Review

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Engineered materials and the cellular microenvironment: a strengthening interface between cell biology and bioengineering

Colin K. Choi*, **Mark T. Breckenridge*** and **Christopher S. Chen**

Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA

Cells constantly probe and respond to a myriad of cues that are present in their local surroundings. The effects of soluble cues are relatively straightforward to manipulate, yet teasing apart how cells transduce signals from the extracellular matrix and neighboring cells has proven to be challenging due to the spatially and mechanically complex adhesive interactions. Over the years, advances in the engineering of biocompatible materials have enabled innovative ways to study adhesion-mediated cell functions, and numerous insights have elucidated the significance of the cellular microenvironment. Here, we highlight some of the major approaches and discuss the potential for future advancement.

Introduction

Cells interact with the surrounding microenvironment by processing various chemical and physical signals. Studies of growth factors, including cytokines and hormones, have clarified mechanisms by which cells transduce soluble extracellular signals. In contrast, the current understanding of how insoluble cues, such as adhesion to the extracellular matrix (ECM) or neighboring cells, are integrated to generate cellular functions is less clear. To understand why this disparity exists, one only needs to appreciate the relative complexity of adhesive interactions, compared to processing soluble cues.

For most growth factors, the primary mechanism for signal transduction is mediated by binding to cell-surface or nuclear receptors. Although there may be effects of nonlinear cooperativity, multivalent ligand-induced avidity or downstream feedback regulation, the basic mechanisms often can be captured using steady-state approximations to describe receptor–ligand kinetics. In this case, the main parameters that one must consider are the concentration of soluble molecules and their binding to receptors, which dictate downstream cascade signaling. By contrast, the signals mediated by cell adhesion are regulated by numerous molecular and mechanical processes; namely, the ligation and clustering of integrins, changes in adhesion dynamics and signaling, cytoskeleton organization, cell shape and polarity, and the generation of myosin-mediated mechanical stress between cells and the ECM. Cells attach via transmembrane integrin receptors that bind to specific motifs on the matrix proteins, such as fibronectin, collagen, and vitronectin [1,2]. Upon ligand binding, the receptors are proposed to undergo activation and clustering to induce intracellular signaling events [3]. Adhesions are also linked to the actin cytoskeleton and over 150 proteins [4,5], which makes them major molecular hubs where mechanical forces and biochemical signals converge for various cellular functions, including tissue organization, migration, and differentiation [6–11]. The coupling to actin and signaling proteins forms a feedback loop that regulates both adhesion dynamics [12–14] and force transmission between the cell and the ECM (Box 1) [15,16]. The substrate parameters, such as composition, architecture and rigidity also serve as input signals to modulate the feedback mechanism. As a result, the spatial organization and mechanical properties of the matrix provide additional layers of control on the cell–ECM interaction, and one of the challenges in cell biology is to investigate this relationship systematically *in vitro*.

In addition to cell–matrix adhesion, it is clear that adhesion between neighboring cells (i.e. cell–cell adhesion) regulates many cellular structures and functions. It has been historically difficult to study the impact of such interactions because of a lack of tools to control or manipulate the spatial organization of cells with respect to each other, or the cell–cell adhesions themselves. As such, there is a growing appreciation for novel technologies that advance our understanding of cell–microenvironment interactions.

Recent progress in the engineering of specialty materials and systems for cell culture has made it possible to begin to tease apart how mechanical forces, cell–matrix adhesion, cell–cell interactions, and multicellular organization might regulate cells. Here, we provide an overview of the major tools that are now being developed within the bioengineering community that have had, or likely will have, a substantial impact on our understanding of cell adhesion and its role in cellular signaling and function. In particular, we focus on engineered surfaces to control

Corresponding author: Chen, C.S. (chrischen@seas.upenn.edu)
* These authors contributed equally to the manuscript.

Box 1. Adhesion dynamics and interplay with the ECM

Cellular responses to soluble cues depend largely on ligand concentration, whereas both the density and the geometric presentations of insoluble ECM ligands are important for regulating cellular functions. Cell adhesion to the microenvironment involves not only the binding of integrin receptors to the underlying ECM, but also integrin clustering and activation, connection to actin, and recruitment of adaptor and signaling proteins (Figure I). When cells make contact with a substrate, activity of the Rho GTPase Rac increases [110], and this leads to actin polymerization at the membrane [111] and small adhesion formation [112,113]. Rac stimulates protrusion by activating WAVE [Wiskott–Aldrich syndrome protein (WASP)-family verprolin-homologous protein], which in turn regulates the Arp 2/3 complex for dendritic actin nucleation [114,115]. The Rho GTPases Rac and Cdc42 also promote actin polymerization by activating p21-activated kinase and mDia2, and Cdc42 can directly bind to WASP proteins. In the lamellipodium, adhesions form initially as diffraction-limited foci [12,116], and their continuous cycle of assembly and disassembly (i.e. turnover) mediates further integrin binding at the leading edge. When the protrusion pauses, nascent adhesions mature by elongating along α-actinin/actin filaments, which emerge centripetally to serve as templates [12]. Adhesion maturation is also regulated by the GTPase Rho, which induces actin stress fiber formation [117,118]. This process involves myosin II, which is activated, in part, when Rho activates Rho-associated kinase (ROCK), and ROCK phosphorylates myosin light chain (MLC) and inhibits MLC phosphatase [115]. Activated myosin II bundles actin filaments and generates contractility, which indicates that tension can modulate adhesion dynamics [119,120]. The morphogenesis of adhesions regulates global cytoskeletal restructuring, cell shape, and the strength of cellular traction on the ECM. As such, it is apparent that homeostasis of the cell with its surroundings depends ultimately on a tightly regulated coupling between integrin-mediated adhesion to the ECM, the actin cytoskeleton, and myosin-mediated forces. Based on this mechanochemical system, it can be appreciated how the composition, structural organization, and mechanics of the ECM can all have an impact on cellular structure, signaling and function. In this review, we provide a brief overview of some of the advances in the engineering of materials that contribute to our understanding of these systems.

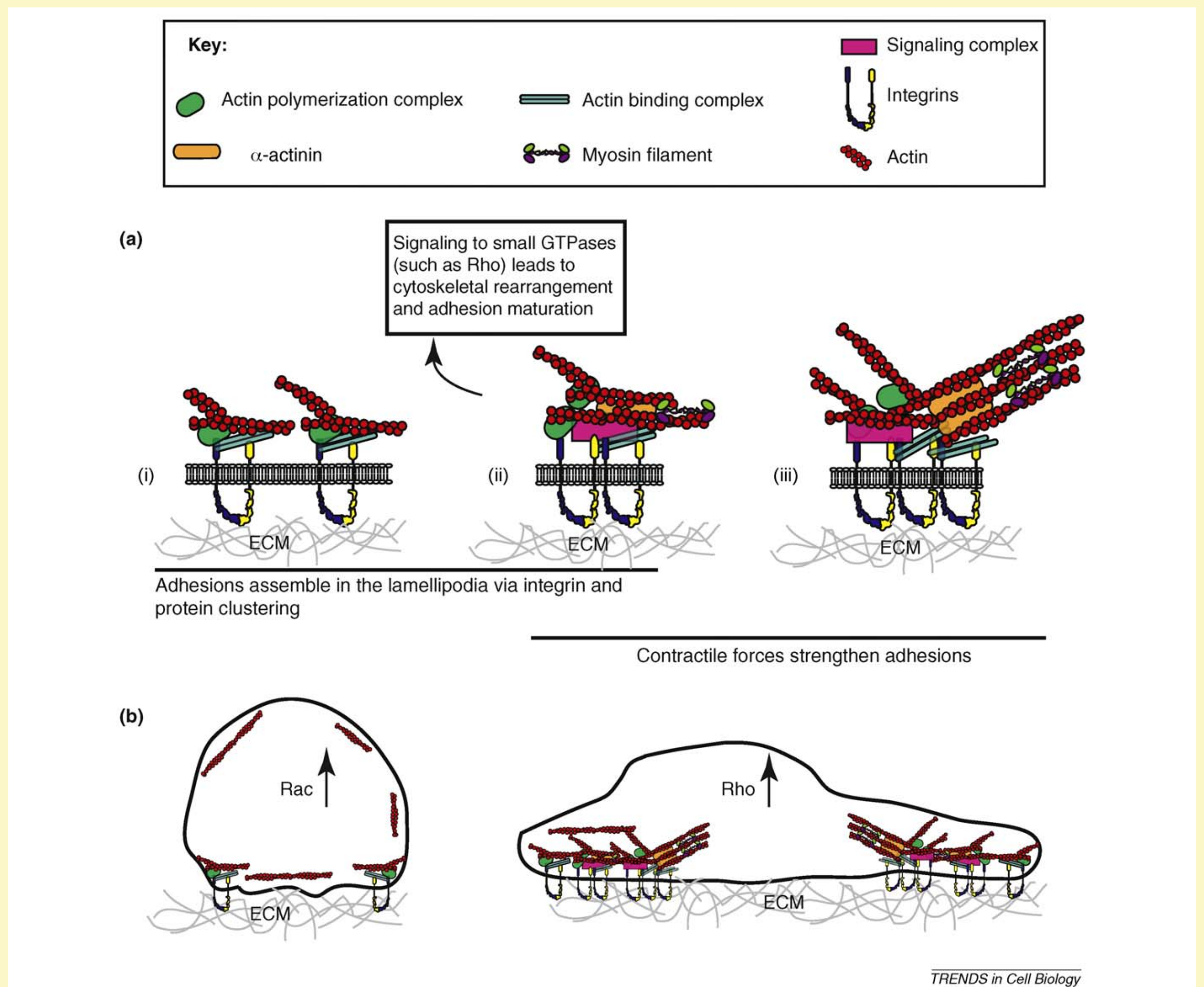

Figure I. Mechanochemical interactions of adhesion maturation and cytoskeletal rearrangement. **(a)** Schematic of adhesion maturation. **(i)** Nascent adhesion formation is initially driven by actin polymerization and consists of a complex of proteins that link integrins with actin. **(ii)** Nascent adhesions elongate in response to actin/α-actinin/myosin II crosslinking. The signaling from adhesions activates small GTPases, such as Rho, that regulate further myosin II contractility and adhesion dynamics. **(iii)** Contractile forces generated by myosin II contribute to the further maturation of adhesions. **(b)** During initial cell spreading, cell shape is constrained, Rac activity increases (Rho decreases), and adhesion formation is driven by actin polymerization (left). During later stages of cell spreading, the cell has spread out and flattened, forming mature adhesions and stress fibers and Rho activity is high (right).

ligand presentation and organization, elastic materials used to manipulate cellular mechanics, and novel specialty biomaterials that are being developed to provide unprecedented control over additional features of native extracellular matrices. We also briefly comment on some of the insights gained to illustrate the utility of these tools. This overview is brief and necessarily incomplete; therefore, we refer to other reviews for more details when necessary.

Engineered ECM surfaces and cell adhesion

Traditionally, cells are grown on tissue culture (plasma-treated) polystyrene in the presence of serum. Cellular attachment is facilitated by the adsorption of ECM proteins such as fibronectin in the serum added to cell culture media. For a more controlled surface treatment, purified matrix proteins are non-covalently adsorbed prior to cell seeding, which produces a coating of specific adhesion-promoting ligands. These proteins often include multiple binding sites for cell surface adhesion receptors and can induce physiological adhesion signaling. ECM proteins often are large and contain multiple binding sites for different cellular receptors, as well as for other ECM proteins. Thus, promoting singular receptor interactions is usually accomplished by using short peptide sequences such as the arginine–glycine–aspartate (RGD) that is found in several ECM proteins [17,18]. Integrins consist of at least 18 types of α and 8 types of β subunits that form about 24 known heterodimers, and each pair can interact with various ECM proteins, such as fibronectin, collagen or vitronectin [2]. When RGD is immobilized on the surface, the number of integrin subtypes involved in the adhesive interaction is limited, and these simplified surfaces are preferred for examining more specific effects of cell adhesion, without additional signaling that might be prompted by other integrin–ECM pairings. The degree of integrin binding and adhesion assembly can be controlled by varying the density of ligands adsorbed to a substrate, and this approach has proven useful to demonstrate that the amount of cell–ECM interaction regulates apoptosis, cell shape, angiogenic morphogenesis, and migration speed [19–21].

On clarifying the role of ECM geometry on cell adhesion and spreading, the uniform coating of ligands has limitations. Cultured cells exhibit diverse adhesion morphology and cytoskeletal organization that are often different from their counterparts *in vivo* [22–24]. Cells remodel the adsorbed ECM and secrete endogenous matrix proteins in several hours to days, which dramatically changes the surface properties in the process and causes the cells to form a mixed population of adhesions with different sizes, molecular compositions, subcellular distributions, and dynamics [25,26]. Such heterogeneity leads to differential signaling activity within adhesions and reorganization of the actin linkage [22,27]. Also, *in vivo* ECM architecture ranges from relatively consistent basement membrane to fibrillar networks and is much more complex than in cultures. Thus, while much of the current understanding of adhesion and related cellular responses has been obtained via simple homogeneous surface coating, how the organizations of adhesions, cell structure, and functions are interconnected remains unclear. These issues call for innovative engineered surfaces with high-resolution spatial patterning and adhesive specificities to control cell–ECM interaction.

One versatile technique that has emerged to pattern ECM proteins at the adhesion scale is based on microcontact printing (Figure 1a). Using methods developed by the semiconductor industry to fabricate lithographically micrometer-scale circuits on silicon wafers, one can similarly generate spatially defined patterns of ECM proteins onto otherwise inert surfaces. This accessible method involves producing stamps made with an inexpensive, tissue-culture-compatible silicone elastomer, poly-dimethylsiloxane (PDMS) [28]. ECM protein can then be inked onto the stamps and printed onto a culture substrate, which leaves behind geometric features that match the micrometer-scale features of the stamp to control where cells can adhere [29,30]. To prevent nonspecific ECM protein adsorption and cell adhesion outside of the printed regions, the unpatterned regions are treated with protein-resistant coatings.

These ECM patterns can guide overall cell geometry, adhesion sizes and location, as well as organization of the actin cytoskeleton, and thus have proven to be an effective tool for studying adhesion-mediated biology [31,32]. For example, a single ECM island or an array of closely spaced dots has been used to constrain or mediate cell spreading, respectively, while maintaining their total area of cell–ECM contact constant. With these substrates, it has been shown that cell shape, or the area of cell spreading, rather than the amount of ECM ligand regulates apoptosis and proliferation [33]. In other words, although integrin binding initiates attachment and signaling, active cytoskeletal remodeling is crucial in regulating cell function. Square ECM protein islands have been utilized to constrain the cell shape, and lamellipodia and filopodia form at the corners, where the adhesion-mediated traction stress is high [34]. In addition, cells plated on anisotropic ECM shapes, such as teardrops or arrowheads, have been found to exhibit directional migration and reorientation of the centrosomes and Golgi, which suggests that spatial segregation of adhesions and actin can determine cell polarity [35,36]. Recently, 1D lines have been used to promote elongated cell morphology and motility along the pattern, which appears to mimic how cells adhere to and migrate on 3D fibrils [37]. Surface patterning has also revealed that, in addition to soluble differentiation factors, the degree of cell spreading or shape regulates cytoskeletal tension and Rho GTPase signaling to guide the lineage of mesenchymal stem cells (MSCs) [38–40]. Taken together, these findings indicate that physical interaction with the ECM modulates adhesive cues in cells, which in turn mediate various cellular processes.

The emergence of these geometric effects on cells has prompted a new focus on understanding how cells interact with the underlying ECM at a more fundamental level. ECM printing can be controlled over different-length scales, which range from the size of a single adhesion complex to large areas for a group of cells (Figure 1b–d). The lower range was explored by varying the pattern size and density to examine the effect on cell spreading, adhesion size and molecular components [31]. In this study,

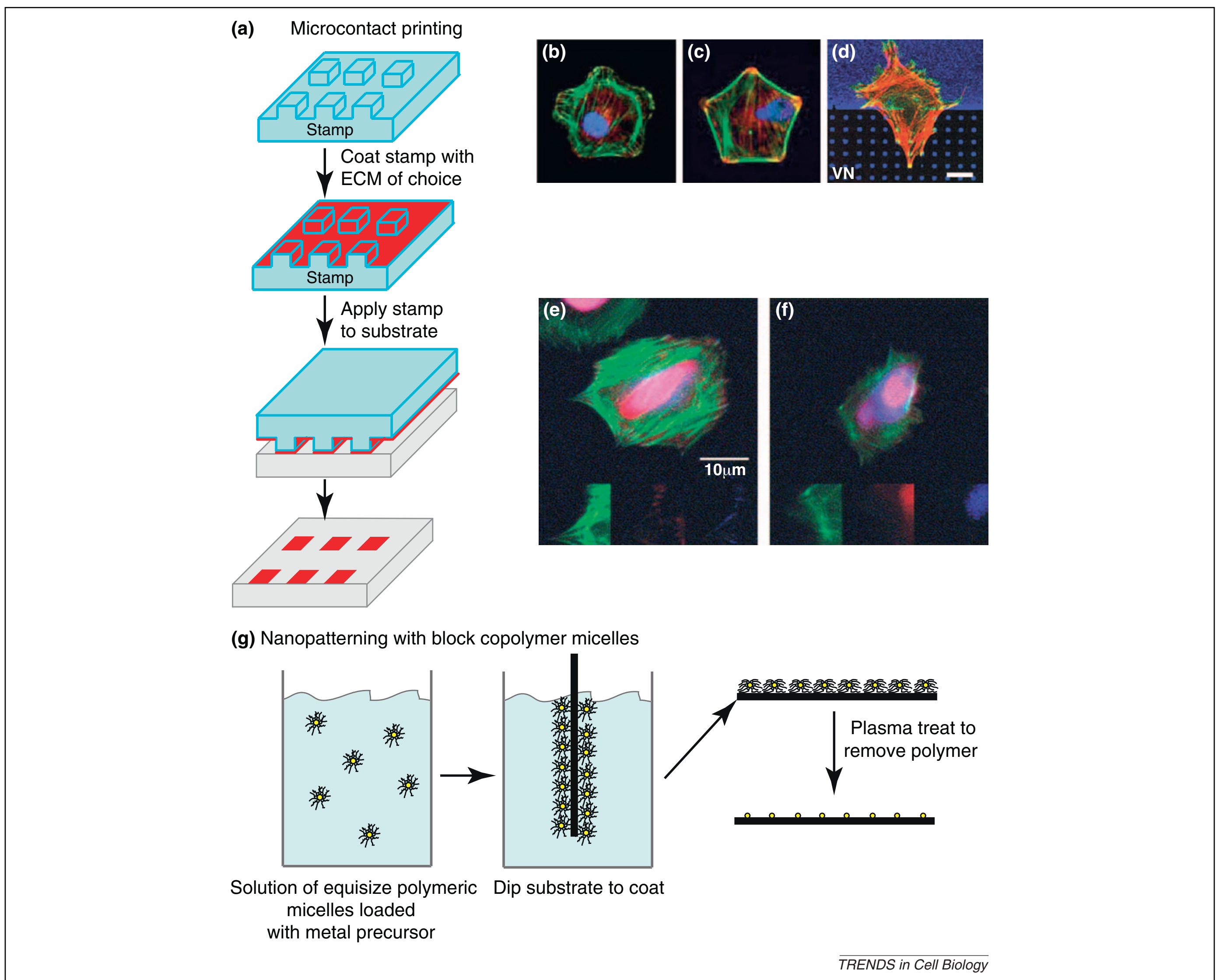

Figure 1. Methods of ECM patterning to control cell shape and adhesions. **(a)** Microcontact printing process. A biomolecule is adsorbed to the PDMS stamp surface. The stamp is then put in contact with the substrate. **(b and c)**. Immunofluorescent images of cells in flower **(b)** and star **(c)** shapes stained for F-actin (green), vinculin (red) and nuclei (blue); reproduced with permission from [40]. **(d)**. B16 cell expressing β3-integrin–green fluorescent protein (GFP) (green) labeled for actin (red) growing on vitronectin (blue), at the border between a uniform and a patterned substratum of 1 μm^2 dots. Note the redistribution of integrin receptors on the patterned substratum. Scale bars: 10 μm. Reproduced with permission from [31]. **(e and f)**. Immunofluorescent micrographs of rat embryonic fibroblasts (REF52) stained for vinculin (red), zyxin (blue) and actin (green). Cell adhering to 58-nm **(e)** and to 110-nm **(f)** nanopatterned surface for 24 h. Small inserts show each labeled protein at 2× magnification of the original images. Vinculin and zyxin can be seen to colocalize on the RGD nanodots spaced 58 nm apart, but not 110 nm apart. Reproduced with permission from [108]. **(g)**. Nanopatterning with block copolymer micelles allows the generation of substrates with a regularized pattern of gold nanoparticles. Block copolymer micelles with a polar core are used to hold a controllable amount of metal precursor in dilute solution. A substrate dipped into the solution comes out with a monolayer of micelles covering its surface. The inter-particle spacing can be controlled by using block copolymers with different lengths of blocks. The polymer is then removed by plasma treatment, leaving a quasi-hexagonal array of particles.

fibronectin dots as small as 0.1 μm^2 mediated cell adhesion and actin linkage, yet cell spreading was inhibited when the spacing of the dots increased to 5 μm. Application of a similar type of ECM patterning has demonstrated that limiting adhesion size regulates α-smooth-muscle-actin-mediated contraction in myofibroblasts, which suggests that adhesion growth is part of a mechanical feedback loop involved in sensing the microenvironment [41]. Larger-scale ECM patterns have been shown to control multicellular organization. Comparisons of single cells on ECM patches with those cultured as doublets (on twice the area) have demonstrated that cell–cell adhesion can induce planar polarity [42] and proliferation [43–45]. Alternatively, patterns that contain multiple cells have demonstrated that cells in multicellular configuration exert greater traction stress at the corners, proliferate more [46], and, in the case of stem cells, alter their differentiation [47].

Recent advances in nanoscale technology have extended patterning techniques to test how the spatial organization of individual ECM proteins, such as fibronectin and collagen, can regulate integrin clustering and adhesion-mediated responses. One study involves functionalizing polymer stars that tether a specific number of RGD peptides on an inert surface to control clustering density of the peptides [48]. Cells grown on the surface with at least five RGD peptides per star develop mature adhesions and actin stress fibers, and they exhibit higher migration speed, compared to those grown on stars with a single peptide. This suggests that increases in local integrin clustering are important for regulating adhesion and cytoskeletal

organization. If it were known that every RGD were bound by an integrin, one could even precisely suggest that a pentameric cluster is important, but because the efficiency of binding is not yet known, we can only conclude that a cluster of five or more is sufficient for this effect.

A separate nanolithography approach involves depositing metal particles of 1–15 nm on a polyethylene glycol (PEG)-treated background using di-block copolymer micelles [49] (Figure 1e–g). Each metal particle (e.g. gold) can be linked to an RGD at a tunable separation distance (up to 200 nm), and the authors have found that cell spreading, leading edge dynamics, and adhesion maturation are optimal at a lateral RGD spacing of <58 nm [49–51]. This demonstrates that the proximity between neighboring ligand–receptor pairs is important for the adhesive function of integrins. More in-depth adhesion signaling has yet to be explored, but the ligation of individual integrins is now recognized as a major parameter in surface engineering to control ECM-mediated cell functions.

Elastic substrates and mechanotransduction

As cells attach and spread onto a substrate, they generate traction against the matrix. Although this phenomenon was reported 30 years ago [52], it has only recently become clear that these mechanical forces are fundamental regulators of cell adhesion and function. Alterations in the density of collagen or fibrin gels have been suggested to have an impact on cell function, and this effect is largely attributed to the changes in the spatial density of ligands presented to cells, even though changing the matrix densities also impacts the mechanics of the scaffolds. To decouple these parameters, polyacrylamide (PA) gel has been adopted as a substrate for cell adhesion studies, which is an elegant way to control substrate rigidity without affecting ligand density.

PA is a well-behaved linear elastic material whose rigidity can be easily manipulated by varying the concentration of acrylamide and bis-acrylamide. By functionalizing the gel surface with immobilized ECM proteins, Pelham and Wang have been able to show that substrate stiffness modulates cell adhesion, spreading, and migration [53]. Building upon this initial observation, later studies have followed to show that rigidity can regulate higher-order cell functions. For example, cell proliferation and survival decreased on relatively soft substrates (4.7 kPa), compared to stiffer substrates (14 kPa) [54]. On collagen-coated PA gels, elongated myotubes displayed actin and myosin striations only on the stiffness that rendered native muscle [55]. Similarly, MSCs cultured on PA gels that have similar stiffness of brain (0.1–1 kPa), muscle (8–17 kPa), or cartilage (25–40 kPa) differentiated specifically to cells of that respective tissue, which indicates that progenitor cells can determine the mechanical property of the ECM and differentiate accordingly [56]. Also, development of a malignant metastatic phenotype has been linked to a hardened microenvironment, which indicates that substrates matched with pathological stiffness can drive disease-related processes [57,58]. Taken together, these data show that cells are capable of sensing a wide range of substrate rigidity and respond accordingly for various cell functions. The mechanisms by which the cell senses stiffness remain to be elucidated, but the mechanotransduction system appears to utilize integrin-mediated adhesions as main force-sensing structures that are capable of integrating bi-directional mechanical loads at the surface level [6,14,59].

As a result of its linear elastic property, PA gel provides a well-defined system to measure the forces that are generated by attached cells (Figure 2). The traction stress applied to the ECM has become a focus of great interest, given the importance of cell-generated forces in sensing stiffness and in modulating adhesion dynamics. Tractions generated by cells cause the substrate material to deform, which can be readily observed by placing fiduciary markers (e.g. fluorescent beads) into the gels [60]. The deformations can then be used to estimate the distribution of forces across the cell–substrate interface [61]. These approaches have been crucial in describing the forces at adhesions and their role in modulating the structure and dynamics of the adhesions and associated cytoskeletal elements [15,16,62,63]. Improvements in resolution have been achieved by using fluorescent beads of two different colors and by more rigorous computational algorithms [64,65]. Also, traction underneath a migrating cell on a planer gel can be measured in 3D by combining a digital volume correlation with confocal image stacks [66,67]. In addition to soft gels, cantilever-based substrates have been developed to measure cellular traction. Instead of relying on heavy computation for the analysis, a substrate that consists of discrete micro-cantilevers can measure local force changes. As a cell crawls over a substrate, the cantilevers lying in the plane can deflect and register the distribution of adhesion-mediated tractions [68]. More recent progression of the technology now uses vertically arrayed polymer cantilevers (microposts) that bend laterally when the cells that attach and spread across their tips exert forces [69,70]. In summary, both the gel-based and micropost-based approaches have begun to provide critical insights into how ECM rigidity, mechanical forces (e.g. contractility and tension), and cellular structures (e.g. adhesions and actin) are interlinked to form a dynamic feedback loop that is central for cells to adapt and respond to adhesive cues from the microenvironment.

It is also important to note that work from several laboratories now suggest that forces affect adherens junctions. Micropost-based substrates have recently been adapted to show that the tugging forces across cell–cell adhesions can regulate their assembly [71]. Two other studies, one using dual micropipets [72], and the other using traditional molecular approaches to modulate myosin-mediated contractility [73], have arrived at the same conclusion. These findings demonstrate that each of these tools provides a unique approach to observe a phenomenon, and, in certain situations, a synergy of different tools is needed to make a conclusive observation.

The parallels between the effects of substrate ligand density, micropatterned surfaces, and substrate stiffness are striking, as all of these manipulations modulate integrin clustering and adhesion assembly [74,75]. They also regulate Rho-mediated traction stresses [41,69], which involves actomyosin contractility and adhesion growth. Decreases in ECM ligand density or substrate stiffness

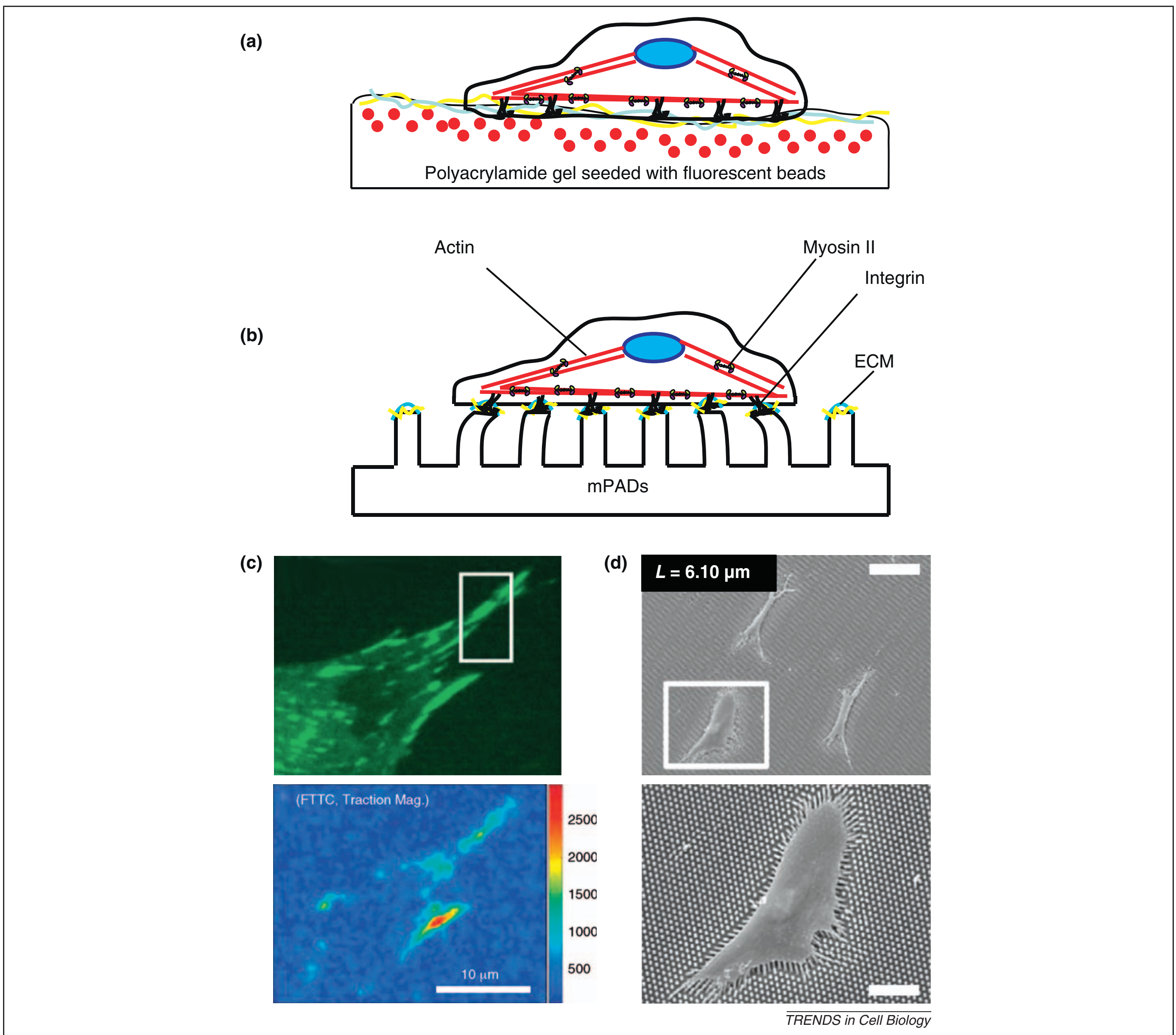

Figure 2. Elastic substrates to study traction forces. **(a)** The displacement of fluorescent beads embedded within a PA gel can be tracked beneath a migrating cell. The displacement can then be used to calculate the stress and strain fields caused by the cell-generated traction forces. **(b)** Similarly, cells can be grown on a bed of microposts (mPADs). Cell-generated traction forces deflect the posts, which allow the stress and strain fields to be calculated. **(c)**. Example of traction force microscopy. Mouse embryo fibroblasts (MEFs) marked with GFP–paxillin (green). Lower image shows a pseudo-colored map of traction magnitude calculated using Fourier-transform traction cytometry (FTTC). Units of color bar given in Pascals. White box indicates a region enlarged in a separate part of the figure (not shown). Reproduced with permission from [65]. **(d)** Scanning electron micrographs of human MSCs plated on PDMS micropost array with a post height of 6.10 μm. Lower image is a magnified version of boxed region. Scale bars are 100 μm in top image and 50 μm in lower image. Reproduced with permission from [109].

cause cells to decrease spreading as well [53,76,77], and this indicates that cell shape is coupled to adhesion-mediated mechanotransduction pathways. Many of these changes can regulate cell proliferation and stem cell differentiation [39,56]. Together, these data suggest that the sensing mechanisms of cellular microenvironment converge to adhesions, where mechanical cues are converted to biochemical signaling events for broader cell functions. Transmission of force via adhesions may physically stretch ECM proteins, such as fibronectin [78], and contraction on the ECM has been shown to release signaling agonists, such as transforming growth factor β1, for myofibroblast differentiation [79]. Similarly, conformation-sensitive adhesion molecules, such as p130CAS [80] and vinculin [81], unfold under tension, and such a mechanism can facilitate specific protein–protein interaction or signaling (e.g. phosphorylation). Thus, a focus of future studies is likely to involve attempts to uncover the underlying cellular machinery using innovative engineered substrates.

The near future: synthetic mimetics of 3D ECM

The study of cell–environment interactions on 2D substrates has provided many useful insights into how adhesive and mechanical cues can drive cell function; however, cell–environment interactions *in vivo* generally occur in 3D, which provides additional contextual stimuli that can affect cell behavior. An important effect of an added dimension is the altered spatial distribution and density of

ECM ligands relative to functionalized 2D substrates. 2D substrates functionalized by incubation with an ECM solution results in a uniform distribution of ligand across the surface, whereas 3D matrix environments are made up of a fibrous mesh with dense clusters of ligands along individual fibers [82]. The ligand distribution and fibrous architecture of 3D matrix provides additional supports for cellular interaction; integrins bound on one face of the cell compared with all around it could have an impact on adhesion clustering, cytoskeletal organization, and the mechanical forces at the cell–ECM interface. In addition to this direct effect on adhesions, the surrounding matrix also imposes new physical constraints on cell shape [37,83,84], limits the diffusion of growth factors to cells [85], and ultimately alters cell function [82,86,87]. Although no synthetic matrix can yet provide direct control over such a diverse set of ECM properties, engineered 3D matrices are now being established to access the biology of these functions in a more controlled manner.

Early experiments with 3D-engineered matrices have focused on modifying the scaffold to physically immobilize growth factors and additional peptides to add functionality. Perhaps the earliest demonstration of such engineered matrices involved the construction of a modified vascular endothelial growth factor (VEGF) that contains a substrate sequence for factor XIIIa, which mediates covalent binding to fibrin gels by its transglutaminating activity during coagulation [88]. For this type of binding, cellular proteolysis of the fibrin matrix releases local VEGF slowly and allows for rapid and sustained neovascularization, without systemic release of large doses of VEGF [89,90]. In addition, direct covalent binding of VEGF to the fibrin provides a vehicle for further functionalization of the matrix. In a recent follow-up to this study, researchers have shown that linking fibronectin domains directly with VEGF causes dramatic synergistic signaling, which suggests that integrins and VEGF receptors operate in close proximity endogenously [91]. Similarly, engineered matrix proteins are also being developed to mediate direct assembly and anchoring into pre-existing collagen scaffolds for new functionalities. One particular strategy takes advantage of collagen mimetic peptides (CMPs), which are short peptides that can non-covalently bind native collagen molecules by entangling into the helical structure. The CMPs can then be conjugated to other bioactive components to add functionality to standard collagen matrices such as cell adhesion peptides [92], immobilizing VEGF [93], or decorating collagen scaffolds with the antiadhesive PEG–CMP [94]. Such a method demonstrates the potential for engineering new functionalities into a native matrix that could direct cell migration, proliferation, and differentiation. These scaffolds offer numerous experimental options to investigate key aspects of multi-dimensional cell–matrix interactions.

A more *de novo* strategy involves starting with a completely artificial scaffold as a backbone polymer to which bioactive ligands can be tethered. Two primary examples are self-assembling peptide systems and bioinert PEG hydrogels. In the former system, the right combination of sequences and secondary structure triggers the short peptides to assemble into filamentous structures. The PEG-based hydrogels have been generated using a variety of different synthetic angles, but they all share common features. The PEG backbone acts as an inert starting material that is then polymerized covalently into a macroscopic polymer. Introduction of proteins or peptides into the gels is straightforward because they are entirely synthetic, and prescribing adhesive properties and proteolytic susceptibility is achievable through inclusion of the appropriate bioactive peptide sequences that make up the hydrogels [95,96]. Hydrogels consist of hydrophilic polymers; thus, they can be used to recapitulate many processes found in natural ECMs, such as diffusive transport and fluid flow. These versatile conditions enable sustained 3D culture for long-term studies, including proliferation, migration, and differentiation.

Although many of these technologies are relatively new and still being optimized, it is apparent that they will soon become an important part of the cell biology toolbox. Synthetic biomaterials are able to mimic biologically important/relevant characteristics of their natural counterparts and provide enhanced levels of control over gel biofunctionality and material properties. This added level of control provides exciting avenues to pursue studies that focus on independent cell–environment interactions, while keeping other parameters constant.

Concluding remarks

We are only now beginning to appreciate the many nuances of adhesive interactions that cells are able to probe and respond to accordingly. Based on many of the examples provided here, one comes to the realization that the advent of new materials has highlighted the importance of geometric and mechanical cues in cell adhesion. Although these are only the first steps in a long journey to understand the underlying mechanisms that control these transduction pathways, their successful application is drawing more engineers into developing the next generation of tools, and more biologists to adopt and capitalize on such approaches. Along with micropatterned surfaces, there is now a robust effort in the microfabrication community towards developing microfluidic technologies for generating *in vitro* platforms that integrate well-defined growth-factor gradients, shear-stress control, or miniaturized, automated culture. Microfluidic networks designed to generate stable, linear or nonlinear gradients of soluble growth factors were pioneered almost a decade ago [97]. Although popular, they require continuous flow of medium over the culture and thereby introduce the confounding variable of shear stress [98]. More recent improvements have led to designs in which gradients are generated without flow in the region of interest and allow the study of chemotaxis in a variety of settings [99–103]. Others are using these microfabrication approaches to examine the effects of culture miniaturization [104] and the development of high-throughput platforms for screening cell culture conditions [105]. Investigators are exploring complex combinations of purified ECM proteins and biomaterials to begin to understand whether complex ECM compositions and their organization elicit unique cellular responses [106,107]. These multidisciplinary efforts to elucidate how cells interact with their microenvironment are likely to continue to accelerate, as long as this growing synergy

between engineering sciences and cell biology continues to unravel these important challenges.

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References

1 Buck, C.A. and Horwitz, A.F. (1987) Cell surface receptors for extracellular matrix molecules. *Annu. Rev. Cell Biol.* 3, 179–205
2 Hynes, R.O. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* 110, 673–687
3 Schwartz, M.A. and Ginsberg, M.H. (2002) Networks and crosstalk: integrin signalling spreads. *Nat. Cell Biol.* 4, E65–68
4 Zaidel-Bar, R. *et al.* (2007) Functional atlas of the integrin adhesome. *Nat. Cell Biol.* 9, 858–867
5 Burridge, K. *et al.* (1988) Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* 4, 487–525
6 Chen, C.S. (2008) Mechanotransduction - a field pulling together? *J. Cell Sci.* 121, 3285–3292
7 Lauffenburger, D.A. and Horwitz, A.F. (1996) Cell migration: a physically integrated molecular process. *Cell* 84, 359–369
8 Berrier, A.L. and Yamada, K.M. (2007) Cell-matrix adhesion. *J. Cell Physiol.* 213, 565–573
9 Gumbiner, B.M. (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* 84, 345–357
10 Discher, D.E. *et al.* (2009) Growth factors, matrices, and forces combine and control stem cells. *Science* 324, 1673–1677
11 Vogel, V. and Sheetz, M. (2006) Local force and geometry sensing regulate cell functions. *Nat. Rev. Mol. Cell Biol.* 7, 265–275
12 Choi, C.K. *et al.* (2008) Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. *Nat. Cell Biol.* 10, 1039–1050
13 Webb, D.J. *et al.* (2004) FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat. Cell Biol.* 6, 154–161
14 Geiger, B. *et al.* (2009) Environmental sensing through focal adhesions. *Nat. Rev. Mol. Cell Biol.* 10, 21–33
15 Balaban, N.Q. *et al.* (2001) Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3, 466–472
16 Beningo, K.A. *et al.* (2001) Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J. Cell Biol.* 153, 881–888
17 Pierschbacher, M.D. and Ruoslahti, E. (1984) Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. *Proc. Natl. Acad. Sci. U. S. A.* 81, 5985–5988
18 Ruoslahti, E. (1996) RGD and other recognition sequences for integrins. *Annu. Rev. Cell Dev. Biol.* 12, 697–715
19 Ingber, D.E. and Folkman, J. (1989) Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro*: role of extracellular matrix. *J. Cell Biol.* 109, 317–330
20 Palecek, S.P. *et al.* (1997) Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* 385, 537–540
21 Re, F. *et al.* (1994) Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.* 127, 537–546
22 Geiger, B. *et al.* (2001) Transmembrane crosstalk between the extracellular matrix—cytoskeleton crosstalk. *Nat. Rev. Mol. Cell Biol.* 2, 793–805
23 Vicente-Manzanares, M. *et al.* (2009) Integrins in cell migration—the actin connection. *J. Cell Sci.* 122, 199–206
24 Gardel, M.L. *et al.* (2010) Mechanical integration of actin and adhesion dynamics in cell migration. *Annu. Rev. Cell Dev. Biol.* 26, 315–333
25 Katz, B.Z. *et al.* (2000) Physical state of the extracellular matrix regulates the structure and molecular composition of cell-matrix adhesions. *Mol. Biol. Cell* 11, 1047–1060
26 Zamir, E. *et al.* (2000) Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. *Nat. Cell Biol.* 2, 191–196
27 Calderwood, D.A. *et al.* (2000) Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J. Biol. Chem.* 275, 22607–22610
28 Whitesides, G.M. *et al.* (2001) Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.* 3, 335–373
29 Mrksich, M. and Whitesides, G.M. (1996) Using self-assembled monolayers to understand the interactions of man-made surfaces with proteins and cells. *Annu. Rev. Biophys. Biomol. Struct.* 25, 55–78
30 Singhvi, R. *et al.* (1994) Engineering cell shape and function. *Science* 264, 696–698
31 Lehnert, D. *et al.* (2004) Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. *J. Cell Sci.* 117, 41–52
32 Thery, M. *et al.* (2006) Cell distribution of stress fibres in response to the geometry of the adhesive environment. *Cell Motil. Cytoskeleton* 63, 341–355
33 Chen, C.S. *et al.* (1997) Geometric control of cell life and death. *Science* 276, 1425–1428
34 Parker, K.K. *et al.* (2002) Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* 16, 1195–1204
35 Jiang, X. *et al.* (2005) Directing cell migration with asymmetric micropatterns. *Proc. Natl. Acad. Sci. U. S. A.* 102, 975–978
36 Thery, M. *et al.* (2006) Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. U. S. A.* 103, 19771–19776
37 Doyle, A.D. *et al.* (2009) One-dimensional topography underlies three-dimensional fibrillar cell migration. *J. Cell Biol.* 184, 481–490
38 Gao, L. *et al.* (2010) Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-cadherin. *Stem Cells* 28, 564–572
39 McBeath, R. *et al.* (2004) Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6, 483–495
40 Kilian, K.A. *et al.* (2010) Geometric cues for directing the differentiation of mesenchymal stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 107, 4872–4877
41 Goffin, J.M. *et al.* (2006) Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. *J. Cell Biol.* 172, 259–268
42 Desai, R.A. *et al.* (2009) Cell polarity triggered by cell-cell adhesion via E-cadherin. *J. Cell Sci.* 122, 905–911
43 Liu, W.F. *et al.* (2006) E-cadherin engagement stimulates proliferation via Rac1. *J. Cell Biol.* 173, 431–441
44 Nelson, C.M. and Chen, C.S. (2002) Cell-cell signaling by direct contact increases cell proliferation via a PI3K-dependent signal. *FEBS Lett.* 514, 238–242
45 Nelson, C.M. and Chen, C.S. (2003) VE-cadherin simultaneously stimulates and inhibits cell proliferation by altering cytoskeletal structure and tension. *J. Cell Sci.* 116, 3571–3581
46 Nelson, C.M. *et al.* (2005) Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11594–11599
47 Ruiz, S.A. and Chen, C.S. (2008) Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells* 26, 2921–2927
48 Maheshwari, G. *et al.* (2000) Cell adhesion and motility depend on nanoscale RGD clustering. *J. Cell Sci.* 113, 1677–1686
49 Arnold, M. *et al.* (2004) Activation of integrin function by nanopatterned adhesive interfaces. *Chemphyschem.* 5, 383–388
50 Cavalcanti-Adam, E.A. *et al.* (2007) Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. *Biophys. J.* 92, 2964–2974
51 Arnold, M. *et al.* (2008) Induction of cell polarization and migration by a gradient of nanoscale variations in adhesive ligand spacing. *Nano Lett.* 8, 2063–2069
52 Harris, A.K. *et al.* (1980) Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* 208, 177–179
53 Pelham, R.J., Jr and Wang, Y. (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13661–13665

54 Wang, H.B. *et al.* (2000) Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. Cell Physiol.* 279, C1345–1350
55 Engler, A.J. *et al.* (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J. Cell Biol.* 166, 877–887
56 Engler, A.J. *et al.* (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126, 677–689
57 Levental, K.R. *et al.* (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 139, 891–906
58 Paszek, M.J. *et al.* (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8, 241–254
59 Ingber, D. (1991) Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol.* 3, 841–848
60 Pelham, R.J., Jr and Wang, Y. (1999) High resolution detection of mechanical forces exerted by locomoting fibroblasts on the substrate. *Mol. Biol. Cell* 10, 935–945
61 Munevar, S. *et al.* (2001) Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. *Biophys. J.* 80, 1744–1757
62 Aratyn-Schaus, Y. and Gardel, M.L. (2010) Transient frictional slip between integrin and the ECM in focal adhesions under myosin II tension. *Curr. Biol.* 20, 1145–1153
63 Gardel, M.L. *et al.* (2008) Traction stress in focal adhesions correlates biphasically with actin retrograde flow speed. *J. Cell Biol.* 183, 999–1005
64 Butler, J.P. *et al.* (2002) Traction fields, moments, and strain energy that cells exert on their surroundings. *Am. J. Physiol. Cell Physiol.* 282, C595–605
65 Sabass, B. *et al.* (2008) High resolution traction force microscopy based on experimental and computational advances. *Biophys. J.* 94, 207–220
66 Hur, S.S. *et al.* (2009) Live cells exert 3-dimensional traction forces on their substrata. *Cell Mol. Bioeng.* 2, 425–436
67 Maskarinec, S.A. *et al.* (2009) Quantifying cellular traction forces in three dimensions. *Proc. Natl. Acad. Sci. U. S. A.* 106, 22108–22113
68 Galbraith, C.G. and Sheetz, M.P. (1997) A micromachined device provides a new bend on fibroblast traction forces. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9114–9118
69 Tan, J.L. *et al.* (2003) Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1484–1489
70 du Roure, O. *et al.* (2005) Force mapping in epithelial cell migration. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2390–2395
71 Liu, Z. *et al.* (2010) Mechanical tugging force regulates the size of cell-cell junctions. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9944–9949
72 Martinez-Rico, C. *et al.* (2010) Integrins stimulate E-cadherin-mediated intercellular adhesion by regulating Src-kinase activation and actomyosin contractility. *J. Cell Sci.* 123, 712–722
73 Brevier, J. *et al.* (2008) The asymmetric self-assembly mechanism of adherens junctions: a cellular push-pull unit. *Phys. Biol.* 5, 016005
74 Cavalcanti-Adam, E.A. *et al.* (2008) Cell adhesion and response to synthetic nanopatterned environments by steering receptor clustering and spatial location. *HFSP J.* 2, 276–285
75 Chen, C.S. *et al.* (2003) Cell shape provides global control of focal adhesion assembly. *Biochem. Biophys. Res. Commun.* 307, 355–361
76 Engler, A. *et al.* (2004) Substrate compliance versus ligand density in cell on gel responses. *Biophys. J.* 86, 617–628
77 Ingber, D.E. (1990) Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Natl. Acad. Sci. U. S. A.* 87, 3579–3583
78 Smith, M.L. *et al.* (2007) Force-induced unfolding of fibronectin in the extracellular matrix of living cells. *PLoS Biol.* 5, e268
79 Wipff, P.J. *et al.* (2007) Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J. Cell Biol.* 179, 1311–1323
80 Sawada, Y. *et al.* (2006) Force sensing by mechanical extension of the Src family kinase substrate p130Cas. *Cell* 127, 1015–1026
81 Grashoff, C. *et al.* (2010) Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* 466, 263–266
82 Even-Ram, S. and Yamada, K.M. (2005) Cell migration in 3D matrix. *Curr. Opin. Cell Biol.* 17, 524–532
83 Cukierman, E. *et al.* (2001) Taking cell-matrix adhesions to the third dimension. *Science* 294, 1708–1712
84 Ochsner, M. *et al.* (2010) Dimensionality controls cytoskeleton assembly and metabolism of fibroblast cells in response to rigidity and shape. *PLoS One* 5, e9445
85 Raghavan, S. *et al.* (2010) Decoupling diffusional from dimensional control of signaling in 3D culture reveals a role for myosin in tubulogenesis. *J. Cell Sci.* 123, 2877–2883
86 Beningo, K.A. *et al.* (2004) Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. *Proc. Natl. Acad. Sci. U. S. A.* 101, 18024–18029
87 Meshel, A.S. *et al.* (2005) Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* 7, 157–164
88 Zisch, A.H. *et al.* (2001) Covalently conjugated VEGF—fibrin matrices for endothelialization. *J. Control. Release* 72, 101–113
89 Ehrbar, M. *et al.* (2004) Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. *Circ. Res.* 94, 1124–1132
90 Ehrbar, M. *et al.* (2008) The role of actively released fibrin-conjugated VEGF for VEGF receptor 2 gene activation and the enhancement of angiogenesis. *Biomaterials* 29, 1720–1729
91 Martino, M.M. *et al.* (2009) Controlling integrin specificity and stem cell differentiation in 2D and 3D environments through regulation of fibronectin domain stability. *Biomaterials* 30, 1089–1097
92 Yamazaki, C.M. *et al.* (2010) A collagen-mimetic triple helical supramolecule that evokes integrin-dependent cell responses. *Biomaterials* 31, 1925–1934
93 Wang, A.Y. *et al.* (2008) Immobilization of growth factors on collagen scaffolds mediated by polyanionic collagen mimetic peptides and its effect on endothelial cell morphogenesis. *Biomacromolecules* 9, 2929–2936
94 Wang, A.Y. *et al.* (2005) Facile modification of collagen directed by collagen mimetic peptides. *J. Am. Chem. Soc.* 127, 4130–4131
95 Gobin, A.S. and West, J.L. (2002) Cell migration through defined, synthetic ECM analogs. *FASEB J.* 16, 751–753
96 Lutolf, M.P. *et al.* (2003) Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5413–5418
97 Dertinger, S.K.W. *et al.* (2001) Generation of gradients having complex shapes using microfluidic networks. *Anal. Chem.* 73, 1240–1246
98 Walker, G.M. *et al.* (2005) Effects of flow and diffusion on chemotaxis studies in a microfabricated gradient generator. *Lab Chip* 5, 611–618
99 Atencia, J. *et al.* (2009) The microfluidic palette: a diffusive gradient generator with spatio-temporal control. *Lab Chip* 9, 2707–2714
100 Saadi, W. *et al.* (2007) Generation of stable concentration gradients in 2D and 3D environments using a microfluidic ladder chamber. *Biomed. Microdevices* 9, 627–635
101 Irimia, D. *et al.* (2007) Polar stimulation and constrained cell migration in microfluidic channels. *Lab Chip* 7, 1783–1790
102 Breckenridge, M.T. *et al.* (2010) A microfluidic imaging chamber for the direct observation of chemotactic transmigration. *Biomed. Microdevices* 12, 543–553
103 Park, E.S. *et al.* (2010) Continuously perfused, non-cross-contaminating microfluidic chamber array for studying cellular responses to orthogonal combinations of matrix and soluble signals. *Lab Chip* 10, 571–580
104 Meyvantsson, I. and Beebe, D.J. (2008) Cell culture models in microfluidic systems. *Annu. Rev. Anal. Chem. (Palo Alto Calif.)* 1, 423–449
105 Melin, J. and Quake, S.R. (2007) Microfluidic large-scale integration: the evolution of design rules for biological automation. *Annu. Rev. Biophys. Biomol. Struct.* 36, 213–231
106 Flaim, C.J. *et al.* (2005) An extracellular matrix microarray for probing cellular differentiation. *Nat. Methods* 2, 119–125
107 Lutolf, M.P. *et al.* (2009) Designing materials to direct stem-cell fate. *Nature* 462, 433–441
108 Cavalcanti-Adam, E.A. *et al.* (2006) Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly. *Eur. J. Cell Biol.* 85, 219–224
109 Fu, J. *et al.* (2010) Mechanical regulation of cell function with geometrically modulated elastomeric substrates. *Nat. Methods* 7, 733–736

110 del Pozo, M.A. *et al.* (2000) Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *EMBO J.* 19, 2008–2014
111 Ridley, A.J. *et al.* (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* 70, 401–410
112 Nobes, C.D. and Hall, A. (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81, 53–62
113 Rottner, K. *et al.* (1999) Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* 9, 640–648
114 Pollard, T.D. and Borisy, G.G. (2003) Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112, 453–465
115 Burridge, K. and Wennerberg, K. (2004) Rho and Rac take center stage. *Cell* 116, 167–179
116 Alexandrova, A.Y. *et al.* (2008) Comparative dynamics of retrograde actin flow and focal adhesions: formation of nascent adhesions triggers transition from fast to slow flow. *PLoS One* 3, e3234
117 Chrzanowska-Wodnicka, M. and Burridge, K. (1996) Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* 133, 1403–1415
118 Ridley, A.J. and Hall, A. (1992) The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70, 389–399
119 Giannone, G. *et al.* (2007) Lamellipodial actin mechanically links myosin activity with adhesion-site formation. *Cell* 128, 561–575
120 Vicente-Manzanares, M. *et al.* (2007) Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. *J. Cell Biol.* 176, 573–580