated function in most studies. Some examples include quantitative and qualitative assessments of mineralization for osteoblasts and the resulting impact on tissue mechanical properties, electrophysiology studies for neurons, and calcium propagation and synchronous contraction for cardiomyocytes. Inconsistencies in the studies to date make consensus difficult to achieve as well, including the use of a wide range of material types, surface chemistries, topographic feature shapes and sizes, matrix elasticities, ligand types (e.g., collagen vs. fibronectin, etc.), and coupling chemistries (e.g., sulfo-SANPAH vs. others). Thus, while certain topographies and substrate elasticities may drive cell differentiation of specific cell types via mechanotransduction, it remains a huge challenge to recommend any particular set of biomaterial parameters for regenerative medicine applications. Nevertheless, the exciting potential of such an approach clearly warrants further study. A better understanding of the mechanisms by which cells respond to ECM cues should also aid efforts to rationally prioritize material properties for therapeutic benefit.

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