Chapter 18
Biomimetic Surfaces for Cell Engineering

John H. Slater, Omar A. Banda, Keely A. Heintz and Hetty T. Nie

Cell behavior, in particular, migration, proliferation, differentiation, apoptosis, and activation, is mediated by a multitude of environmental factors: (i) extracellular matrix (ECM) properties including molecular composition, ligand density, ligand gradients, stiffness, topography, and degradability; (ii) soluble factors including type, concentration, and gradients; (iii) cell–cell interactions; and (iv) external forces such as shear stress, material strain, osmotic pressure, and temperature changes (Fig. 18.1). The coordinated influence of these environmental cues regulate embryonic development, tissue function, homeostasis, and wound healing as well as other crucial events in vivo [1–3]. From a fundamental biology perspective, it is of great interest to understand how these environmental factors regulate cell fate and ultimately cell and tissue function. From an engineering perspective, it is of interest to determine how to present these factors in a well-controlled manner to elicit a desired cell output for cell and tissue engineering applications. Both biophysical and biochemical factors mediate intracellular signaling cascades that influence gene expression and ultimately cell behavior [4–7], making it difficult to unravel the hierarchy of cell fate stimuli [8]. Accordingly, much effort has focused on the fabrication of biomimetic surfaces that recapitulate a single or many aspects of the in vivo microenvironment including topography [9–12], elasticity [5], and ligand presentation [13–17], and by structured materials that allow for control over cell shape [18–23], spreading [18, 23–25], and cytoskeletal tension [26–28]. Controlled presentation of these properties to develop a desired microenvironment can be harnessed to guide cell fate decisions toward chosen paths and has provided a wealth of knowledge concerning which cues regulate apoptosis [29–34], proliferation [35–39], migration, lineage-specific stem cell differentiation [7, 8, 15, 18, 23, 37, 40–44], and immune cell activation to name a few. This chapter focuses on the implementation of biomimetic surfaces that recapitulate and control one or more aspects of the cellular microenvironment to induce a desired cell response. More specifically, biomimetic

J. H. Slater (✉) · O. A. Banda · K. A. Heintz · H. T. Nie
Department of Biomedical Engineering, University of Delaware, Newark, DE 19716, USA
e-mail: jhslater@udel.edu

© Springer International Publishing Switzerland 2016

M. Zhang et al. (eds.), *Carbon Nanomaterials for Biomedical Applications,*
Springer Series in Biomaterials Science and Engineering 5,
DOI 10.1007/978-3-319-22861-7_18

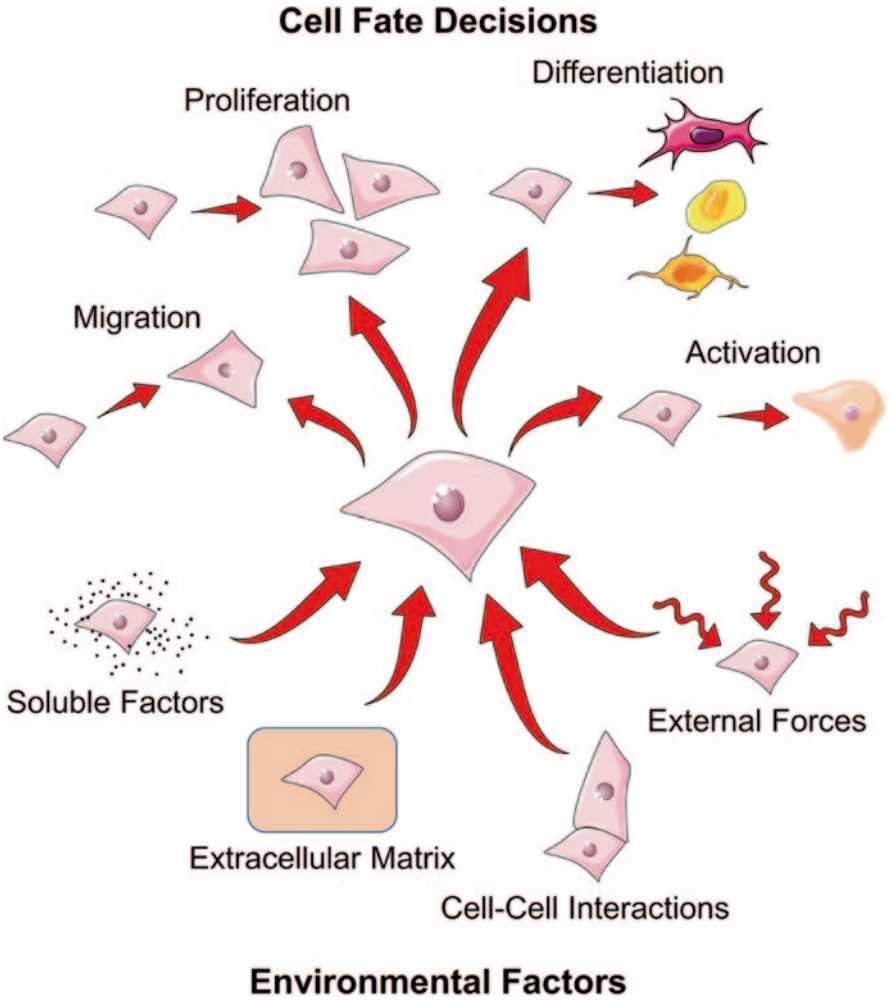

Fig. 18.1 Environmental stimuli regulate cell fate decisions. A multitude of environmental factors regulates cell fate decisions. Cells respond to both soluble and matrix-bound growth factors, extracellular matrix properties including composition, ligand density and spacing, stiffness, and topography, cell–cell interactions, and the application of external forces including shear stress, material strain, osmotic pressure, and temperature changes. The culmination of these environmental inputs regulates cell behavior with respect to migration, proliferation, differentiation, and activation to name a few. The major focus of much research involves the fabrication and implementation of biomimetic surfaces that allow for direct modulation over one or more of these environmental factors to elicit a desired cellular output.

surfaces that mimic in vivo ECM composition, density, gradients, stiffness, or topography; those that allow for control over cell shape, spreading, or cytoskeletal tension; and those that mimic cell surfaces are discussed.

18.1 Influence of ECM Properties on Cell Behavior

Many mammalian cells are adherent-dependent, meaning that they must adhere to ECM to maintain viability and to function properly. Accordingly, much research has focused on manipulating ECM properties to investigate its influence on cell fate. To understand how ECM properties influence cell behavior, it is important to understand how cells adhere and the process of adhesion maturation. Cell adhesion to the ECM is regulated by integrins; alpha/beta heterodimeric transmembrane glycoproteins that recognize short peptide sequences residing within ECM proteins [45]. Specific combinations of alpha/beta subunits recognize different ligands; some integrins recognize more than one ligand and some ligands ligate more than one integrin [46]. Upon activation, integrins interact with talin to form a small cluster that acts as an initiation site for adhesion formation [47]. Adhesions mature from small nascent adhesions, to focal complexes, to focal adhesions through force-mediated actomyosin contraction, and eventually fibrillar adhesions, if cultured on fibronectin [48]. This coordinated maturation process is regulated by both ECM composition and the transmission of force to adhesion plaques [49]. During the maturation process, the molecular composition, size, and location of adhesions change in addition to conformational changes in force- sensitive adhesion site proteins [47, 48, 50–53]. These molecular alterations induce changes in adhesion-mediated signaling events that regulate gene expression [49, 54, 55]. As anchoring units to the ECM, adhesions are also key mediators of migration. The coordinated formation of a lamellipodium and new adhesions at the leading edge, actomyosin contraction within the cell, and disassembly of mature adhesions at the trailing end are responsible for allowing cells to migrate on surfaces [56]. Migration is a critical function involved in wound healing, angiogenesis, immune responses, and embryonic development to name a few [57]. When integrin function is impaired or unregulated [58], adhesion properties can change drastically as observed in a number of disease states including cancer metastasis, arthritis, asthma, thrombosis [59], muscular dystrophy [60, 61], skin-blistering disease [62–64], and leukocyte adhesion deficiency [65]. A number of environmental cues influence these events including ECM composition, integrin/ligand affinity [66], presentation/orientation of integrin-ligating domains [44, 67–69], ligand gradients [70], and underlying material properties including wettability [71], stiffness [72, 73], topography [74], and degradability [75]. Cells also sense ligand and stiffness gradients that induce haptotaxis [76, 77] and durotaxis [78, 79], respectively.

Since adhesion formation and maturation play a large role in intracellular signaling of adherent-dependent cells, there has been much interest in fabricating engineered surfaces that control these processes. In many situations, it is advantageous to utilize ECM-derived peptides rather than full ECM proteins. In 1984, Pierschbacher and Ruoslahti discovered the $\alpha_v\beta_3$ ligating peptide RGD [80]. Since its discovery within fibronectin [80], additional proteins containing the RGD sequence have been identified including vitronectin [81], osteopontin [82], laminin [83], and collagen [84], although not all of these RGD sequences are readily accessible by cells [85].

In addition to RGD, a multitude of integrin-ligating peptide sequences have been discovered [85, 86] including IKVAV and YIGSR in laminin that ligate $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_6\beta_1$ integrins [87]; PHSRN [88] and REDV [89] in fibronectin that ligate $\alpha_5\beta_1$ and $\alpha_4\beta_1$, respectively, and GFOGER [88], a collagen-mimetic helical peptide [90], that ligates $\alpha_2\beta_1$ to name a few. As differential integrin ligation can significantly influence cell behavior [91], it is important to choose the proper ECM proteins or peptides for mimicking the tissue of interest. Generally, peptide sequences are not as efficacious as their native proteins, therefore cyclic peptides [92] and peptide fragments that span the entire binding domain have been developed [85]. Peptides have the advantage of being more stable and specific compared to the multiple ligation sites present in full proteins [93]. To demonstrate that a peptide behaves as expected, scrambled peptides are commonly used as nonadhesive controls [94, 95]. When using peptides, it is important to make them accessible for integrin ligation. By incorporating short spacers between the surface and conjugated peptide or protein, the biomolecule can become more accessible allowing efficient cell adhesion and spreading [95]. Interactions between poly(ethylene glycol) (PEG) spacers and peptides or proteins should be considered to prevent aggregation and maintain peptide and protein accessibility [67].

In addition to ECM proteins, various growth factors including epidermal growth factor (EGF) [96], basic fibroblast growth factor (bFGF) [97, 98], and vascular endothelial growth factor (VEGF) [99] have been studied as they can enhance proliferation, migration, and angiogenesis. In vivo, growth factors are continuously bound and released from the ECM [100]; therefore, the impacts of adsorption and tethering of these elements to surfaces to determine if efficacy and bioactivity can be retained have been investigated. Gradients of morphogenetic proteins are also involved in differential gene expression that determines cell fate [101]. The proteins tenascin, thrombospondin-1, and osteonectin/secreted protein acidic and rich in cysteine (SPARC) are of interest as they are known to control cell detachment from the ECM in wound healing and are also implicated in tumorigenesis [102–104]. Although not currently studied in conjunction with bioactive surfaces, these proteins could be useful in promoting and controlling cell migration and should be considered when creating biomimetic materials [86].

18.2 Surface Functionalization

As discussed in the previous section, many aspects of the ECM influence cell behavior. To create biomimetic surfaces, one must be able to functionalize surfaces with peptides and proteins of interest. There are countless methods and techniques used to link biomolecules including ECM-associated peptides, proteins, and growth factors to material surfaces [105, 106]. Even the simplest studies investigating the influence of ECM properties on cell behavior often involve advanced fabrication techniques. Biomimetic surfaces created using laser scanning lithography (LSL) [107–111], microcontact printing (μCP) [112], microfluidic devices [113, 114], and

block copolymer micelle nanolithography (BCMN) [115] as well as others have allowed researchers to study the influence of various surface-displayed biomolecules on cell behavior. Peptides are often covalently linked to surfaces via an amide bond between a carboxylic group on the substrate, and the N-terminus of the peptide [93], although many other bioconjugate methods applying various functional groups exist [116]. To investigate the spacing or size of ECM components on integrin spacing or adhesion site growth and maturation, it is crucial to possess subcellular control over how the ECM is displayed. Many techniques for high-resolution control of patterned ECM components have been developed and a few are discussed here. For sub-100-nm resolution patterning of proteins, dip-pen nanolithography allows alkanethiol self-assembled monolayers (SAMs) to be formed on a gold surface by direct writing with a SAM-coated atomic force microscope (AFM) tip. The self-assembled monomers are delivered to the surface via transport, the surrounding non-patterned areas functionalized with a nonadhesive SAM, typically a poly(ethylene oxide) (PEO)-terminated SAM, and protein is adsorbed to the patterned SAM providing a means to create high-resolution, subcellular-sized patterns of ECM components [117, 118]. Nanosphere lithography also allows for sub-100-nm to micrometer-sized patterning of ECM proteins and direct control over adhesion site growth, spacing between adhesions, and adhesion site density [119, 120]. BCMN is a method for patterning gold nanoparticles, which can be functionalized with single cyclic RGD peptides allowing for ligation of single integrins in desired locations. LSL can be applied to pattern a wide variety of proteins and peptides, works in 2D and 3D applications, and relies on laser-induced photocoupling of biomolecules to hydrogels or photothermal decoupling of SAMs on metallic surfaces [107–111]. Regardless of the method used when adsorbing or linking biomolecules to the surface of a material, it is important to note how these processes impact the structure and presentation of the proteins and ligands as these elements can further impact binding events [67, 68].

18.3 ECM Mimetic Surfaces

The physical spacing and clustering of ligands is of importance to cells. To vary the magnitude of cell adhesion, one can vary integrin expression level, integrin–ECM binding affinity, or ECM ligand surface density [66] and spacing. In vivo, different ligand densities and spacing can be optimal for different cellular processes, as integrin ligation controls intracellular signaling events [49, 54, 55]. Furthermore, if ligands are clustered at the appropriate spacing, integrins will cluster, thereby allowing stable adhesion formation and maturation [48]. Along with specific mechanical and chemical composition, it is important to note that the ECM can contain various structural features that correspond to the type of cells it is supporting [121]. The scale of these structural features varies from the nano- to microscale, with the arrangement and orientation of fibrillar fibronectin and collagen networks largely impacting how the ECM interacts with cells in tissues [122]. As an example of

how surface mimicry is important to cell migration, collagen-coated surfaces with and without D-periodicity were studied [123]. Collagen contains a banding pattern every 67 nm called the D-period; collagen prepared in the absence of potassium ions forms without banding. Cell orientation and direction of motion depend on the D-period where cells move parallel to the collagen fibers with the D-period, while cells cultured on collagen fibers without the D-period exhibit random motion. Thus, the D-period is an essential structural feature of the ECM for directional fibroblast migration [123]. To mimic these nanoscale structural elements in ECM proteins, several approaches have been developed for altering ligand density, cluster size, and integrin spacing including PEO bioconjugation [124, 125], BCMN [69, 92, 115], and nanopatterning [119, 120]. Using these structured materials, one can investigate the influence of integrin spacing or adhesion site growth on cell adhesion, migration, differentiation, and proliferation. To obtain surfaces with various spacing of RGD-modified PEO in a brush layer, the ratio of polystyrene-PEO-maleimide and polystyrene-PEO in a polystyrene homopolymer can be varied. The maleimide group can be reacted with cysteine-GRGDS or cysteine-GRGES peptides to vary the surface density of RGD to investigate the influence of RGD spacing on cell fate. Migration rates for human bone marrow mesenchymal stem cells (hMSCs) were fastest when the RGD peptides were spaced at 50 nm [125]. As lateral spacing of RGD was increased, cells displayed a more adipogenic morphology, while at smaller lateral spacing, the cells were more osteogenic [125]. These results indicate that simply altering integrin packing density can have a profound influence on cell fate. A similar bioconjugation technique varied the ratio of peptide-modified, YIGSR in this case, star-PEO molecules, to unmodified star-PEO molecules, and the number of peptides per star-PEO to obtain surfaces with varying ligand densities and spacing to control integrin clustering [124]. Using this approach, the impacts of peptide spacing and density can be decoupled. By increasing the cluster size of the adhesive ligand, similar cell migration rates can be obtained with a lower overall surface density. The ligand clusters created in this manner, however, are randomly spaced, and thus the surfaces can have areas with larger ligand clusters if two or more functionalized star-PEO molecules are in close proximity [124]. In a more controlled fashion, integrin spacing can be altered by depositing gold nanoparticles onto a surface using BCMN [115]. BCMN relies on the self-assembly of diblock copolymer micelles around small gold particles; these micelles are then dip-coated onto a substrate. After the micelle-coated substrate is subjected to plasma treatment, the gold particles remain on the surface [115]. After gold nanoparticle deposition, the open spaces on the surface are backfilled with PEG, and the particles functionalized with a cyclic RGD. The backfilling of PEG accounts for any topographical features on the surface, as the thickness of the PEG layer can be set to match the diameter of the nanoparticles to create a patterned surface free of topography [115]. Cells on these surfaces migrate along ligand gradients toward areas with denser ligand concentration [92]. This patterning approach was also implemented to determine the critical distance between individual RGD groups and subsequently integrin spacing for mesenchymal stem cell (MSC) adhesion. It was determined that a minimum integrin spacing of approximately 70 nm is needed for cell adhesion and spreading with

cells unable to effectively spread above that threshold [44]. The same integrin spacing was previously reported for both osteoblasts and fibroblasts indicating a universal distance needed for tight integrin packing to support adhesion formation [126]. It is suspected that this spacing reflects the distance between the integrin β-subunit binding domains of the talin1 protein [127]. BCMN can also be used to create disordered and ordered arrays of RGD functionalized gold nanoparticles against a non-adhesive background. Disordered arrays of cyclic RGD were able to support cell adhesion above the 70-nm interligand distance. When ligand spacing is ordered, and above 70 nm, minimal potential exists for integrin clustering; however, when ligand spacing is disordered, and above 70 nm, there is more potential for ligands to randomly be close enough for integrin clustering and stable adhesion formation [69]. Similarly, gold palladium nanodots can be deposited in defined clusters using nanoimprint lithography [128]. These cyclic RGD functionalized nanodots were utilized to investigate both the spacing between integrins and number of integrins per adhesion on cell migration. For 3T3 fibroblasts, it was determined that at least four nanodots per adhesion were required to achieve almost a threefold increase in the number of adherent cells compared to when only two or three nanodots existed per cluster. The optimal interdot spacing in a cluster was determined to be approximately 60 nm. The global density of nanodots did not impact cell spreading when the distance between nanodots in each cluster was held constant at 60 nm, indicating that the ability to cluster integrins is more important than the global ligand density in inducing stable adhesion and cell spreading [128]. Similar results were achieved using nanopatterned surfaces created with nanosphere lithography. It was demonstrated that local ligand density is more important than global ligand density in supporting cell adhesion and proliferation using nanopatterned surfaces displaying patterned fibronectin [120]. Interestingly, while nanopatterns ranging from ~ 100 to 1500 nm all supported adhesion and proliferation, cell migration was 4.8-fold faster on 100-nm-sized patterns [119]. Additionally, an exponential decay in cell migration with increased pattern size was observed indicating that stable adhesion formation retards migration [119]. All of these studies indicate that the formation of stable adhesions is crucial in cell attachment, spreading, and proliferation and that inducing unstable adhesion via increased integrin spacing or limiting adhesion site growth can induce highly migratory cell behavior. Findings from these high-resolution, nanopatterned biomimetic materials provide much insight into the influence of ECM nanoscale structure and organization on cell behavior and a means to locally control cell function via adhesion site manipulation. Using these highly structured surfaces, one may be able to spatially control cell migration, proliferation, and differentiation providing a single platform to investigate many aspects of cell fate as a function of ECM properties.

ECM composed of fibrillary proteins often contains well-organized topography that cells recognize and respond too. Various approaches to mimic these native protein structures exist. When grooves exist on the surface of a material, cell migration in the direction parallel to the grooves is often significantly higher than migration perpendicular to the grooves; cells migrating on grooved structures prefer to orient and migrate in the direction of the grooves; thus, grooves can be seen as guiding

cell motion [74, 129, 130]. Cells typically orient more with increasing groove depth and orient less with increasing groove width and pitch [131]. Using this knowledge, topographic features have been implemented to guide vascular organization of human endothelial colony forming cells (ECFCs) by creating fibronectin patterned polydimethylsiloxane (PDMS) micropillars. The micropillars induce the cells to align in a single-cell chain manner [132]. Different cell types are not equally sensitive to topographical features; endothelial cells (ECs), human fibroblasts cells (FCs), and smooth muscle cells (SMCs) all behave differently on identical grooved structures in PDMS. FCs show much higher alignment and directional migration than ECs and SMCs [133]. These results indicate that surface topography acts as another parameter that must be controlled or that can be utilized in inducing desired cell behavior.

While the influence of ECM proteins, ECM-derived peptides, or topography on cell behavior has been discussed, it has been shown that ECM-bound growth factors play a role in cell fate also. Methods to increase wound healing and endothelial tubule formation for tissue vascularization have been developed using combinations of ECM proteins and ECM-associated growth factors [99, 134]. ECs cultured on hydrogel surfaces co-patterned with RGDS and VEGF in 35–70-μm-wide strips undergo more tubulogenesis compared to hydrogels patterned with RGDS alone, indicating that matrix-bound VEGF plays a significant role in EC function [99]. The strips of ligand and protein are important for cells to align and form cords or prevascular structures [134]. To improve wound-healing capabilities, fibrin matrices have been engineered to contain a multifunctional recombinant fragment of fibronectin that has the ability to bind fibrin for incorporation into a fibrin matrix, interact with integrins for cell adhesion, and promiscuously bind growth factors [135]. Migration of SMCs, ECs, and MSCs were all enhanced when the growth-factor-binding component was incorporated [135], indicating that bound or conjugated growth factors can also be considered in cell engineering applications with biomimetic materials. These matrices were also tested for their wound-healing capabilities in skin and bone; cell staining showed rapid cell migration into the enhanced fibrin matrices and significant improvement in wound healing than when growth factors were delivered in a standard fibrin matrix [135]. A similar study using the growth-factor-binding component was completed using a composite PEG synthetic fibrin-mimetic matrix beneficial for its more likely straightforward path to clinical approval [136]. Due to the likely engineering benefits of incorporating matrix-bound growth factors, some growth factors have been genetically engineered to contain a domain of placenta growth factor-2 (PlGF-2) to increase affinity for ECM proteins [137]. The ability to incorporate surface-bound growth factors with desired ECM properties opens new avenues for cell engineering research.

Aside from substrate biochemical properties, the mechanical properties of a material have a profound influence on cell behavior [72, 73, 79, 138–145]. In vivo, cells experience regions of various stiffness corresponding to associated tissues [73]; muscle ranges from 3 to 6 kPa, and arteries range from 24 to 45 kPa [146], whereas brain tissue has stiffness values at several hundred pascals, and tendon and cartilage in the megapascal range [139]. Furthermore, stiffness gradients exist at the

interface between ligaments, articular cartilage, and bone; bone composition itself is also highly variant with sections ranging from compact to spongy [70]. In order to control cell behavior on biomimetic materials, it is important to consider mechanical properties as well as chemical properties. Investigating substrate stiffness on cell behavior, however, has found difficulty in decoupling stiffness from other material properties, as altering the substrate stiffness of a native ECM material can also influence ligand availability and pore size [8, 138]. Fortunately, the use of synthetic hydrogel matrices has allowed scientists to overcome some of these limitations; synthetic hydrogels allow for decoupling of matrix stiffness and ligand density but they cannot overcome variations of pore size and density associated with materials of variant stiffness [8, 138, 142]. It has been demonstrated that substrate stiffness can influence a number of cell fate decisions including migration and stem cell differentiation. For example, stiff substrates (>40 kPa) prove to induce osteogenesis, medium stiffness substrates (~10 kPa) induce myogenesis, and soft substrates induce adipogenesis and neurogenesis [27, 42]. However, surface porosity may alter and attenuate the effects of substrate stiffness. To modulate substrate elasticity of gels, the substrate cross-linking can be modified by adjustments in molecular weight or weight percent [147, 148]. On gel substrates, increased elastic modulus decreases the porosity, which may increase cell contact area [8, 149]. However, soft porous substrates have much larger pores than their stiff counterparts and therefore allow for less cell contact area [8]. Conversely, on nonporous substrates, the cell contact area is preserved and all samples tend to induce osteogenesis regardless of stiffness [8]. This indicates that cell contact area also influences stem cell differentiation and that materials used for such experiments must be well characterized to determine which parameters are influencing cell fate decisions. Although stiffness and porosity may conflict, cells patterned with high aspect ratios on soft substrates still tend toward osteogenesis [150]. Earlier methods approaching this problem involved the combination of synthetic and native substrates [151]. By using shaded patterns laser-printed on transparency slides [152] or a microfluidic gradient maker [153], polyacrylamide hydrogels were photopolymerized with graded stiffness. Using a layer of collagen covalently linked to the top of the gradient hydrogel, cells are exposed to a surface with uniform molecular composition, but they can sense the stiffness gradient of the underlying material. Thicknesses of various layers do have an influence; studies have been performed to determine how deeply cells can sense their substratum [154, 155]. Bovine vascular SMCs cultured on these combination surfaces migrate slightly faster on softer gels than on stiffer gels; however, cells migrate toward and accumulate in stiffer regions [151]. Normal rat kidney epithelial cells, NIH 3T3 fibroblasts, and bovine pulmonary arterial ECs also show increased motility on soft surfaces and accumulate in stiffer regions [72, 140, 156, 157]. Likewise, using combinations of soft and stiff materials, pieces of glass have been embedded in polyacrylamide hydrogels of varying thickness [146]. The thickness of the overlying hydrogel determines how stiff the material appears to cells, due to the close proximity of the underlying glass. When cells are cultured on thicker hydrogels, the cells lose the ability to sense the glass, and the material appears more compliant. One can also vary the substrate geometry to create gradients and

steps in stiffness. Using this approach, the overall pore size and material density remain constant while the apparent stiffness is altered. This assay could be applied to study cell migration due to substrate stiffness with a wide range of hydrogel materials and cell types [146]. Material stiffness can also be studied in how it relates to cancer cell migration. Explained by metastasis, mammary epithelial cells have been shown to be more ignorant of material stiffness as they transition from being non-transformed to fully transformed [158]. These results indicate that cells sense both biochemical and mechanical properties of ECM mimetic materials providing a large set of parameters researchers can utilize to develop engineered materials to guide cell fate decisions.

18.4 Utilizing Single-Cell Patterns to Regulate Spreading, Shape, and Cytoskeletal Tension

While the previous section discussed biomimetic materials that recapitulate in vivo ECM properties, this section discusses the implementation of patterned ECM for the creation of single-cell patterns that can be implemented to induce a desired cellular response. Culturing cells on engineered surfaces that present an array of a patterned ECM protein in a user-controlled, well-defined geometry against a biologically inert background has proven to be a viable method to create a more homogenous cell population during in vitro culture [159, 160]. These surfaces not only allow for the manipulation and investigation of individual cells but also, through arraying, allow for the generation of a large population of single cells to be investigated simultaneously [109, 159–161]. Culturing cells on ECM patterns of varying size or geometry allows for a high level of control over many cell properties including the extent of spreading [159, 162], shape [163, 164], and adhesion site arrangement with respect to size, spacing, and shape [120, 165–168]. Manipulation of these properties has been implemented to influence cell morphology [163, 164], cytoskeletal organization [169], the distribution of intracellular components (nucleus, centrosome, Golgi apparatus) [163], and cell polarity [163, 164], and to manipulate the extent of cytoskeletal tension generated in cells in order to modulate their behavior [159, 160, 162, 166]. For example, modulation of the extent of cell spreading was used to regulate hepatocyte function [162] and to direct lineage-specific MSC differentiation [159]. Similarly, regulation of cell shape, while maintaining a constant spread area, was implemented to guide lineage-specific MSC differentiation [160]. Variations of contact area and cell shape influence stem cell differentiation independently. Cells with large spread areas lead to greater osteogenesis [37] or myogenesis [18], while those with restricted cell spreading lead to greater adipogenesis [37] or chondrogenesis [18]. However, differences in lineage commitment are also seen in different shapes with the same spread area. Cells with high aspect ratios tended toward osteogenesis or neurogenesis, while those with the same spread area but an aspect ratio closer to one tended toward adipogenesis [37]. Additionally, cells of matching spread area with concave shapes and protruding

features also tend toward osteogenesis, while their rounded counterparts tended toward adipogenesis [23]. Protruding features create a cusp that allows for anchorage of many stress fibers, resulting in a potential increase in contractility and isometric cell tension than those with rounded features [170, 171]. Increased cellular tension alters cell signaling through force-mediated pathways [37] which act largely through myosin II isoforms [42]. Force-mediated differentiation is also the center of the "differential adhesion hypothesis" which suggests that different cell lineages have different numbers and compositions of adhesion proteins [172]. Cells cultured on substrates of matching elastic moduli and shape can differentiate into distinct mature lineages through use of different adhesive ligands [43]. Collagen induces greater neurogenesis, while laminin induces greater adipogenesis [173]. Surface chemistry alters cell fate as adhesive proteins ligate different integrins resulting in the activation of different signaling cascades [15, 43]. Cell shape, tension, substrate elasticity, and adhesive ligands are all able to dictate stem cell lineage commitment individually, under different conditions. However, these various factors may have some crosstalk. Therefore, biochemical and biophysical cues must work synergistically to create microenvironments to induce lineage-specific stem cell differentiation [174].

In addition to stem cell differentiation, patterned surfaces that regulate single-cell properties have been implemented to regulate cell proliferation. Cell–cell interactions were originally thought to arrest cell proliferation as cells plated at low density proliferated, while high-density populations arrested proliferation [37]. Although accurate, this observation overlooked the ability of low-density populations of cells to spread, while high-density populations cannot [37]. In cells with restricted spread area, having multiple cell–cell interactions arrested proliferation [36]. However, cells with restricted spread area but only one cell–cell interaction proliferated [39] revealing a regulatory role for cell–cell interactions in proliferation. Cell–cell interactions regulate proliferation first through rearrangement of cell cytoskeleton, as adherens junctions serve as cytoskeletal anchorage points for the similar to adhesion sites for cell–substrate interactions [175]. Likewise, these adherens junctions stimulate signaling cascades some of which, inclusive of RhoA [36], PI3K [38], and Rac1 [175], are known to induce proliferation. Wells that restrict cell spreading can be created by using capillary action to wick a polymer precursor solution between a PDMS master and a functionalized surface [36, 38, 39]. The precursors are thermally polymerized and the PDMS master removed to reveal wells. To alter the shape of the wells, new PDMS must be fabricated, either using replica molding or traditional photolithography [176]. Wells with simple geometries will collect one cell per well with passive seeding. For complex patterns where passively seeded cells will overpopulate the pattern, small electrodes can be included to act as electrical traps and ensure the collection of the desired number of cells [36]. Similar to observations of high and low plating density [37], cells cultured on adhesive surfaces tend to proliferate when spread, but arrest proliferation when cell spreading is restricted [25, 177]. Similar to cell spreading area, restricting adhesive area also arrests proliferation [24, 44]. Adhesion sites not only act as the interface between surfaces and cells but also aid in tension generation, and activate kinases [178], such as focal

adhesion kinase [25, 179, 180] and src [181] under high adhesive states and stimulate cell proliferation. The ability to modulate cell behavior through regulation of cell spreading or shape stems from differences in the amount of cytoskeletal tension generated in cells as dictated by the pattern size or geometry [159, 160]. While the specific force-sensitive proteins that regulate cytoskeletal tension-mediated control over intracellular signaling remain elusive, it is known that RhoA's influence over ROCK-mediated myosin contraction of actin stress fibers is the main regulator of tension generation [159], and cells with higher levels of intracellular tension display larger adhesion sites [159, 160, 182], more prominent actin stress fibers [159, 160, 182], increased JNK and ERK activation [160], and elevated Wnt signaling [160]. These results indicate the importance of cytoskeletal tension in determining cell behavior and that modulation of cellular tension can make cells more sensitive to [160] or override soluble signals [159]. Following this train of thought, a new biomimetic, cell-derived patterning technique was recently developed that allows for direct recapitulation of the morphology or adhesion site arrangement of user-chosen cells of interest [109]. Rather than implementing simple geometries, cell-derived patterning implements images of the cells of interest as templates for pattern formation resulting in direct recapitulation of the shape, adhesion site arrangement, and cytoskeletal architecture of user-chosen cells of interest [109]. As single-cell patterns become more sophisticated, tighter control over cell shape, adhesion, and cytoskeletal tension will allow for more thorough investigations of these properties on cell fate decisions and potentially lead to biomimetic surfaces that allow for direct control over cell phenotype.

18.5 Surfaces that Mimic Cell Surfaces

Another prevalent signaling source that regulates cell fate is communication between cells in close proximity: juxtacrine signaling. These signals are transmitted through interactions of transmembrane or membrane-bound proteins on adjacent cells. Tight junctions between adjacent epithelial cells serve as highly impermeable adhesions and help separate fluids on the apical side from the underlying basal membrane, thereby promoting cell polarization and control over soluble factor transport [183]. Cadherins, cell–cell adhesion proteins that are intracellularly linked to actin, are responsible for forming adherens junctions between adjacent cells that mediate cell–cell recognition, maintain structural integrity, and assist in some forms of cell migration [184]. More specialized juxtacrine signals include immunoglobulins and their ligands, such as major histocompatibility complex (MHC)/T cell receptor (TCR) complexes between T cells and their target antigen presenting cells (APCs) [185], and gap junction proteins, such as connexins used in synaptic communication between neurons [186]. Each of these communication modalities has been shown to initiate signaling cascades within cells leading to changes in cell morphology or differentiation states [187–191] and each bring their own set of challenges when attempting to model them in vitro. A major challenge in designing

a system that mimics the surface of a cell at a cell–cell junction is the presentation of the membrane proteins that regulate junction formation such that their function is preserved. This is due, in part, to the fact that many of these membrane proteins contain extracellular, transmembrane, and intracellular domains, and therefore may misfold and lose functionality when on a surface. To address this issue, a variety of conjugation methods have been developed to present truncated versions of these membrane proteins such that their activity is preserved and active domains remain accessible [192–195]. As an alternative, synthetic lipid-based membranes have also been developed to more closely mimic the presentation of these proteins in vivo [190, 196–198].

18.6 Biomimicry of Cell–Cell Contacts on Substrates

These methods involve passive adsorption or covalent linking of a protein or proteins of interest onto a substrate with controlled concentration and/or spatial presentation. One of the most straightforward methods to pattern protein is μCP [176]. An elastomer stamp, typically PDMS, is fabricated by curing against a photolithographically generated master. The surface of the stamp is inked with the desired protein solution, rinsed, dried, and brought into contact with the substrate, creating a pattern of protein matching the features on the stamp [176]. Successful patterning resolutions of several microns [199] up to several nanometers have been reported [200]. The nature of this method often promotes the use of truncated proteins containing only the extracellular domains of the original construct linked to an Fc region derived from an antibody; this minimizes the possibility of generating misfolded or inactivated ligands. The included Fc region allows for controlled and specific binding to the target surface as long as an Fc binding protein, such as Protein A or Protein G, is present. This methodology has been implemented to examine N-cadherin, E-cadherin, Neurexin, and many other extracellular domains of cell–cell contacts [194, 196, 201, 202]. In some cases, the spatial organization of multiple proteins in cell–cell junctions has been shown as a necessary precursor to some signaling cascades; therefore, it is often of interest to generate surfaces that allow for spatial patterning of multiple proteins in close proximity with each protein confined to its own pattern. Multiple rounds of μCP have been employed to spatially pattern multiple proteins to examine competitive effects or spatial display on cell–cell junction formation and cell activation [199]. For example, patterns of poly-L-lysine for neuron adhesion have been patterned along with the juxtacrine factors L1 or N-cadherin, which are essential for axon and neuron development, to compare the effects of both [203]. Additionally, laser-based methods have been implemented to pattern multiple proteins [107, 204, 205]. These approaches utilize digital masks to control laser position during patterning rather than the physical masters needed for μCP, allowing for quick changes to pattern design. Additionally, complex and image-derived patterns can be achieved [107, 204]. All of the aforementioned patterning methods are fundamentally limited for use in cell–cell contact studies since

the patterned proteins are not mobile as would occur in a cell membrane in their non-ligated states.

One major limitation of many patterning methods is the inability to mimic the more complex functionalities of membrane-associated proteins at cell–cell junctions. At these junctions, receptor–ligand pairs not only respond to each other but also often undergo conformational or spatiotemporal changes made possible by the fluidity and support of a lipid bilayer. Supported lipid bilayers (SLBs) are biologically inspired lipid-based surfaces that can mimic the fluidity of the cell membrane [206]. SLBs allow for the exploration of directed accumulation and dissipation of membrane-bound proteins involved in cellular processes, such as cadherin-dependent directed cell adhesion and migration [196], T cell activation [190, 199], and other membrane-dependent events. In the simplest lipid bilayer system, two separate chambers of an aqueous phase (most often a saline buffer solution) share an edge containing a small aperture (100 μm–1 mm) upon which the membrane will be deposited. The first synthetic lipid bilayers were created by "painting" a lipid solution (typically a suspension of phospholipids in some nonpolar organic solvent, such as decane) over the aperture [207]. This "painting" can be accomplished using a small painter's brush or by generating a lipid suspension over the entire aqueous phase and subsequently lowering and raising the aqueous phase about the aperture; each cycle performed in this way adds an additional monolayer to the membrane. Inclusion of electrodes within the saline chambers allows for the measure of changes in an electrical resistance of the membrane when a current passes through aperture. In this way, the lipid membrane—often referred to as black lipid membranes due to their appearance under the microscope—can be probed to investigate the adsorption of a variety water-soluble macromolecules. Although not all additions affect the resistance of the membrane, this is a common method to test for successful membrane modification, especially for ion-permeable additions such as channel proteins. The implementation of black lipid membranes is somewhat hindered due to their relative instability and to the limited scope of analytical tools available when compared to other methods utilizing synthetic membranes. SLBs address these shortcomings and often provide a much broader range of applications. SLBs are most commonly produced by the deposition and subsequent fusion of unilaminar or multilaminar lipid vesicles on the surface of a well. A variety of methods to generate SLBs exist as well as substrates on which to generate them [208–211], but they all share the same important features. To generate an SLB, the supporting surface must be smooth, clean, and hydrophilic. Contaminants or defects on the surface not only complicate characterization of the membranes but also compromise the integrity of the membranes themselves. As with the black lipid membranes, SLBs have a liquid phase on either side. Once the supported membrane has formed, a thin layer of water is suspended between the substrate and membrane. The depth of this layer typically falls between 20 and 40 Å [212]. As a result, the incorporation of transmembrane proteins with membrane protrusions larger than 20–40 Å may lead to contact between the protein and substrate, potentially resulting in misfolded intracellular or extracellular domains. To address this issue, SLBs have been generated on substrates functionalized with a polymer scaffold [213, 214]. The scaffold serves

as a cushion for the bilayer and provides a less rigid contact surface for proteins suspended within the membrane. Phospholipids chemically cross-linked to the polymer cushion can provide additional stability for supported bilayers. Much of the novelty of supported bilayer systems is generated from the plethora of compositions and chemistries available. Within the cell membrane, diversity is present not only in the associated proteins but also in lipid composition and organization. This diversity is necessary for cellular activity, and its organization plays roles in processes spanning from cell division to apoptosis. As a result, synthetic membranes must often involve the incorporation of lipids based on the process being studied. Lipids have been designed specifically for facilitated incorporation of truncated proteins. One example is biotin-conjugated lipids, commonly called "biotinylated" lipids. Biotin, also vitamin H or coenzyme R, is a cell-derived metabolic cofactor involved in several critical metabolic pathways. Its use in synthetic membranes, however, stems from a high non-covalent binding affinity to the protein avidin. Functionalizing a lipid with biotin and fusing the target protein with avidin facilitate the incorporation of select proteins to the cell membrane. The same outcome can be achieved through incorporation of metal-chelating lipids, such as nitrilotriacetic acid, which binds ionic nickel. A histidine-tagged protein can then form a non-covalent attachment to nickel allowing for tethering of the His-tagged protein to the membrane [215, 216].

18.7 T Cell Activation Using Biomimetic Surfaces for Cell–Cell Contacts

The activation of T cells using biomimetic surfaces that mimic APCs is of great interest in immunology. One of the goals of this research is developing a method to prime T cells in order to jump-start the immune system of patients who are unable to sufficiently mount the appropriate response to an infection [217, 218]. T cell activation itself occurs naturally when migrating T cells encounter an APC presenting antigen on extracellular MHC [219]. T cells bind MHC with TCR, and this binding triggers a T cell activation event leading to the release of various chemokines and growth factors, thereby initiating an immune response. Theoretically, new therapies could be developed for patients involving transplantation of activated T cells, assisting the patient's immune system and promoting natural defenses against a particular pathogen. Although many of the steps and components involved in this process have already been elucidated [220, 221], the exact mechanisms by which the process initiates and proceeds are not well understood. Immediately upon binding antigen presenting MHC, both the T cell and APC begin a process of actin-mediated accumulation of various membrane receptors to the binding site [221]. This site has been characterized into two distinct regions that form a bull's-eye-like pattern. The inner region, referred to as the central supramolecular activation complex (cSMAC), contains the TCR/MHC complex, as well as other associated proteins, and is surrounded by an outer region, the peripheral SMAC (pSMAC), which contains the ligand/integrin complex ICAM-1/LFA-1 (APC/T cell) used for T cell

adhesion to the APC [198, 220–222]. It has been shown that both regions play a role in T cell activation, and that certain components are relocated from the cSMAC to the pSMAC during the activation process. In recent years, several groups have used various methods to study various elements of the complex juxtacrine signaling that occurs during T cell activation. Using a μCP technique on a solid substrate, it was demonstrated that the organization of pSMAC components, the ligand–receptor pair CD80 (APC)/CD28 (T cell) had a major influence on the strength of a T cell response [199]. An Fc-coupled activating anti-CD28 was stamped onto the substrate in controlled patterns. The spacing of these patterned followed the scale of patterns typical of cSMAC and pSMAC spacing within the immunological synapse [199]. It was shown that when TCR and CD28 on the T cell were activated and IL-2 secretion was observed only when CD28 was deposited in regions analogous to a pSMAC, indicating spatial presentation of these ligand/receptor pairs plays a role in T cell activation. It was then shown that the stiffness of the substrate on which these ligand–receptor pairs were presented had a positive correlation with IL-2 secretion within the 10–200-kPa range [223]. Building on these findings, it was hypothesized that the mechanism dictating the spatial dependence of these ligands was the lateral mobility of proteins in the membrane involved in downstream signaling, such as Lck [224]. Using a BCMN method [225] to control presentation of an anti-CD3 activating antibody, it was shown that controlling the concentration of the ligands present during the immunological synapse formation also plays a role in the strength of an immune response, as seen by increasing expression of IL-2 and CD69 [226]. Later, another group designed an SLB system for high-throughput single T cell activation by creating a chip with an array of nanowells of approximately 30 μm [190]. Within these SLBs, they utilized biotin chemistry to incorporate anti-CD3 and MHC and incorporated ICAM-1 using a his-tag. T cells deposited in these nanowells formed T cell activation complexes, and the group was able to observe the process of this activation on an SLB. The ability to mimic cell surface fluidity and molecular composition adds another layer of control in generating biomimetic surfaces for cell engineering. These cell-surface mimetic materials will continue to increase our knowledge of how cell–cell interactions regulate cell fate, potentially impacting many areas of cell engineering.

18.8 Summary

The implementation of biomimetic surfaces to regulate cell function is still in its infancy. While a multitude of environmental factors that regulate cell function exist, it still remains a mystery as to which ones dominate basic cell function. From a fundamental biology perspective, biomimetic materials have shed light on which factors regulate cell fate decisions and will continue to provide insight into these relationships. From a cell engineering perspective, there are many environmental factors that can be recapitulated in in vitro environments, but knowing which ones to utilize for specific applications remains difficult. As biomimetic materials advance

further, new insights into both fundamental biology and cell engineering applications will be discovered leading to revolutionary new treatments and in vitro models for disease management.

References

1. H. Fujiwara, M. Ferreira, G. Donati, D.K. Marciano, J.M. Linton, Y. Sato, A. Hartner, K. Sekiguchi, L.F. Reichardt, F.M. Watt, The basement membrane of hair follicle stem cells is a muscle cell niche. Cell **144**, 577–589 (2011)
2. T. Sato, J.H. van Es, H.J. Snippert, D.E. Stange, R.G. Vries, M. van den Born, N. Barker, N.F. Shroyer, M. van de Wetering, H. Clevers, Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature **469**, 415–8 (2011)
3. A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation. Science **281**, 1305–1308 (1998)
4. M.-H. Kim, M. Kino-oka, Switching between self-renewal and lineage commitment of human induced pluripotent stem cells via cell-substrate and cell-cell interactions on a dendrimer-immobilized surface. Biomaterials **35**, 5670–5678 (2014)
5. F. Chowdhury, Y. Li, Y.-C. Poh, T. Yokohama-Tamaki, N. Wang, T.S. Tanaka, Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions. PloS ONE **5**, e15655 (2010)
6. F. Guilak, D.M. Cohen, B.T. Estes, J.M. Gimble, W. Liedtke, C.S. Chen, Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell **5**, 17–26 (2009)
7. J. Lee, A.A. Abdeen, K.A. Kilian, Rewiring mesenchymal stem cell lineage specification by switching the biophysical microenvironment. Sci. Rep. **4**, 5188 (2014)
8. B. Trappmann, J.E. Gautrot, J.T. Connelly, D.G.T. Strange, Y. Li, M.L. Oyen, M.a. Cohen Stuart, H. Boehm, B. Li, V. Vogel, J.P. Spatz, F.M. Watt, W.T.S. Huck, Extracellular-matrix tethering regulates stem-cell fate. Nat. Mater. **11**, 642–649 (2012)
9. I.D. de Souza, M.A.E. Cruz, A.N. de Faria, D.C. Zancanela, A.M.S. Simão, P. Ciancaglini, A.P. Ramos, Formation of carbonated hydroxyapatite films on metallic surfaces using dihexadecyl phosphate-lb film as template. Coll. Surf. B, Biointerfaces **118**, 31–40 (2014)
10. F. Benazzo, L. Botta, M.F. Scaffino, L. Caliogna, M. Marullo, S. Fusi, G. Gastaldi, Trabecular Titanium can induce in vitro osteogenic differentiation of human adipose derived stem cells without osteogenic factors. J. Biomed. Mater. Res. A. **102**, 2061–2071 (2014)
11. X. Deng, J. Lahann, Orthogonal surface functionalization through bioactive vapor-based polymer coatings. J. Appl. Polym. Sci. 131, 40315–40323 (2014)
12. S.J. Liliensiek, S. Campbell, P.F. Nealey, C.J. Murphy, The scale of substratum topographic features modulates proliferation of corneal epithelial cells and corneal fibroblasts. J. Biomed. Mater. Res. **79**, 185–192 (2006)
13. W.P. Daley, S.B. Peters, M. Larsen, Extracellular matrix dynamics in development and regenerative medicine. J. Cell Sci. **121**, 255–264 (2008)
14. C.M. Metallo, J.C. Mohr, C.J. Detzel, J.J. de Pablo, B.J. Van Wie, S.P. Palecek, Engineering the stem cell microenvironment. Biotechnol. Prog. **23**, 18–23 (2007)
15. K.Y. Tan, H. Lin, M. Ramstedt, F.M. Watt, W.T.S. Huck, J.E. Gautrot, Decoupling geometrical and chemical cues directing epidermal stem cell fate on polymer brush-based cell micropatterns. Integr. Biol. (Camb.). **5**, 899–910 (2013)
16. C. Mas-Moruno, R. Fraioli, F. Albericio, J.M. Manero, F.J. Gil, Novel peptide-based platform for the dual presentation of biologically active peptide motifs on biomaterials. Appl. Mater. Interfaces **6**, 6525–6536 (2014)
17. S.J. Ellis, G. Tanentzapf, Integrin-mediated adhesion and stem-cell-niche interactions. Cell Tissue Res. **339**, 121–130 (2010)

18. L. Gao, R. McBeath, C.S. Chen, Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-Cadherin. Stem Cells **28**, 564–572 (2010)
19. S.R. Neves, P. Tsokas, A. Sarkar, E.A. Grace, P. Rangamani, S.M. Taubenfeld, C.M. Alberini, J.C. Schaff, R.D. Blitzer, I.I. Moraru, Iyengar, R. cell shape and negative links in regulatory motifs together control spatial information flow in signaling networks. Cell **133**, 666–680 (2008)
20. J. Meyers, J. Craig, D.J. Odde, Potential for control of signaling pathways via cell size and shape. Curr. Biol. **16**, 1685–1693 (2006)
21. S.A. Ruiz, C.S. Chen, Emergence of patterned stem cell differentiation within multicellular structures. Stem Cells **26**, 2921–2927 (2008)
22. J. Eyckmans, G.L. Lin, C.S. Chen, Adhesive and mechanical regulation of mesenchymal stem cell differentiation in human bone marrow and periosteum-derived progenitor cells. Biol. Open. **1**, 1058–1068 (2012)
23. K.A. Kilian, B. Bugarija, B.T. Lahn, M. Mrksich, Geometric cues for directing the differentiation of mesenchymal stem cells. Proc. Natl. Acad. Sci. U S A. **107**, 4872–4877 (2010)
24. Z.A. Cheng, O.F. Zouani, K. Glinel, A.M. Jonas, M.-C. Durrieu, Bioactive chemical nanopatterns impact human mesenchymal stem cell fate. Nano Lett. **13**, 3923–3929 (2013)
25. D.M. Pirone, W.F. Liu, S.A. Ruiz, L. Gao, S. Raghavan, C.A. Lemmon, L.H. Romer, C.S. Chen, An inhibitory role for FAK in regulating proliferation: a link between limited adhesion and rhoA-ROCK signaling. J. Cell Biol. **174**, 277–288 (2006)
26. J.H.C. Wang, B.P. Thampatty, Mechanobiology of adult and stem cells. Int. Rev. Cell Mol. Biol. **271**, 301–346 (2008)
27. D.E. Discher, P. Janmey, Y.-L. Wang, Tissue cells feel and respond to the stiffness of their substrate. Science **310**, 1139–1143 (2005)
28. S. Khetan, M. Guvendiren, W.R. Legant, D.M. Cohen, C.S. Chen, J.A. Burdick, Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. Nat. Mater. **12**, 458–465 (2013)
29. H.B. Wang, M. Dembo, Y.L. Wang, Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. Am. J. Physiol. Cell Physiol. **279**, C1345–1350 (2000)
30. K. Rana, J.L. Liesveld, M.R. King, Delivery of apoptotic signal to rolling cancer cells: a novel biomimetic technique using immobilized trail and e-selectin. Biotechnol. Bioeng. **102**, 1692–1702 (2009)
31. K. Rana, C.A. Reinhart-King, M.R. King, Inducing apoptosis in rolling cancer cells: a combined therapy with aspirin and immobilized trail and e-selectin. Mol. Pharm. **9**, 2219–2227 (2012)
32. S.M. Frisch, H. Francis, Disruption of epithelial cell-matrix interactions induces apoptosis. J. Cell Biol. **124**, 619–626 (1994)
33. A.J. Hale, C.A. Smith, L.C. Sutherland, V.E. Stoneman, V. Longthorne, A.C. Culhane, G.T. Williams, Apoptosis: molecular regulation of cell death. Eur. J. Biochem. **237**, 884 (1996)
34. J.L. Leight, M.A. Wozniak, S. Chen, M.L. Lynch, C.S. Chen, Y. Wang, Matrix rigidity regulates a switch between Tgf-B1—induced apoptosis and epithelial Â€″. Mesenchymal. Transit. **23, 781–791** (2012)
35. E. Hadjipanayi, V. Mudera, R.A. Brown, Close dependence of fibroblast proliferation on collagen scaffold matrix stiffness. J. Tissue Eng. Regen. Med. **3**, 77–84 (2009)
36. D.S. Gray, W.F. Liu, C.J. Shen, K. Bhadriraju, C.M. Nelson, C.S. Chen, Engineering amount of cell-cell contact demonstrates biphasic proliferative regulation through RhoA and the actin cytoskeleton. Exp. Cell Res. **314**, 2846–2854 (2008)
37. R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and Rhoa regulate stem cell lineage commitment. Dev. Cell. **6**, 483–495 (2004)
38. C.M. Nelson, C.S. Chen, Cell-cell signaling by direct contact increases cell proliferation via a Pi3k-dependent signal. FEBS Lett. **514**, 238–242 (2002)
39. C.M. Nelson, C.S. Chen, VE-cadherin simultaneously stimulates and inhibits cell proliferation by altering cytoskeletal structure and tension. J. Cell Sci. **116**, 3571–3581 (2003)
40. Z.a. Cheng, O.F. Zouani, K. Glinel, A.M. Jonas, M.-C. Durrieu, Bioactive chemical nanopatterns impact human mesenchymal stem cell fate. Nano Lett. **13**, 3923–3929 (2013)

41. D.E. Discher, D.J. Mooney, P.W. Zandstra, Growth factors, matrices, and forces combine and control stem cells. Science **324**, 1673–1677 (2009)
42. A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification. Cell **126**, 677–689 (2006)
43. J. Lee, A.A. Abdeen, D. Zhang, K.A. Kilian, Directing stem cell fate on hydrogel substrates by controlling cell geometry, matrix mechanics and adhesion ligand composition. Biomaterials **34**, 8140–8148 (2013)
44. X. Wang, C. Yan, K. Ye, Y. He, Z. Li, J. Ding, Effect of RGD nanospacing on differentiation of stem cells. Biomaterials **34**, 2865–2874 (2013)
45. R.O. Hynes, Integrins: versatility, modulation, and signaling in cell adhesion. Cell **69**, 11–25 (1992)
46. A.S. Goldstein, In *Tissue Engineering,* ed. by J.P. Fisher, A.G. Mikos, J.D. Bronzino. Cell adhesion. (CRC Press, Boca Raton, 2007), pp. 5–1 to 5–17
47. R. Zaidel-Bar, C. Ballestrem, Z. Kam, B. Geiger, Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. J. Cell Sci. **116**, 4605–4613 (2003)
48. R. Zaidel-Bar, M. Cohen, L. Addadi, B. Geiger, Hierarchical assembly of cell-matrix adhesion complexes. Biochem. Soc. Trans. **32**, 416–420 (2004)
49. B. Geiger, A. Bershadsky, R. Pankov, K.M. Yamada, Transmembrane crosstalk between the extracellular matrix and the cytoskeleton. Nat. Rev. Mol. Cell. Biol. **2**, 793–805 (2001)
50. B. Geiger, K.M. Yamada, Molecular architecture and function of matrix adhesions. Cold Spring Harb. Perspect. Biol. (2011). doi:10.1101/cshperspect.a005033
51. R. Zaidel-Bar, R. Milo, Z. Kam, B. Geiger, A paxillin tyrosine phosphorylation switch regulates the assembly and form of cell-matrix adhesions. J. Cell Sci. **120**, 137–148 (2007)
52. C. Ballestrem, N. Erez, J. Kirchner, Z. Kam, A. Bershadsky, B. Geiger, Molecular mapping of tyrosine-phosphorylated proteins in focal adhesions using fluorescence resonance energy transfer. J. Cell Sci. **119**, 866–875 (2006)
53. B. Zimerman, T. Volberg, B. Geiger, Early molecular events in the assembly of the focal adhesion-stress fiber complex during fibroblast spreading. Cell Motil. Cytoskeleton. **58**, 143–159 (2004)
54. C.M. Longhurst, L.K. Jennings, Integrin-mediated signal transduction. Cell. Mol. Life Sci. **54**, 514–526 (1998)
55. F.G. Giancotti, E. Ruoslahti, Integrin signaling. Science **285**, 1028–1033 (1999)
56. M.P. Sheetz, D. Felsenfeld, C.G. Galbraith, D. Choquet, Cell migration as a five-step cycle. Biochem. Soc. Symp. **65**, 233–243 (1999)
57. D.A. Lauffenburger, A.F. Horwitz, Cell migration: a physically integrated molecular process. Cell **84**, 359–369 (1996)
58. A. van der Flier, A. Sonnenberg, Function and interactions of integrins. Cell Tissue Res. **305**, 285–298 (2001)
59. S. Huveneers, H. Truong, E.H.J. Danen, Integrins: signaling, disease and therapy. Int. J. Radiat. Biol. **83**, 743–751 (2007)
60. U. Mayer, G. Saher, R. Faessler, A. Bornemann, F. Echtermeyer, H. von der Mark, N. Miosge, E. Poesch, K. von der Mark Absence of integrin alpha 7 causes a novel form of muscular dystrophy. Nat. Genet. **17**, 318–323 (1997)
61. Y.K. Hayashi, F.-L. Chou, E. Engvall, M. Ogawa, C. Matsuda, S. Hirabayashi, K. Yokochi, B.L. Ziober, R.H. Kramer, S.J. Kaufman, E. Ozawa, Y. Goto, I. Nonaka, T. Tsukahara, J. Wang, E.P. Hoffman, K. Arahata, Mutations in the integrin alpha 7 gene cause congenital myopathy. Nat. Genet. **19**, 94–97 (1998)
62. F. Vidal, D. Aberdam, C. Miquel, A.M. Christiano, L. Pulkkinen, J. Uitto, J.-P. Ortonne, G. Meneguzzi, Integrin Beta 4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. Nat. Genet. **10**, 229–234 (1995)
63. C.M. Niessen, L.M.H. van der Raaij-Helmer, E.H.M. Hulsman, R. van der Neut, M.F. Jonkman, A. Sonnenberg, Deficiency of the integrin beta 4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties. J. Cell Sci. **109**, 1695–1706 (1996)

64. L. Ruzzi, L. Gagnoux-Palacios, M. Pinola, S. Belli, G. Meneguzzi, M. D'Alessio, G. Zambruno, A homozygous mutation in the integrin alpha6 gene in junctional epidermolysis bullosa with pyloric atresia. J. Clin. Invest. **99**, 2826–2831 (1997)
65. N. Hogg, P.A. Bates, Genetic analysis of integrin function in man: lad-1 and other syndromes. Matrix Biol. **19**, 211–222 (2000)
66. S.P. Palecek, J.C. Loftus, M.H. Ginsberg, D.A. Lauffenburger, A.F. Horwitz, Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature **385**, 537–540 (1997)
67. Y. Xue, M.L. O'Mara, P.P.T. Surawski, M. Trau, A.E. Mark, Effect of poly(ethylene glycol) (peg) spacers on the conformational properties of small peptides: a molecular dynamics study. Langmuir **27**, 296–303 (2011)
68. S. Rao, K.W. Anderson, L.G. Bachas, Oriented immobilization of proteins. Mikrochimica Acta **128**, 127–143 (1998)
69. J. Huang, S.V. Graeter, F. Corbellini, S. Rinck, E. Bock, R. Kemkemer, H. Kessler, J. Ding, J.P. Spatz, Impact of order and disorder in RGD nanopatterns on cell adhesion. Nano Lett. **9**, 1111–1116 (2009)
70. S. Sant, M.J. Hancock, J.P. Donnelly, D. Iyer, A. Khademhosseini, Biomimetic gradient hydrogels for tissue engineering. Can. J. Chem. Eng. **88**, 899–911 (2010)
71. J.H. Lee, G. Khang, J.W. Lee, H.B. Lee, Interaction of different types of cells on polymer surfaces with wettability gradient. J. Coll. Interface Sci. **205**, 323–330 (1998)
72. R.J. Pelham, Y. Wang, Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc. Natl. Acad. Sci. U. S. A. **94**, 13661–13665 (1997)
73. D.E. Discher, P. Janmey, Y. Wang, Tissue cells feel and respond to the stiffness of their substrate. Science **310**, 1139–1143 (2005)
74. J.-P. Kaiser, A. Reinmann, A. Bruinink, The effect of topographic characteristics on cell migration velocity. Biomaterials **27**, 5230–5241 (2006)
75. J.L. West, J.A. Hubbell, Polymeric biomaterials with degradation sites for proteases involved in cell migration. Macromolecules **32**, 241–244 (1999)
76. J.B. McCarthy, S.L. Palm, L.T. Furcht, Migration by haptotaxis of a schwann cell tumor line to the basement membrane glycoprotein laminin. J. Cell Biol. **97**, 772–777 (1983)
77. S.B. Carter, Haptotaxis and the mechanism of cell motility. Nature **213**, 256–260 (1967)
78. B. Harland, S. Walcott, S.X. Sun, Adhesion dynamics and durotaxis in migrating cells. Phys. Biol. **8**, 015011 (2011)
79. L.G. Vincent, Y.S. Choi, B. Alonso-Latorre, J.C. del Alamo, A.J. Engler, Mesenchymal stem cell durotaxis depends on substrate stiffness gradient strength. Biotechnol. J. **8**, 472–484 (2013)
80. M.D. Pierschbacher, E. Ruoslahti, Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature **309**, 30–33 (1984)
81. I. Schvartz, D. Seger, S. Shaltiel, Vitronectin. Int. J. Biochem. Cell Biol. **31**, 539–544 (1999)
82. L. Liaw, V. Lindner, S.M. Schwartz, A.F. Chambers, C.M. Giachelli, osteopontin and beta 3 integrin are coordinately expressed in regenerating endothelium in vivo and stimulate Arg-Gly-Asp–dependent endothelial migration in vitro. Circ. Res. **77**, 665–672 (1995)
83. K.M. Malinda, H.K. Kleinman, The laminins. Int. J. Biochem. Cell. Biol. **28**, 957–959 (1996)
84. A.V. Taubenberger, M.A. Woodruff, H. Bai, D.J. Muller, D.W. Hutmacher, The effect of unlocking RGD-motifs in collagen I on Pre-osteoblast adhesion and differentiation. Biomaterials **31**, 2827–2835 (2010)
85. E. Ruoslahti, RGD and other recognition sequences for integrins. Annu. Rev. Cell. Dev. Biol. **12**, 697–715 (1996)
86. M.C. Farach-Carson, R.C. Wagner, K.L. Kiick, In *Tissue Engineering,* ed. by J.P. Fisher, A.G. Mikos, J.D. Bronzino, Extracellular matrix: structure, function, and applications to tissue engineering. (CRC Press, Boca Raton, 2007), pp. 3–1 to 3–22
87. J.E. Frith, R.J. Mills, J.E. Hudson, J.J. Cooper-White, Tailored integrin-extracellular matrix interactions to direct human mesenchymal stem cell differentiation. Stem Cells Dev. **21**, 2442–2456 (2012)

88. T.H. Barker, The role of ecm proteins and protein fragments in guiding cell behavior in regenerative medicine. Biomaterials **32**, 4211–4214 (2011)
89. S.P. Massia, J.A. Hubbell, Vascular endothelial cell adhesion and spreading promoted by the peptide redv of the iiics region of plasma fibronectin is mediated by integrin alpha 4 beta 1. J. Biol. Chem. **267**, 14019–14026 (1992)
90. A.M. Wojtowicz, A. Shekaran, M.E. Oest, K.M. Dupont, K.L. Templeman, D.W. Hutmacher, R.E. Guldberg, A.J. Garcia, Coating of biomaterial scaffolds with the collagen-mimetic peptide GFOGER for bone defect repair. Biomaterials **31**, 2574–2582 (2010)
91. Y.Q. Liu, D.R. Senger, Matrix-specific activation of src and rho initiates capillary morphogenesis of endothelial cells. Faseb. J. **18**, 457–468 (2004)
92. M. Arnold, V.C. Hirschfeld-Warneken, T. Lohmueller, P. Heil, J. Bluemmel, E.A. Cavalcanti-Adam, M. López-García, P. Walther, H. Kessler, B. Geiger, J.P. Spatz, Induction of cell polarization and migration by a gradient of nanoscale variations in adhesive ligand spacing. Nano Lett. **8**, 2063–2069 (2008)
93. U. Hersel, C. Dahmen, H. Kessler, RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials **24**, 4385–4415 (2003)
94. G.A. Monteiro, A.V. Fernandes, H.G. Sundararaghavan, D.I. Shreiber, Positively and negatively modulating cell adhesion to type i collagen via peptide grafting. Tissue Eng. Part A. **17**, 1663–1673 (2009)
95. D.L. Hern, J.A. Hubbell, Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J. Biomed. Mater. Res. **39**, 266–276 (1997)
96. A.S. Gobin, J.L. West, Effects of epidermal growth factor on fibroblast migration through biomimetic hydrogels. Biotechnol. Prog. **19**, 1781–1785 (2003)
97. C.L. Jackson, M.A. Reidy, Basic fibroblast growth factor: its role in the control of smooth muscle cell migration. Am. J. Pathol. **143**, 1024–1031 (1993)
98. S.A. DeLong, J.J. Moon, J.L. West, Covalently immobilized gradients of Bfgf on hydrogel scaffolds for directed cell migration. Biomaterials **26**, 3227–3234 (2005)
99. J.E. Leslie-Barbick, C. Shen, C. Chen, J.L. West, Micron-scale spatially patterned, covalently immobilized vascular endothelial growth factor on hydrogels accelerates endothelial tubulogenesis and increases cellular angiogenic responses. Tissue Eng. Part A. **17**, 221–229 (2011)
100. G.S. Schultz, A. Wysocki, Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen. **17**, 153–162 (2009)
101. H.L. Ashe, J. Briscoe, The interpretation of morphogen gradients. Development **133**, 385–394 (2006)
102. K.S. Midwood, G. Orend, The role of tenascin-C in tissue injury and tumorigenesis. J. Cell. Commun. Signal. **3**, 287–310 (2009)
103. Z. Lopez-Dee, K. Pidcock, L.S. Gutierrez, Thrombospondin-1: multiple paths to inflammation. Mediat. Inflamm. **2011**, 1–10 (2011)
104. S.A. Arnold, R.A. Brekken, Sparc: a matricellular regulator of tumorigenesis. J. Cell. Commun. Signal. **3**, 255–273 (2009)
105. A.E. Rodda, L. Meagher, D.R. Nisbet, J.S. Forsythe, Specific control of cell-material interactions: targeting cell receptors using ligand-functionalized polymer substrates. Prog. Polym. Sci. **39**, 1312–1347 (2014)
106. R. Vasita, K. Shanmugam, D.S. Katti, Improved biomaterials for tissue engineering applications—surface modification of polymers. Curr. Top. Med. Chem. **8**, 341–353 (2008)
107. J.H. Slater, J.S. Miller, S.S. Yu, J.L. West, Fabrication of multifaceted micropatterned surfaces with laser scanning lithography. Adv. Funct. Mater. **21**, 2876–2888 (2011)
108. M.S. Hahn, J.S. Miller, J.L. West, Laser scanning lithography for surface micropatterning on hydrogels. Adv. Mater. **17**, 2939–2942 (2005)
109. J.H. Slater, J.C. Culver, B.L. Long, C.W. Hu, J. Hu, T.F. Birk, A.A. Qutub, M.E. Dickinson, J.L. West, Recapitulation and modulation of the cellular architecture of a user-chosen cell of interest using cell-derived, biomimetic patterning. ACS Nano **9**, 6128–6138 (2015)

110. J.H. Slater, J.L. West, Fabrication of multifaceted, micropatterned surfaces and image-guided patterning using laser scanning lithography. Methods. Cell. Biol. **119**, 193–217 (2014)
111. J.C. Culver, J.C. Hoffmann, R.A. Poché, J.H. Slater, J.L. West, M.E. Dickinson, Three-dimensional biomimetic patterning in hydrogels to guide cellular organization. Adv. Mater. **24**, 2344–2348 (2012)
112. S.R.K. Vedula, H. Hirata, M.H. Nai, A. Brugues, Y. Toyama, X. Trepat, C.T. Lim, B. Ladoux, Epithelial bridges maintain tissue integrity during collective cell migration. Nat. Mater. **13**, 87–96 (2013)
113. J. Goulpeau, B. Lonetti, D. Trouchet, A. Ajdari, P. Tabeling, Building up longitudinal concentration gradients in shallow microchannels. Lab. Chip. **7**, 1154–1161 (2007)
114. S. Allazetta, S. Cosson, M.P. Lutolf, Programmable microfluidic patterning of protein gradients on hydrogels. Chem. Commun. **47**, 191–193 (2011)
115. R. Glass, M. Moeller, J.P. Spatz, Block copolymer micelle nanolithography. Nanotechnology **14**, 1153–1160 (2003)
116. G.T. Hermanson, *Bioconjugate Techniques* (Academic Press, Waltham, 2008), p. 1323
117. K.-B. Lee, S.-J. Park, C.A. Mirkin, J.C. Smith, M. Mrksich, Protein nanoarrays generated by dip-pen nanolithography. Science **295**, 1702–1705 (2002)
118. D.S. Ginger, H. Zhang, C.A. Mirkin, The evolution of dip-pen nanolithography. Angew. Chem. Int. Ed. Eng. **43**, 30–45 (2004)
119. J.H. Slater, P.J. Boyce, M.P. Jancaitis, H.E. Gaubert, A.L. Chang, M.K. Markey, W. Frey, Modulation of endothelial cell migration via manipulation of adhesion site growth using nanopatterned surfaces. ACS. Appl. Mater. Interfaces. **7**, 4390–4400 (2015)
120. J.H. Slater, W. Frey, Nanopatterning of fibronectin and the influence of integrin clustering on endothelial cell spreading and proliferation. J. Biomed. Mater. Res. Part A. **87A**, 176–195 (2008)
121. V. Vogel, M. Sheetz,Local force and geometry sensing regulate cell functions. Nat. Rev. Mol. Cell. Biol. **7**, 265–275 (2006)
122. S.F. Badylak, D.O. Freytes, T.W. Gilbert, Extracellular matrix as a biological scaffold material: structure and function. Acta. Biomater. **5**, 1–13 (2009)
123. K. Poole, K. Khairy, J. Friedrichs, C. Franz, D.A. Cisneros, J. Howard, D. Mueller, Molecular-scale topographic cues induce the orientation and directional movement of fibroblasts on two-dimensional collagen surfaces. J. Mol. Biol. **349**, 380–386 (2005)
124. G. Maheshwari, G. Brown, D.A. Lauffenburger, A. Wells, L.G. Griffith Cell adhesion and motility depend on nanoscale RGD clustering. J. Cell. Sci. **113**, 1677–1686 (2000)
125. J.E. Frith, R.J. Mills, J.J. Cooper-White, Lateral spacing of adhesion peptides influences human mesenchymal stem cell behaviour. J. Cell. Sci. **125**, 317–327 (2012)
126. M. Arnold, E.A. Cavalcanti-Adam, R. Glass, J. Bluemmel, W. Eck, M. Kantlehner, H. Kessler, J.P. Spatz, Activation of integrin function by nanopatterned adhesive interfaces. ChemPhysChem **5**, 383–388 (2004)
127. D.R. Critchley, A.R. Gingras, Talin at a glance. J. Cell. Sci. **121**, 1345–1347 (2008)
128. M. Schvartzman, M. Palma, J. Sable, J. Abramson, X. Hu, M.P. Sheetz, S.J. Wind, Nanolithographic control of the spatial organization of cellular adhesion receptors at the single-molecule level. Nano Lett. **11**, 1306–1312 (2011)
129. K.A. Diehl, J.D. Foley, P.F. Nealey, C.J. Murphy, Nanoscale topography modulates corneal epithelial cell migration. J. Biomed. Mater. Res. A. **75A**, 603–611 (2005)
130. E. Lamers, J.T. Riet, M. Domanski, R. Luttge, C.G. Figdor, J.G.E. Gardeniers, X.F. Walboomers, J.A. Jansen, Dynamic cell adhesion and migration on nanoscale grooved substrates. Eur. Cell. Mater. **23**, 182–194 (2012)
131. J.Y. Lim, H.J. Donahue Cell sensing and response to micro- and nanostructured surfaces produced by chemical and topographic patterning. Tissue. Eng. **13**, 1879–1891 (2007)
132. L.E. Dickinson, D.R. Rand, J. Tsao, W. Eberle, S. Gerecht, Endothelial cell responses to micropillar substrates of varying dimensions and stiffness. J. Biomed. Mater. Res. A. **100A**, 1457–1466 (2012)

133. S.A. Biela, Y. Su, J.P. Spatz, R. Kemkemer, Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano-micro range. Acta. Biomater. **5**, 2460–2466 (2009)
134. J.J. Moon, M.S. Hahn, I. Kim, B.A. Nsiah, J.L. West, Micropatterning of poly (ethylene glycol) diacrylate hydrogels with biomolecules to regulate and guide endothelial morphogenesis. Tissue. Eng. Part A. **15**, 579–585 (2008)
135. M.M. Martino, F. Tortelli, M. Mochizuki, S. Traub, D. Ben-David, G.A. Kuhn, R. Mueller, E. Livne, S.A. Eming, J.A. Hubbell, Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. Sci. Transl. Med. **3**, 100ra89 (2011)
136. M.M. Martino, P.S. Briquez, A. Ranga, M.P. Lutolf, J.A. Hubbell, Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix. Proc. Natl. Acad. Sci. U S A. **110**, 4563–4568 (2013)
137. M.M. Martino, P.S. Briquez, E. Guc, F. Tortelli, W.W. Kilarski, S. Metzger, J.J. Rice, G.A. Kuhn, R. Mueller, M.A. Swartz, J.A. Hubbell, Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. Science **343**, 885–888 (2014)
138. B. Trappmann, C.S. Chen, How cells sense extracellular matrix stiffness: a material's perspective. Curr. Opin. Biotechnol. **24**, 948–953 (2013)
139. R.G. Wells, The role of matrix stiffness in regulating cell behavior. Hepatology **47**, 1394–1400 (2008)
140. C.-M. Lo, H.-B. Wang, M. Dembo, Y. Wang, Cell movement is guided by the rigidity of the substrate. Biophys. J. **79**, 144–152 (2000)
141. S.C. Wei, L. Fattet, J.H. Tsai, Y. Guo, V.H. Pai, H.E. Majeski, A.C. Chen, R.L. Sah, S.S. Taylor, A.J. Engler, J. Yang, Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a twist1-G3bp2 mechanotransduction pathway. Nat. Cell. Biol. **17**, 678–688 (2015)
142. J.H. Wen, L.G. Vincent, A. Fuhrmann, Y.S. Choi, K.C. Hribar, H. Taylor-Weiner, S. Chen, A.J. Engler, Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat. Mater. **13**, 979–987 (2014)
143. J.R. Tse, A.J. Engler, Stiffness gradients mimicking in vivo tissue variation regulate mesenchymal stem cell fate. PLoS ONE **6**, 9 (2011)
144. F. Rehfeldt, A.J. Engler, A. Eckhardt, F. Ahmed, D.E. Discher, Cell responses to the mechanochemical microenvironment–implications for regenerative medicine and drug delivery. Adv. Drug. Deliv. Rev. **59**, 1329–1339 (2007)
145. A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification. Cell **126**, 677–689 (2006)
146. C.-H.R. Kuo, J. Xian, J.D. Brenton, K. Franze, E. Sivaniah, Complex stiffness gradient substrates for studying mechanotactic cell migration. Adv. Mater. **24**, 6059–6064 (2012)
147. J.Hc Wang, B. Li, in *Microscopy: Science, Technology, Applications, and Education,* vol.1, ed. A. Mendez-Vilas, J.Diaz The principles and biological applications of cell traction force microscopy. (2010), 449–458
148. H. Zhang, L. Wang, L. Song, G. Niu, H. Cao, G. Wang, H. Yang, S. Zhu, Controllable properties and microstructure of hydrogels based on crosslinked poly (ethylene glycol) diacrylates with different molecular weights. J. Appl. Polym. Sci. **121**, 531–540 (2011)
149. J.H. Sung, M.-R. Hwang, J.O. Kim, J.H. Lee, Y.I. Kim, J.H. Kim, S.W. Chang, S.G. Jin, J.A. Kim, W.S. Lyoo, S.S. Han, S.K. Ku, C.S. Yong, H.-G. Choi, Gel characterisation and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int. J. Pharm. **392**, 232–240 (2010)
150. G.M. Harris, M.E. Piroli, E. Jabbarzadeh, Deconstructing the effects of matrix elasticity and geometry in mesenchymal stem cell lineage commitment. Adv. Funct. Mater. **24**, 2396–2403 (2014)
151. J.Y. Wong, A. Velasco, P. Rajagopalan, Q. Pham, Directed movement of vascular smooth muscle cells on gradient-compliant hydrogels. Langmuir **19**, 1908–1913 (2003)

152. S. Nemir, H.N. Hayenga, J.L. West, Pegda hydrogels with patterned elasticity: novel tools for the study of cell response to substrate rigidity. Biotechnol. Bioeng. **105**, 636–644 (2010)
153. N. Zaari, P. Rajagopalan, S.K. Kim, A.J. Engler, J.Y. Wong, Photopolymerization in microfluidic gradient generators: microscale control of substrate compliance to manipulate cell response. Adv. Mater. **16**, 2133–2137 (2004)
154. S. Sen, A.J. Engler, D.E. Discher, Matrix strains induced by cells: computing how far cells can feel. Cell. Mol. Bioeng. **2**, 39–48 (2009)
155. J. Maloney, E. Walton, C. Bruce, K. Van Vliet, Influence of finite thickness and stiffness on cellular adhesion-induced deformation of compliant substrata. Phys. Rev. E. **78**, 041923 (2008)
156. D.S. Gray, J. Tien, C.S. Chen, Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's Modulus. J. Biomed. Mater. Res. A. **66**, 605–614 (2003)
157. S. Kidoaki, T. Matsuda, Microelastic gradient gelatinous gels to induce cellular mechanotaxis. J. Biotechnol. **133**, 225–230 (2008)
158. E.L. Baker, J. Srivastava, D. Yu, R.T. Bonnecaze, M.H. Zaman, Cancer cell migration: integrated roles of matrix mechanics and transforming potential. PLoS ONE **6**, 3700–3711 (2011)
159. R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and rhoa regulate stem cell lineage commitment. Dev. Cell. **6**, 483–495 (2004)
160. K.A. Kilian, B. Bugarija, B.T. Lahn, M. Mrksich, Geometric cues for directing the differentiation of mesenchymal stem cells. Proc. Natl. Acad. Sci. U S A. **107**, 4872–4877 (2010)
161. M. Thery, Micropatterning as a tool to decipher cell morphogenesis and functions. J. Cell Sci. **123**, 4201–4213 (2010).
162. R. Singhvi, A. Kumar, G.P. Lopez, G.N. Stephanopoulos, D.I.C. Wang, G.M. Whitesides, D.E. Ingber, Engineering cell-shape and function. Science **264**, 696–698 (1994)
163. M. Thery, V. Racine, M. Piel, A. Pepin, A. Dimitrov, Y. Chen, J.B. Sibarita, M. Bornens, Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. Proc. Natl. Acad. Sci. U S A. **103**, 19771–19776 (2006)
164. J. James, E.D. Goluch, H. Hu, C. Liu, M. Mrksich, Subcellular curvature at the perimeter of micropatterned cells influences lamellipodial distribution and cell polarity. Cell. Motil. Cytoskeleton. **65**, 841–852 (2008)
165. N. Xia, C.K. Thodeti, T.P. Hunt, Q.B. Xu, M. Ho, G.M. Whitesides, R. Westervelt, D.E. Ingber, Directional control of cell motility through focal adhesion positioning and spatial control of rac activation. Faseb. J. **22**, 1649–1659 (2008)
166. J.M. Goffin, P. Pittet, G. Csucs, J.W. Lussi, J.J. Meister, B. Hinz, Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. J. Cell Biol. **172**, 259–268 (2006)
167. D. Lehnert, B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B.A. Imhof, M. Bastmeyer, Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. J. Cell Sci. **117**, 41–52 (2004)
168. J.A. Deeg, I. Louban, D. Aydin, C. Selhuber-Unkel, H. Kessler, J.P. Spatz, Impact of local versus global ligand density on cellular adhesion. Nano. Lett. **11**, 1469–1476 (2011)
169. M. Thery, A. Pepin, E. Dressaire, Y. Chen, M. Bornens, Cell distribution of stress fibres in response to the geometry of the adhesive environment. Cell. Motil. Cytoskeleton. **63**, 341–355 (2006)
170. W.F. Liu, C.S. Chen, Cellular and multicellular form and function. Adv Drug Deliv. Rev. **59**, 1319–1328 (2007)
171. S. Huang, D.E. Ingber, The structural and mechanical complexity of cell-growth control. Nat. Cell. Biol. **1**, E131–138 (1999)
172. R.A. Foty, M.S. Steinberg, The differential adhesion hypothesis: a direct evaluation. Dev. Biol. **278**, 255–263 (2005)
173. J. Lee, A.A. Abdeen, D. Zhang, K.A. Kilian, Directing stem cell fate on hydrogel substrates by controlling cell geometry, matrix mechanics and adhesion ligand composition. Biomaterials **34**, 8140–8148 (2013)

174. P.C.D.P. Dingal, D.E. Discher, Combining insoluble and soluble factors to steer stem cell fate. Nat. Mater. **13**, 532–537 (2014)
175. W.F. Liu, C.M. Nelson, D.M. Pirone, C.S. Chen, E-cadherin engagement stimulates proliferation via Rac1. J. Cell. Biol. **173**, 431–441 (2006)
176. Y. Xia, G.M. Whitesides, Soft lithography. Annu. Rev. Mater. Sci. **28**, 153–184 (1998)
177. C.J. Shen, S. Raghavan, Z. Xu, J.D. Baranski, X. Yu, M.a. Wozniak, J.S. Miller, M. Gupta, L. Buckbinder, C.S. Chen, Decreased cell adhesion promotes angiogenesis in a Pyk2-dependent manner. Exp. Cell. Res. **317**, 1860–1871 (2011)
178. N.Q. Balaban, U.S. Schwarz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, B. Geiger, Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat. Cell. Biol. **3**, 466–472 (2001)
179. S.M. Frisch, K. Vuori, E. Ruoslahti, P.Y. Chan-Hui, Control of adhesion-dependent cell survival by focal adhesion kinase. J. Cell. Biol. **134**, 793–799 (1996)
180. A. Hamadi, M. Bouali, M. Dontenwill, H. Stoeckel, K. Takeda, P. Rondé, Regulation of focal adhesion dynamics and disassembly by phosphorylation of fak at tyrosine 397. J. Cell. Sci. **118**, 4415–4425 (2005)
181. C. Lieu, S. Kpoetz, The Src family of protein tyrosine kinases: a new and promising traget for colorectal cancer therapy. Clin. Colorectal. Cancer. **9**, 89–94 (2010)
182. S.-Y. Tee, J. Fu, C.S. Chen, P.A. Janmey, Cell shape and substrate rigidity both regulate cell stiffness. Biophys. J. **100**, L25–27 (2011)
183. K. Shin, V.C. Fogg, B. Margolis, Tight junctions and cell polarity. Annu. Rev. Cell. Dev. Biol. **22**, 207–235 (2006)
184. B.M. Gumbiner, Regulation of cadherin-mediated adhesion in morphogenesis. Nat. Rev. Mol. Cell. Biol. **6**, 622–634 (2005)
185. I. Gutcher, B. Becher, Apc-derived cytokines and t cell polarization in autoimmune inflammation. J. Clin. Invest. **117**, 1119–1127 (2007)
186. J.I. Nagy, F.E. Dudek, J.E. Rash, Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. Brain. Res. Brain. Res. Rev. **47**, 191–215 (2004)
187. K. Sugimoto, N. Ichikawa-Tomikawa, S. Satohisa, Y. Akashi, R. Kanai, T. Saito, N. Sawada, H. Chiba, The tight-junction protein claudin-6 induces epithelial differentiation from mouse F9 and embryonic stem cells. PloS ONE **8**, e75106 (2013)
188. N. Borghi, M. Lowndes, V. Maruthamuthu, M.L. Gardel, W.J. Nelson, Regulation of cell motile behavior by crosstalk between cadherin- and integrin-mediated adhesions. Proc. Natl. Acad. Sci. U S A. **107**, 13324–13329 (2010)
189. C. Rüffer, V. Gerke, The C-terminal cytoplasmic tail of claudins 1 and 5 but not its Pdz-binding motif is required for apical localization at epithelial and endothelial tight junctions. Eur. J. Cell. Biol. **83**, 135–144 (2004)
190. A.J. Torres, R.L. Contento, S. Gordo, K.W. Wucherpfennig, J.C. Love, Functional single-cell analysis of T-cell activation by supported lipid bilayer-tethered ligands on arrays of nanowells. Lab. Chip. **13**, 90–99 (2013)
191. L.K. Buehler, K.A. Stauffer, N.B. Gilula, N.M. Kumar, Single channel behavior of recombinant beta 2 gap junction connexons reconstituted into planar lipid bilayers. Biophy. J. **68**, 1767–1775 (1995)
192. R. Tsuruta, R.R. Cobb, M. Mastrangelo, E. Lazarides, P.M. Cardarelli, C. Ltc, Soluble vascular cell adhesion molecule (VCAM)-Fc fusion protein induces leukotriene C4 secretion in platelet-activating factor-stimulated eosinophils abstract: eosinophil adhesion to vascular cell adhesion molecule-1 (Vcam-1) is important for cell. J Leukoc Biol. **65**, 71–79 (1999)
193. J. Gavard, J.S. Gutkind, Vegf controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of Ve-cadherin. Nat. Cell. Biol. **8**, 1223–1234 (2006)
194. F. Drees, A. Reilein, W.J. Nelson, Cell-adhesion assays: fabrication of an E-cadherin substratum and isolation of lateral and basal membrane patches. Methods. Mol. Biol. **294**, 303–320 (2005)

195. T. Ozawa, M. Horii, E. Kobayashi, A. Jin, H. Kishi, A. Muraguchi, The binding affinity of a soluble Tcr-Fc fusion protein is significantly improved by crosslinkage with an anti-Cb antibody. Biochem. Biophys. Res. Commun. **422**, 245–249 (2012)
196. T.D. Perez, W.J. Nelson, S.G. Boxer, L. Kam, E-cadherin tethered to micropatterned supported lipid bilayers as a model for cell adhesion. Langmuir **21**, 11963–11968 (2005)
197. C.-J. Huang, N.-J. Cho, C.-J. Hsu, P.-Y. Tseng, C.W. Frank, Y.-C. Chang, Type I collagen-functionalized supported lipid bilayer as a cell culture platform. Biomacromolecules **11**, 1231–1240 (2010)
198. J.T. Groves, M.L. Dustin, Supported planar bilayers in studies on immune cell adhesion and communication. J. Immunol. Methods. **278**, 19–32 (2003)
199. K. Shen, V.K. Thomas, M.L. Dustin, L.C. Kam, Micropatterning of costimulatory ligands enhances Cd4 + T cell functio*n.* Proc. Natl. Acad. Sci. U S A. **105**, 7791–7796 (2008)
200. L. Geppert, Semiconductor lithography for the next millennium. Spectrum, IEEE 33–38 (1996)
201. D. Fichtner, B. Lorenz, S. Engin, C. Deichmann, M. Oelkers, A. Janshoff, A. Menke, D. Wedlich, C.M. Franz, Covalent and density-controlled surface immobilization of e-cadherin for adhesion force spectroscopy. PloS One **9**, e93123 (2014)
202. K. Czöndör, M. Garcia, A. Argento, A. Constals, C. Breillat, B. Tessier, O. Thoumine, Micropatterned substrates coated with neuronal adhesion molecules for high-content study of synapse formation. Nat. Commun. **4**, 2252 (2013)
203. P. Shi, K. Shen, L.C. Kam, Local presentation of L1 and N-cadherin in multicomponent, microscale patterns differentially direct neuron function in vitro. Dev. Neurobiol. **67**, 1765–1776 (2007)
204. A.D. Doyle, F.W. Wang, K. Matsumoto, K.M. Yamada, One-dimensional three-dimensional underlies topography fibrillar cell migration. J. Cell. Biol. **184**, 481–490 (2014)
205. J.H. Slater, J.L. West, *Fabrication of Multifaceted, Micropatterned Surfaces and Image-Guided Patterning Using Laser Scanning Lithography*, 1st ed., vol. 119 (Elsevier Inc., Amsterdam, 2014), p 193–217
206. E.T. Castellana, P.S. Cremer, Solid supported lipid bilayers: from biophysical studies to sensor design. Surface. Sci. Rep. **61**, 429–444 (2006)
207. P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature **194**, 979–980 (1962)
208. K.L. Weirich, J.N. Israelachvili, D.K. Fygenson, Bilayer edges catalyze supported lipid bilayer formation. Biophys. J. **98**, 85–92 (2010)
209. K. Funakoshi, H. Suzuki, S. Takeuchi, Lipid bilayer formation by contacting monolayers in a microfluidic device for membrane protein analysis. Anal. Chem. **78**, 8169–8174 (2006)
210. J.T. Groves, N. Ulman, S.G. Boxer, Micropatterning fluid lipid bilayers on solid supports. Science **275**, 651–653 (1997)
211. B.A. Suarez-isla, K. Wan, J. Lindstrom, M. Montal, Bio. Single-channel recordings from purified acetylcholine receptors. 10 634–636 22(1983)
212. S.J. Johnson, T.M. Bayerl, D.C. McDermott, G.W. Adam, A.R. Rennie, R.K. Thomas, E. Sackmann Structure of an adsorbed dimyristoylphosphatidylcholine bilayer measured with specular reflection of neutrons. Biophys. J. **59**, 289–294 (1991)
213. T. Wang, D. Li, X. Lu, A. Khmaladze, X. Han, S. Ye, P. Yang, G. Xue, N. He, Z. Chen, Single lipid bilayers constructed on polymer cushion studied by sum frequency generation vibrational spectroscopy. J. Phys. Chem. C Nanomater. Interfaces. **115**, 7613–7620 (2011)
214. E. Sackmann, M. Tanaka, Supported membranes on soft polymer cushions: fabrication, characterization and applications. Trends. Biotechnol. **18**, 58–64 (2000).
215. S. Chevalier, C. Cuestas-Ayllon, V. Grazu, M. Luna, H. Feracci, J.M. de la Fuente, Creating biomimetic surfaces through covalent and oriented binding of proteins. Langmuir **26**, 14707–14715 (2010)
216. S.F. Evans, D. Docheva, A. Bernecker, C. Colnot, R.P. Richter, M.L. Knothe, Biomaterials solid-supported lipid bilayers to drive stem cell fate and tissue architecture using periosteum derived progenitor cells. Biomaterials **34**, 1878–1887 (2013)

217. D.J. Powell, B.L. Levine, Adoptive T-cell therapy for malignant disorders. Haematologica **93**, 1452–1456 (2008).
218. C.H. June, Adoptive T cell therapy for cancer in the clinic. J. Clin. Invest. **117**, 1466–1476 (2007)
219. E.R. Unanue, Antigen-presenting function of the macrophage. Annu. Rev. Immunol. **2**, 395–428 (1984)
220. A. Grakoui, S.K. Bromley, C. Sumen, M.M. Davis, The immunological synapse: a molecular machine controlling T cell activation. Science **285**, 221–227 (1999)
221. M.M. Davis, M. Krogsgaard, J.B. Huppa, C. Sumen, M.a. Purbhoo, D.J. Irvine, L.C. Wu, L. Ehrlich, Dynamics of cell surface molecules during T cell recognition. Annu. Rev. Biochem. **72**, 717–742 (2003)
222. C.R.F. Monks, B.A. Freiberg, H. Kupfer, N. Sciaky, A. Kupfer, Three-dimensional segregation of supramolecular activation clusters in T cells. Nature **340**, 764–766 (1998)
223. E. Judokusumo, E. Tabdanov, S. Kumari, M.L. Dustin, L.C. Kam Mechanosensing in T lymphocyte activation. Biophys. J. **102**, L5–7 (2012)
224. K.T. Bashour, J. Tsai, K. Shen, J.-H. Lee, E. Sun, M.C. Milone, M.L. Dustin, L.C. Kam, Cross talk between Cd3 and Cd28 is spatially modulated by protein lateral mobility. Mol. Cell. Biol. **34**, 955–964 (2014)
225. R.G. Spatz, M. Martin, P. Joachim, Block copolymer micelle nanolithography. Nanotechnology **14**, 1153 (2003)
226. J. Matic, J. Deeg, A. Scheffold, I. Goldstein, J.P. Spatz, Fine tuning and efficient T cell activation with stimulatory Acd3 nanoarrays. Nano Lett. **13**, 5090–5097 (2013)