Nanotechnology Usages for Cellular Adhesion and Traction Forces

Sangyoon J. Han and Nathan J. Sniadecki

Abstract Cell mechanobiology studies have incorporated micro- and nanotechnology-based tools to understand the interaction between cells and their surrounding environment. These tools have helped to uncover findings that physical factors in the extracellular matrix can strongly affect important cell functions like proliferation, migration, differentiation, and survival. Here, we review the nanotechnologies that have been used for cellular adhesions and traction forces and the findings that have come at the molecular and protein level.

1 Introduction

Prior to revolutions in the life sciences, there have been innovations in technology that helped unlock the limitations in scientific understanding. For example, development of the microscope led to the discovery of cells and automated DNA sequencing and information storage has both made it possible to map and better understand the human genome. Likewise, adoption of nanotechnology in cell biology has led to a new era where it is now possible to control the physical interactions between a cell and its surroundings. A typical eukaryotic cell has a diameter that can range between 10 and 100 μm, but the protein structures inside it are even smaller. Understanding the mechanics of a cell is critical because it plays a role in orchestrating cell function. It is now possible to use approaches that have

S. J. Han and N. J. Sniadecki (✉)
Department of Mechanical Engineering, University of Washington, Seattle, WA, USA
e-mail: nsniadec@uw.edu; syhan@u.washington.edu

N. J. Sniadecki
Department of Bioengineering, University of Washington, Seattle, WA, USA

Stud Mechanobiol Tissue Eng Biomater (2011) 4: 177–200
DOI: 10.1007/8415_2010_26

Published Online: 22 September 2010

the same length scale as the structures of interest in a cell to learn how they work in the overall system. In particular, it has become apparent that spatial domains, structural compositions, and mechanical forces influence the adhesive binding interactions between cells and their underlying substrates.

The environment that surrounds a cell is its extracellular matrix (ECM). It is composed of matrix proteins such as collagen and elastin that serve as tension-bearing structures. These proteins form strong fibers that are mixed with a gel-like substance that consists of proteoglycans. By their affinity with water, proteoglycans provide resistance against compressive forces as well as regulate cell behavior through interactions with other enzymes. The structure of the ECM can also take a sheet-like mesh, referred to as the basement membrane, which consists of laminin and collagen networks. In addition to these proteins, there are others such as fibronectin and vitronectin that help cells adhere by having binding sites for adhesions receptors. Mechanical and structural features of the ECM such as stiffness, content, and density of available ligands can influence cellular functions through mechanosensation at integrins [40].

Integrins are heterodimeric, transmembrane receptors that bind to ligands found in the ECM [48]. In particular, integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ bind to a short peptide sequence (Arg-Gly-Asp, RGD) found in ECM proteins. They have a width of 10 nm and are 10–100 times more abundant on a cell's surface than other receptor types [2]. Integrins are freely dispersed within a cell's membrane, but become tightly bound when they encounter a ligand. A conformational change occurs upon binding within the cytoplasmic domains of integrins that allow for the recruitment of proteins at the cytoplasmic side of the membrane. These proteins are known as focal adhesion proteins and they link integrins to the actin cytoskeleton. At the same time, these focal adhesion proteins can act as signaling transducers that interpret the cell's environment and influence cellular decisions like migration, apoptosis, proliferation, or differentiation. As more focal adhesion proteins and integrins are recruited, the size, strength and signaling activity of the structure becomes larger. These aggregated clusters are known as focal complexes and have are approximately a 100 nm in diameter and contain over a 100 different proteins that associate within the adhesion site [4, 18, 39]. Focal complexes are transient structures that can vanish or develop into mature focal adhesions. Although the molecular nature of ligand binding is relatively well-established, the changes that allow the focal complex to transition into a focal adhesion remain elusive. Yet, the size, strength, assembly, and disassembly of focal adhesions appear to be key mediator of many cellular functions.

One aspect of focal adhesion maturation is that mechanical forces acting on the structure are essential for its regulation. Focal adhesions are elongated and localized at the terminating end of fiber bundles containing actin, myosin, II, and α-actinin, which are known as stress fibers [81]. Interestingly, the formation and growth of focal adhesions relies on myosin, II, especially its isoform myosin IIA [33, 101, 102]. It is increasingly evident that traction forces from the actin–myosin contractile machinery mediate the assembly and disassembly of focal adhesions [36, 88]. Traction forces are generated by the cross-bridge cycling between myosin and actin and allow a cell to spread, migrate, and maintain its shape. Since focal adhesions act as points

where tension in stress fibers are transmitted to the surrounding microenvironment, they are essential for a cell's ability to migrate, but also to pull and rearrange proteins in the ECM or reshape tissue during morphogenesis [59, 74, 75, 96].

While cells can perform many functions by applying traction forces at their focal adhesions, much interest has been focused on how cells sense and respond to forces. In vivo, mechanical forces are transmitted to cells through the tissue during physiological events: For example, hemodynamic shear forces acts on endothelial cells in the vasculature and fibroblasts experience loads during the movement of tendons and ligaments. These forces that cells experience are of great importance in tissue formation, maintenance, and growth [3, 49]. Cells use traction forces to respond to their surroundings [27, 93]. In particular, cells can sense and respond to rigidity of a substrate by using traction forces to probe the elasticity and by using feedback from mechanosignaling at the focal adhesions [27]. The stiffness of the ECM can change if the tissue has reached a severe diseased state. Increased stiffness of mammary gland tissue can promote malignancy by increasing focal adhesions, Rho activity, traction forces, and migration [64, 79]. On the other hand, the geometry of a cell's spread area affects differentiation in human mesenchymal stem cells (MSCs) by driving osteogenesis or adipogenesis [55, 73]. Matrix rigidity is also found to be a key factor that directs stem cell differentiation [30]. Since both substrate stiffness and spread area of a cell change a state of cellular function (e.g. differentiation) through Rho-mediated cytoskeletal contractility [8, 73, 86], there has been a growing number of tools developed for studying traction forces which we highlight in this chapter.

The binding interactions between cells and their surroundings have been investigated over the last half-century through the development of micro- and nano-engineered tools and techniques. The first representative example is the internal reflection microscopy which enabled a closer scrutiny of the cell–substrate contact area [100]. Chick heart fibroblasts were observed to have non-uniform, discrete adhesion points at their peripheral regions of contact, whereas the center of cell was separated from the substrate by a distance up to a few hundred nanometers [22]. This observation of the existence of focal adhesions was also later confirmed by electron microscopy [1]. Micropatterning of ECM proteins on a surface is the second example. By spatially controlling the available area of ligand on which a cell can spread, it was found that whether a cell proliferates or dies can be strongly influenced [17]. As mentioned previously, the same technique was used to discover that cells differentiate into specific lineages depending on available area [73]. As a last example, optical tweezers trap nanosize objects with focused light and have been used to study the formation and maturation of focal adhesions by trapping ECM-coated beads at a cell's surface where integrins can bind [19]. It was found that by increasing the trapping force of the optical tweezer, the adhesion strength between a cell and a bead also grew stronger. This strengthening of the adhesion site implicates that cells can sense forces at their integrins and recruit additional focal adhesion proteins to reinforce the connection. Together, these examples underline that with the advent of new technology, the fundamental mechanism behind a cell's behavior can be revealed.

In this chapter, we review nanotechnology-based tools that have been used for cell–surface interactions, major discoveries through using the tools, and the needed areas for new technology. Specifically, we will examine achievements attained by surface control over the chemical presentation of the ECM and tools for measuring and analyzing cellular traction forces.

2 Surface Control

Cell adhesion is the essential mechanism that guarantees the structural and functional integrity of tissue. It involves different biochemical signaling pathways that mediate the integrin-ligand transduction response [111]. However, knowledge has been somewhat limited by the biochemical composition of a substrate used for cell culture, which is typically a polystyrene dish. There have been efforts to tailor the surface chemistry of polystyrene for better attachment of cells by modulating the ability of the surface of polystyrene to capture soluble ECM proteins in culture media [23, 43]. It was found that the surface that promotes the best adhesion of cultured cells had a contact angle of 40° with water, which is relatively hydrophobic. By forming a gradient of the wettability on a surface using a nano-film of metal, it was observed that cells migrated toward regions of higher adhesivity, a process referred to as haptotaxis [14, 15]. Likewise, cells have been found to spread to a larger extent when in contact with a hydrophobic surface [44]. Moreover, by adjusting the adhesivity of a surface by applying a hydrophilic hydrogel coating of poly(2-hydroxyethyl methacrylate), it was found that spread area, DNA synthesis, and proliferation of cells increased with the degree of surface adhesivity [34]. These early findings on adhesion, migration, and proliferation established a framework that has been used to engineer surfaces that control the geometrical area, spacing, and density of ligands.

Self-assembled monolayers (SAMs) of alkanethiols on gold have become a model surface to study cell adhesion due to its fine control over protein adsorption and its ability to spatially pattern regions of adhesion. Alkanethiols have a general structure of $SH(CH_2)_nX$ in which the sulfur atom coordinates with a gold atom of underlying film, n is the length of the hydrocarbon spacers, and X is the terminal group that is presented on the surface (Fig. 1a). Alkanethiols self-assemble on gold surface in a side-by-side fashion that is dense, stable, and highly ordered, creating a semi-crystalline monolayer that is approximately 2–3 nm in thickness (Fig. 1b). The terminal group (X) and the length of alkyl chain spacer ($(CH_2)_n$) can be tailored to control the surface properties of the monolayer [7]. If the terminal group is hydrophobic such as methyl or carboxyl, it readily adsorbs a number of ECM proteins, whereas terminal groups such as poly(ethylene glycol) (PEG) prevent protein adsorption [83]. Furthermore, the adhesivity of the model surface can be also tuned by altering the ratio of terminal groups between methyl and PEG.

Using SAMs, different densities and gradients of ECM ligands could be made to study cell growth and migration. By controlling the adsorption time of carboxyl

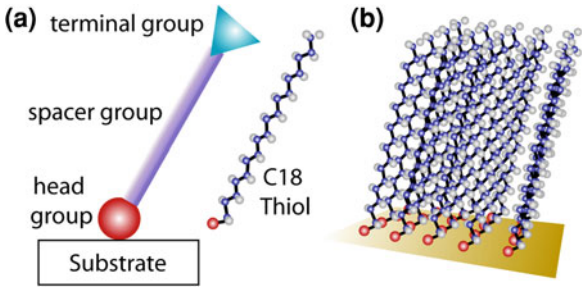

Fig. 1 Typical alkanethiol molecules on a gold substrate form self-assembled monolayers (SAMs). **a** Simplified illustration of a single C18 thiol molecule shows a head group (sulfuric atom), spacer group (alkyl chain), and terminal group. **b** Alkanethiols form a tightly-packed SAM on a gold substrate that can be used to study cell adhesion by tailoring the degree of protein adsorption. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

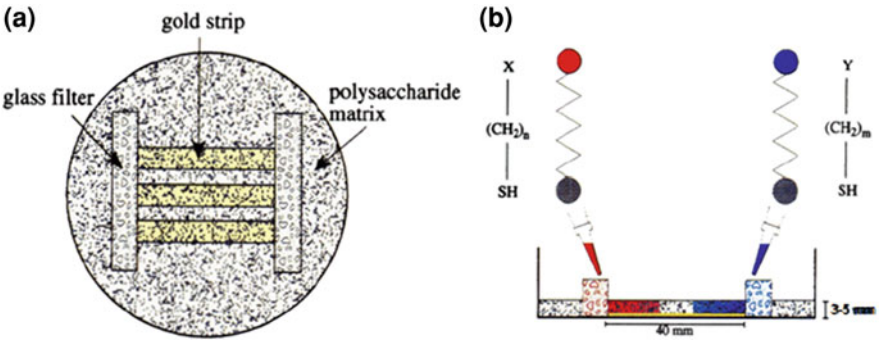

Fig. 2 Cross-diffusion device used for the preparation of the ligand gradients: **a** top view and **b** side view. The polysaccharide matrix is used to diffuse different alkanethiol solutions from either end to create a concentration gradient on top of a gold strip. Adapted from [66]. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

terminal group that had been conjugated with RGD, it was found that more cells attached to the surface and had larger spread areas [50]. Likewise, on gradients of fibronectin, it was observed that cells migrated faster in the direction of the highest gradient [91, 92]. To make the gradients, a cross-diffusion method was used (Fig. 2), where inert and reactive alkanethiols were simultaneously added to either end of filtration gel and allowed to diffuse across the gel to produce a gradient of carboxyl terminal groups on a gold-coated surface. In addition to studying the haptotactic migration speed of cells, it was also found that cells polarized their morphological structure in the direction of the steepest gradient. Previously, cells had been seen to migrate in the direction of steepest gradient of chemo-attractants, a process known as chemotaxis. Interestingly, when haptotaxis was compared against chemotaxis by preparing a gradient of fibronectin in a direction

perpendicular to a gradient of VEGF, it was found that directional migration was influence by both factors, but the influence of chemotaxis was stronger [67]. This result supports that insoluble factors like ligand density gradients have a role in cell migration, but may not be as dominant as soluble factors.

A powerful advantage of using SAMs has been the ability to incorporate microscale spatial control by microcontact printing. Here, an elastomeric stamp is coated with alkanethiols and placed in contact with a gold substrate to form regions of SAMs (Fig. 3a). The region that did not contact the stamp was treated subsequently with PEG-based alkanethiols to block cell adhesion. The stamp is made from a silicone rubber and formed with features as small as 0.2 μm through a technique known as soft lithography [76, 106]. By having square patterns of different area on a stamp (Fig. 3b), it was found that spread area determines whether cells proliferate or undergo apoptosis [17]. Different patterns of small dots were also used to limit the available ECM area for a cell, but still allow them to spread to the same area as the square patterns. It was found that whether a cell proliferations or commits suicide did not depend on the extent of its cell–ECM contact area but on the extent of its projected spread area. Cell spreading was also found to control whether stems cells undergo osteogenesis or adipogenesis [73]. Since then, it has been observed that cells patterned onto a wider variety of shapes such as triangles, pentagons, hexagons, and trapezoids formed lamellipodial extensions used for migration at the sharp corners of each pattern (Fig. 3c) [11]. Similar patterns have been found to play a role in stem cell differentiation [55]. The work on cell polarity has progressed beyond the use of symmetric geometries

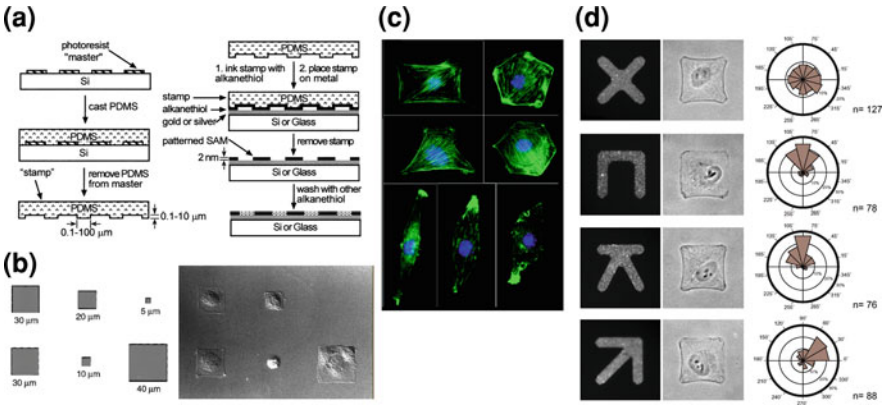

Fig. 3 Microcontact printing of SAMs. **a** Schematic showing microcontact printing procedure of alkanethiol molecules. Reproduced from [105]. **b** Cells attached and spread on printed islands of square patterns (shown in phase image at *right*) with several different areas. Adapted from [17]. **c** Patterned cells on various shapes have more actin filaments at the sharp corners. *Green* actin, *blue* nucleus. Reproduced from [11]. **d** Cells (*middle column*) were plated on anisotropic adhesive ligands (*left column*). Polarity vectors from nucleus–centrosome locations were plotted as angular distribution (*right column*). Reproduced from [99]. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

by patterning anisotropic shapes (X, C, K and arrows), which all had the same projected area for the cells (Fig. 3d) [99]. It was found that cells defined their polarity by positioned their centrosomes in the direction of greatest adhesivity. These works suggest that projected spread area determines cell fate in both growth and differentiation and adhesive geometry determines cell polarity and migration, but there may be cross-interactions between the two.

Microcontact printing typically confines a cell from migrating, but using electrochemistry, however, it is possible to switch a non-adhesive surface to an adhesive one by electrically desorbing PEG-terminated SAMs from a surface. This approach has been used to patterned cells of two different cell types to look at co-culture interactions [109]. The switchable SAMs also allowed cells to migrate out of confined region after a voltage pulse, providing a nondestructive alternative to scratch-wound assays to study cell migration [53]. With this electrochemical approach, it was possible to study the effect of cell polarization on the direction of migration. Fibronectin was printed onto a substrate in an asymmetric, teardrop shape (Fig. 4a, top left) [54]. Before release, the lamellipodia of the cells preferentially formed at the blunt end, but with a lesser degree at the sharp end (Fig. 4a, bottom left). After release, the cells migrated in the same direction as their blunt ends (Fig. 4a, right), suggesting that asymmetry of lamellipodia extension induced biased migration. Building from this work, the persistence of biased migration was studied with arrays of teardrop patterns formed in a ring (Fig. 4b, top) [57]. When blunt ends of the teardrops were adjacent to the sharp ends of the next pattern, cells preferentially migrated in the direction of the blunt

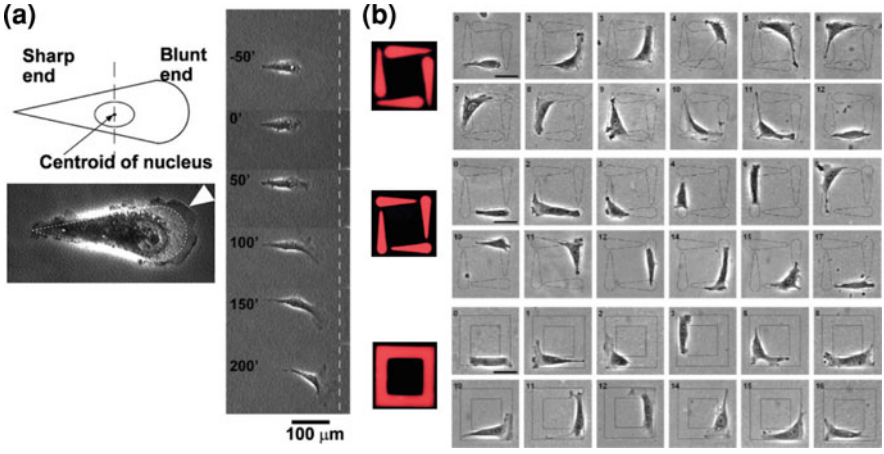

Fig. 4 Cells show directed migration on micropatterned surfaces. **a** A 3T3 fibroblast patterned by a teardrop shape (*top left*) showed ruffling activity of lamellipodia (*arrowhead*, *bottom left*) and migration in the direction of the blunt end upon release of confinement (*right*). Adapted from [54]. **b** Arrays of teardrop patterns direct cell migration. NIH 3T3 fibroblasts migrate from the blunt ends to the sharp ends of the next island (*top*). In contrast, cells migrate from the sharp end to the blunt end of an adjacent island (*middle*). Cells on continuous rectangular islands (*bottom*) showed no preferential orientation of cell migration. Adapted from [57]

ends. However, when the sharp ends were adjacent to blunt ends, the direction of migration was reversed (Fig. 4b, middle). These works demonstrate that directional migration depends on the polarity of the cell, but also on the availability of ECM in the direction of lamellipodia formation.

Cell polarization from geometric anisotropy and migration along the polarized direction implicates that cells are polarized before migration. Since haptotaxis was observed on gradients of ECM ligand, cells might be polarized in a confined, but gradient condition. This question was evaluated using microfluidic networks and SAMs [82]. To prepare the surface, SAMs were deposited as patterned strips using an arrangement of parallel microfluidic channels (Fig. 5A, a,b). A Y-shaped microfluidic serpentine channel was placed on top of the treated surface and active RGD peptide and inactive RDG peptide were flowed over the SAMs from two entrance ports to form a diffusion-based gradient (Fig. 5A, c). The remaining unexposed regions were also blocked in a solution of nonadhesive peptides, rendering square patterns in which there was a gradient in adhesivity (Fig. 5A, d). Cells formed focal adhesions predominantly in the direction of the gradient (Fig. 5A, e). Interestingly, despite the presence of a gradient, the geometric effect of sharp corners persisted in these cells, which indicates that nanoscale geometries may have a stronger effect than adhesive gradients.

Instead of using continuous confinement of a certain pattern shape, microcontact printing at the length scale of focal adhesions can provide more insight into

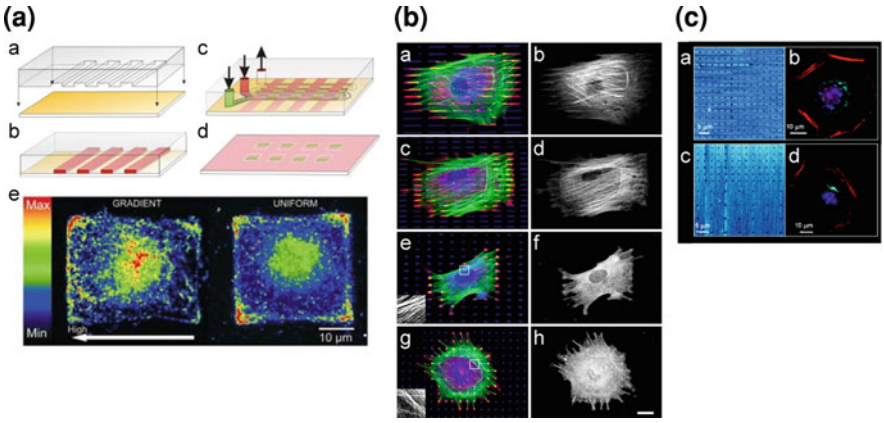

Fig. 5 Various micropatterning approaches. **A** Micropatterning combined with microfluidics creates a pattern with gradient (*a–d*). Vinculin heat maps (*e*) of stacked images of cells on gradient islands and uniform islands show focal adhesion density distribution. Reproduced from [82]. **B** Myofibroblasts plated on arrays of islets of different lengths (*a*, *b* 20 μm, *c*, *d* 10 μm, *e*, *f* 6 μm, and *g*, *h* 2 μm). *Green* F-actin, *red* vinculin, *blue* fibronectin, *right column image* α-SMA. Reproduced from [42]. **C** Dip-pen nanolithography was used to form symmetric (*a*) and asymmetric (*c*) nanoarrays. Images of the patterns were observed under lateral force microscopy (*a*, *c*). Fibroblasts attached to asymmetric nanoarrays (*d*) showed polarized cytoskeletal structure whereas those on the symmetric pattern showed no polarity. *Green* Golgi apparatus, *red* actin, *blue* nuclei. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

how cells sense and respond to the ECM environment. Cells plated onto substrates micropatterned with arrays of fibronectin islets were used to verify the hypothesis that only large focal adhesion can support high tension from stress fibers [42]. Incorporation of α-smooth muscle actin (α-SMA) into stress fibers was the hallmark used to indicate a high cytoskeletal tension. Cells plated on the long islets arrays formed "supermature" focal adhesions and only those cells showed stress fibers that had α-SMA (Fig. 5B, a,d). In contrast, cells grown on the small islet arrays showed no α-SMA incorporation in their stress fibers (Fig. 5B, e,h). Furthermore, when the cells were stretched mechanically, their focal adhesions grew in size, allowing α-SMA to be recruited to the stress fibers, but this response only occurred for adhesions that were stretch above a critical size to support the higher cytoskeletal tension.

Dip-pen nanolithography (DPN) is another approach to patterned adhesive islands on which cells can form focal adhesions. DPN uses an atomic force microscope tip that is inked with SAMs or proteins to deposit these molecules at defined locations on a surface [61]. Although there have been few studies that used this powerful tool to study cell–ECM interaction, one was conducted on how asymmetric peptide nanoarray affect cell polarization [47]. Cells were plated on either symmetric arrays having 3 μm pitch and 500 nm RGD peptide spots (Fig. 5C, a) or asymmetric arrays consisting of two regions—high density (3 μm pitch) and low density (6 μm pitch) regions with the same 500 nm spot size (Fig. 5C, c). Cells on the asymmetric array were observed to be polarized toward the higher density region as evidenced by the relative position of Golgi apparatus with respect to the nucleus (Fig. 5C, d). Cells on the symmetric arrays showed non-uniform distribution of their Golgi, indicating they were not polarized (Fig. 5C, b). Although this polarization result is in agreement with the result found in ligand gradients using microfluidics, the DPN study demonstrated that nanotechnology affords the opportunity to conduct more quantitative studies by tailoring the spatial pattern—ligand size, spacing between adhesive islands, aspect ratios, and shapes.

Efforts to study how individual integrins interact with each other in mechanosensing have led to the development multidomain colloidal gels to control spacing between individual integrin receptors. The colloidal gels are made from a large polymer molecule that has a brush-like structure (Fig. 6A, a) [51]. Poly(methyl methacrylate) (PMMA) forms the backbone of the molecule and polyethylene oxide (PEO) forms the side chains. A portion of the ends of the side chains are functionalized with RGD peptides to allow cells to adhere, but those side chains without peptides block cell adhesion. The diameter of the brush-like molecule was approximately 32 nm and the spacing between RGD peptides varied between 14 and 25 nm, which depended upon the number of peptides bound to the molecule (Fig. 6A, b). The nanoscale spacing between RGD peptides allowed for control over integrin clustering, independent of the overall RGD surface density. For colloidal gels with small spacing between RGD peptides, the clustering of integrins permitted the cells to remain attached to the surface when subjected to a centrifugal detachment force. This result indicates that integrin clustering is more essential for the mechanical stability of cell adhesion than ligand surface density. Similar colloidal

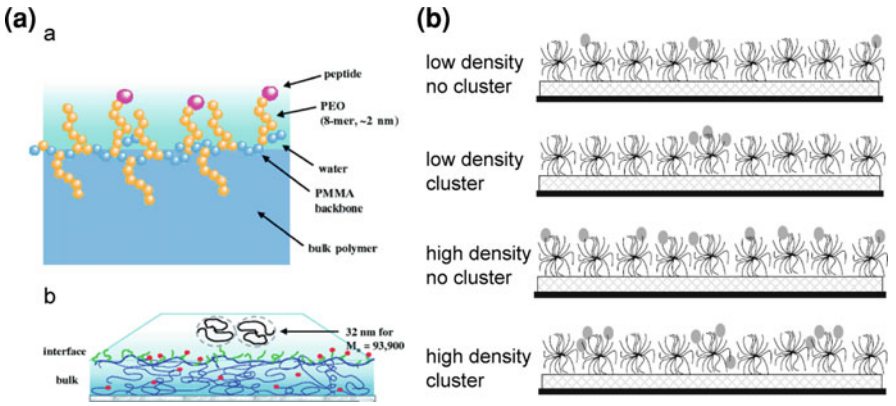

Fig. 6 Multidomain peptide colloidal gel system. **A** Schematic of RGD-comb structure (*a*) shows RGD peptides attached on short PEO side chains that are tethered to the PMMA backbone. RGD islands (*b*) are formed in a quasi-2D configuration at the water–polymer interface. Reproduced from [56]. **B** On a film of star polymers, ligands (*shaded oval*) are tethered with controlled density and degree of spacing to control integrin clustering. Adapted from [71]

gels were used to study how integrin clustering affects cell migration [71]. Cells were plated on PEO gel substrates that either permitted or restricted clustering and had either high or low density of RGD peptide (Fig. 6B). As expected, cells on substrates that had high RGD surface density had increased motility. However, migration speeds were significantly reduced if the integrins were restricted from clustering. Furthermore, ligand clustering reduced the average ligand density required for cell migration and also allowed more stress fibers to form. In contrast, non-clustered integrin did not allow full spreading or motility, but only weak adhesion. Together, the studies using colloidal gels implicate that integrin clustering enables recruitment of cytoplasmic regulatory and structural proteins at focal adhesion to activate signaling pathway that regulate cell motility and traction forces.

Patterning gold nanodots is a technique that corroborates the findings that used colloidal gels to investigate integrin clustering, but affords a higher degree of spatial control and precision in the placement of the adhesion sites. Each gold nanodot has a diameter of 6–8 nm whereas that of the integrin head domain is 9 nm [107], resulting in only a single integrin binding to a deposited gold particle. Arrays of gold nanodots were made by self-assembled polymer micelles, which contained a single gold particle at their center (Fig. 7A, top). The micelles form a monolayer on a substrate that begins the seeding process for the gold particle arrays. When the micelle-coated substrate is exposed to oxygen plasma, the polymer capsule is removed and only the naked gold particles are left on the surface (Fig. 7A, bottom). By using different diameters of micelles, the spacing between the particles could be varied between 30 and 140 nm [95]. Examples of gold nanodot patterns with 50 and 90 nm inter-particle spacing are shown in Fig. 7B. Gold particles are biofunctionalized with RGD-containing peptide, and rest of the region on a substrate is covered with PLL-g-PEG to prevent integrin adhesion to the glass. Taking

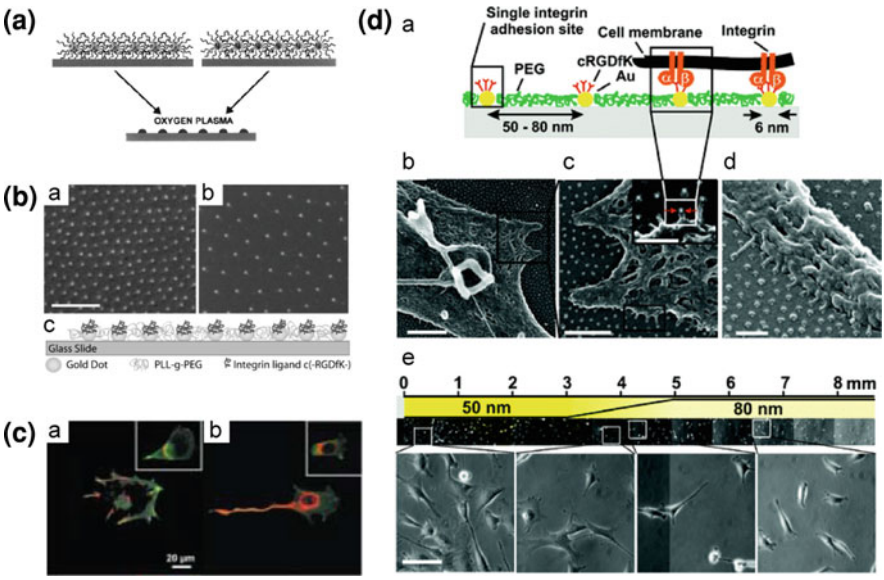

Fig. 7 Gold dots for controlling integrin interactions and clustering. **A** Gold clusters (*bottom*) are formed via oxygen plasma treatment by degrading copolymer micelles completely (*top*). Reproduced from [95]. **B** Nanopatterned glass substrates have hexagonally arranged gold particles with inter-particle spacings of 50 nm (*left*) and 90 nm (*right*). The illustration on the *bottom* shows the schematic of a nanopatterned substrate with gold particles coated with RGD peptide for integrin adhesion and space between the gold dots filled with PLL-g-PEG for integrin blocking. **C** Cells on 58-nm spaced RGD-coated nanodots (*a*) showed β_3-integrins (*green*) colocalized with focal adhesion kinase (FAK, *red*) whereas cells on 73-nm spaced nanopattern (*b*) showed poor spreading and no colocalization with FAK. Reproduced from [5]. **D** Schematic (*a*) and SEM images (*b–d*) of cells on a gradient of nanodots across an 8-mm long substrate (*e*). Reproduced from [6]. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

advantage of the nanoscale control over ligand spacing, it was found that cells on substrates with adhesive spacing greater than 73 nm showed poor adhesion and spreading, whereas cells on smaller spacings spread as well as they do on glass surfaces coated uniformly with RGD peptide [5]. Cells on nanodots with 58 nm spacings (Fig. 7C, a) were observed to have focal complexes that were well formed because β_3 integrin and focal adhesion kinase (FAK) co-localized with each other. On the other hand, cells on 73 nm spacing (Fig. 7C, b) had poor spreading because β_3 integrin and FAK failed to co-localize. These results demonstrate that the distance between ligands can restrict the ability for integrin to cluster together and thus prevent the maturation of an adhesion. Interestingly, integrin clustering was found to be more important than the amount of ligand deposited on a surface. Cells spread better on hybrid arrays of $2 \times 2\ \mu m^2$ squares, where each square consisted of 58-nm spaced gold dots, than on uniform arrays of 73-nm spaced dots that had more ligand content. The influence of ligand spacing on focal adhesion formation was further confirmed by a study where integrins that formed on 108-nm spaced

nanodots showed rapid turnover of focal adhesions and reduced amounts of zyxin at the focal adhesions, which is a protein that is recruited to the adhesion complex upon mechanical force [16, 62]. In addition to cell spreading, studies on cell polarization have benefitted from this nanopatterning technique. A gradient of nanodot spacings from 50 to 80 nm (Fig. 7D, e) was used to find that cells reorient their polarization towards closer spaced patterns and are able to sense spatial differences as small as 1 nm in spacing [6]. Cells on gradients of gold dots also showed membrane protrusions toward individual gold particles at the nanometer scale, which suggests that membrane protrusion activity is essential for restructuring the polarity of a cell (Fig. 7D, b,d).

3 Force Measuring Tools

Use of surface control techniques at the nanoscale has helped to reveal the structure and underlying mechanisms that influences cell adhesion, polarization, and migration. However, the same intracellular proteins involved in adhesion are also essential for actin–myosin generated traction forces that are transmitted at focal adhesions. For this reason, traction forces can strongly contribute to cellular responses such as adhesion strength, cytoskeletal morphology, and cell motility. During cell migration, for example, cells need to pull themselves forward to move. Traction forces had been hypothesized to drive cell migration due to actin–myosin cross-bridges, but they had been difficult to measure at the nanoscale. Development of deformable substrates has provided approaches to observe and quantify cellular traction forces by the extent of physical distortion in a substrate.

The first method for measuring traction forces was to use a thin film of silicone rubber that wrinkled under the load from traction forces exerted on them [45].

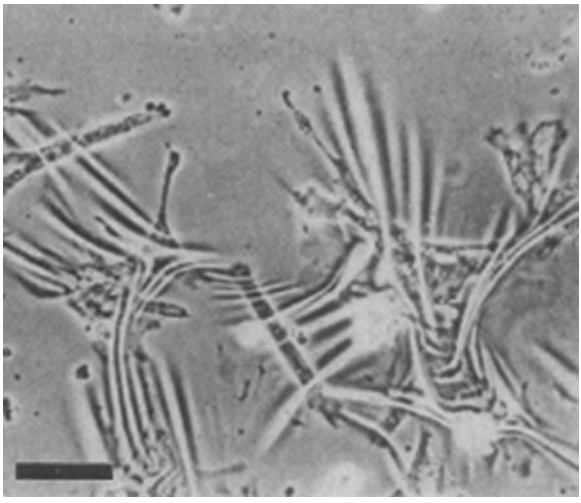

Fig. 8 Silicone rubber membrane wrinkles due to the traction forces from several chick heart fibroblasts. Reproduced from [45]. *Bar* 100 μm

This wrinkling substrate was made by briefly exposing silicone fluid to a flame that crosslinked only the surface of the silicone, creating a skin of 1 μm thickness. When cells were plated on the surface, they spread out and pulled tangentially on the rubber sheet, producing visible wrinkles in the rubber (Fig. 8). This result was significant in that traction forces could be observed and compared for the first time. Wrinkling membranes were used to confirm that signaling pathways such as the small GTPase RhoA or Ca^{2+}/calmodulin pathways regulate traction forces through stress fiber formation and focal adhesion formation [20, 46]. Despite the immense significance of this technique to the field, it provided a limited measurement because it could only be used to assess cells by the number of wrinkles they produced and not the exact amount of force. Some improvements have been made to provide a semi-quantitative measurement of the traction forces using wrinkling membranes [12, 13]. The mechanics of wrinkling however is nonlinear in its relationship between force and deformation so it is inherently inaccurate to find an exact force measurement. Moreover, wrinkles are formed rather chaotically so there is limited temporal and spatial resolution possible to measure forces acting at individual focal adhesions [25].

The first, non-wrinkling, but still deformable substrate was made by crosslinking the silicone rubber in a glass chamber using a glow discharge device (Fig. 9a) [60]. Small beads that were a micrometer in diameter were embedded on the elastomer surface. As a cell produced traction forces, the position of the beads on the silicone

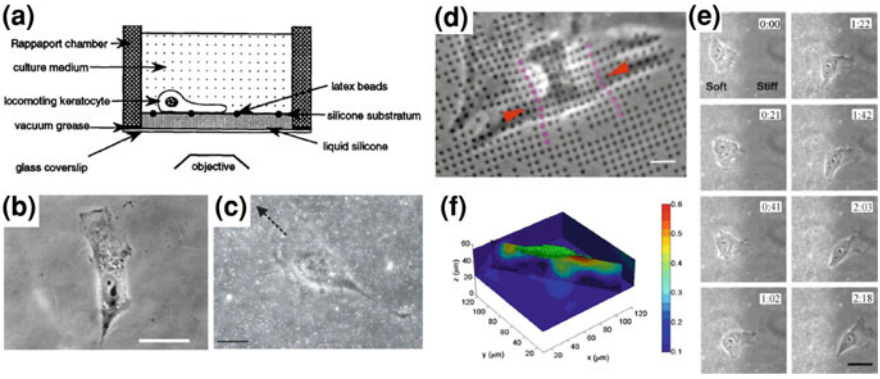

Fig. 9 Traction force microscopy (TFM) is used to measure cellular forces. **a** Silicone rubber-based traction force microscopy has latex beads embedded on the surface of silicone film to report the distortion from traction forces. Reproduced from [60]. **b** A fibroblast on a compliant polyacrylamide (PA) gel. Reproduced from [80]. *Bar* 10 μm. **c** Traction forces of a fibroblast migrating in the direction of the *arrow* were measured by the displacement of fluorescent nanobeads (0.2 μm diameter) which are seen as *white dots* embedded in the gel. Reproduced from [77]. *Bar* 20 μm. **d** Regular array of micropatterned dots on an elastomeric substrate shows contraction of a fibroblast (indicated by *arrowheads*). Reproduced from [8]. *Dots pitch* 2 μm, *bar* 6 μm. **e** A fibroblast plated on a soft region of a gel migrated towards the rigid region. Reproduced from [69]. *Bar* 40 μm. **f** 3D forces of a cell are measured by the displacement contour slice along the depth of a gel. *Color bar* Magnitude of total 3D displacement. Reproduced from [72]. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

rubber changed due to deformation of the film. From the bead displacements, the force vectors of cells could be measured. At first, the local traction force was assumed to be linearly related to bead displacement. This incorrect assumption was later rectified by using elasticity theory about the deformation of a compliant film [24]. This technique was only able to measure traction forces for highly contractile cells and so a limited range of cultured cells were able to be studied. Nonetheless, there were interesting findings on the migration of fish keratocytes using this early elastic film. These cells are highly motile and had been studied extensively to understand the mechanisms and mechanics of cell migration. Using the non-wrinkling silicone films, small bead displacements were observed at the leading edge of the cells and larger bead displacements at the rearward region.

The introduction of polyacrylamide gel to measure traction forces was a breakthrough in addressing the limitations of silicone film substrates by providing a way to control the stiffness by varying the mixing ratios between the acrylamide monomer and bisacrylamide cross-linker (Fig. 9b) [80, 104]. The technique measured the movement of fluorescent nanobeads embedded in the gel and was referred to as traction force microscopy (TFM) (Fig. 9c). The displacement vectors are calculated by subtracting the positions of beads as they are deformed by a cell's traction forces from their original, undeformed positions once the cell has been removed. The traction stress field is then calculated from the displacement vectors using elasticity theory [10, 58]. Since the beads are randomly seeded into the gels, there can be uncertainty in solving for the appropriate traction forces at regions of low bead density. To address this, orthogonal arrays of fluorescent beads were made by electron beam lithography and then patterned onto an elastomeric substrate (Fig. 9d) [8]. Due to the even distribution of the bead markers, the number of possible solutions for the traction force vector field for a measured displacement field was significantly reduced.

Despite some of the difficulties in analyzing traction forces using TFM, it has become widely used to study cells during migration and contraction. The traction stress distribution of migrating fibroblasts was found to have high forces at the leading edge of a cell whereas the middle and posterior of a cell had lower forces (Fig. 9c) [25]. This non-uniform distribution of forces indicates that cells are pulled forward by contractile forces at the front of a cell. The role of high forces at the leading edge were also found to determine the change in direction during migration [77]. Since the gels can have finely controlled stiffness, this has led to an important finding that cells sense and respond to the rigidity of the ECM [27]. Cells on soft gels have less spread area, increased rates of motility and lamellipodia extension, small focal adhesions, and less phosphorylation of focal adhesion proteins as compared to those on rigid gels [80]. Building from this finding, gels were created that contained both soft and rigid regions that were adjacent to each other [69]. Cells were able to migrate from the soft region to the rigid region (Fig. 9e). However, cells refused to cross from the rigid region into the soft region because they had higher traction forces and more spreading on the rigid side. This phenomenon of directed migration due to substrate rigidity is called durotaxis. It was later found that durotaxis, like haptotaxis and chemotaxis, is sensitive to

gradients in substrate stiffness [52]. In addition, ECM stiffness has been found to affect the contractile development of cardiomyocytes [31], differentiation of stem cells [30], and invasiveness of breast tumor cells [64, 79].

TFM has also been used to study the effect of ligand density and the involvement of traction forces in spreading. The spread areas of cells were tightly influenced by the ligand density on a surface, but had different increasing relationships depending on cell type and ECM ligand type [29, 38, 84]. Interestingly, traction forces also increased with spread area and ligand density, indicating that traction forces play a role in a cell's ability to extend its structure and maintain stable contact with a surface [84, 85]. These findings are in agreement with those from previous studies using surface adhesivity control, where cell spreading and adhesion strength were enhanced with increased ligand density [5, 44, 50]. Since integrins need high ligand density in order to cluster, it is likely that ligand density allows focal adhesions to stabilize in such a way that multiple integrins work together to support a larger degree of traction force. Furthermore, focal adhesion size, a measure of the degree of recruitment of cytoplasmic proteins and integrins, was found to increase with the local traction force acting at an individual adhesion [8]. In light of these findings, it is likely that the high force required to detach cells on colloidal gels and gold nanodots is because these surfaces promote integrin clustering so that large focal adhesions are able to form [71, 89].

TFM has elucidated the two-dimensional (2D) distribution of cellular contractility, but cells often reside in a three-dimensional (3D) context. Cell motility in 3D is slower and the molecular composition of focal adhesions are smaller as compared to those in 2D [21, 32, 110]. Thus, traction forces of cells are expected to be different in 3D environments. Some early works into measuring traction forces in 3D have been implemented using TFM, but have not found dramatic differences are compared to traction forces in 2D [9, 72]. One approach was to overlay a second TFM gel on top of a cell to look at tangential traction forces on the dorsal and ventral surfaces [9]. It was found that the strength of these traction forces were similar to those observed in 2D culture. To analyze traction forces that were normal to a surface, the distortion of beads in a gel underneath a cell were measured in all three directions using confocal microscopy [72]. Interestingly, the normal displacements were similar to or slightly greater than tangential displacements for the cells (Fig. 9f). The forces that cells generate in 3D imply that they can use them to explore or remodel their microenvironment using both normal and tangential forces. Although these works provided an early framework to analyze 3D traction forces, there are still many challenges and obstacles to create a feasible and analyzable assay to study the physical interactions of cells in 3D [26, 32].

Continuous, deformable substrates such as silicone membranes or TFM gels have an inherent disadvantage in that a local force at a focal adhesion can cause a wide distortion of the substrate, which can in turn cause weaker, secondary forces that act externally at adjacent focal adhesions. Thus, the continuous nature of the substrates makes it difficult to isolate the local force at each focal adhesion. The need for tools to measure the local traction force in an independent manner brought about the development of the microfabricated cantilevers [28, 35, 36, 97]. The first cantilever

used for a traction force study was a horizontal cantilever which was fabricated on a silicon wafer using surface micromachining fabrication (Fig. 10a) [35]. The cantilever is deflected laterally when a cell pulls on the tip of the cantilever. The local traction force can be determined by multiplying the displacement of the tip by the stiffness of the cantilever. Interestingly, a migrating cell showed much larger force at its tail than those found at the front [35]. This result is similar to traction forces of keratocytes measured on silicone rubber [60], but somewhat different from findings by TFM [25, 77]. Nonetheless, the study with this micromachined device provided deeper understanding that forces at the front of the cell need to overcome those at the rear in order for a cell to migrate [90].

One of the limitations of the horizontal cantilever was a fixed, single location of force sensing which only allowed for a force measurement across a straight-line trajectory of a migrating cell. Unlike TFM, it did not provide a 2D map of traction forces acting at all adhesions of a cell. To address this issue, vertical arrays of elastomeric cantilevers were developed through a soft lithography and replica casting with a silicone rubber [28, 97]. Cells could spread out by attaching to the tips of individual posts (Fig. 10b) [63, 97]. Like the horizontal cantilever, posts deflect independent of each other and thus could report the local traction force based upon the stiffness of the posts. The use of this array of vertical cantilevers has helped confirm that RhoA signaling and focal adhesion size affect the strength of traction forces, as seen previously with wrinkling silicone membranes and TFM [97]. Moreover, the stiffness of a post can be easily tuned by tailoring dimensions of a post, so traction forces of a cell could be tested in response to different stiffness. As seen with 2D TFM, traction forces increased with the stiffness of the posts in an array, which indicates that the displacement at a focal adhesion is constant, but the local force increases in accordance to the stiffness of the

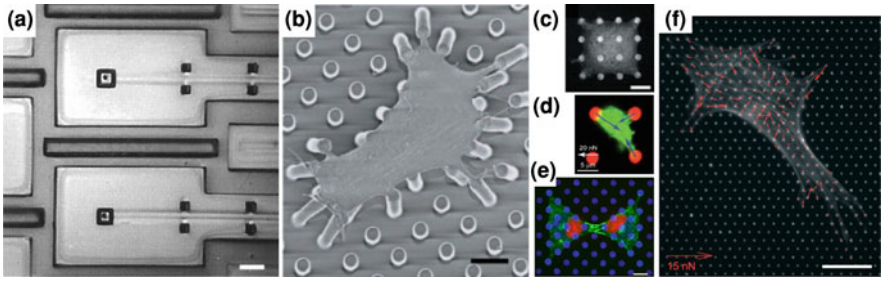

Fig. 10 Microfabricated cantilevers for traction force study. **a** MEMS-based horizontal cantilever force sensor. The end of the cantilever is positioned below the *square* opening in the surface of the substrate. Reproduced from [35]. *Bar* 10 μm. **b** Scanning electron micrograph of a smooth muscle cell attached on tips of vertical post arrays. **c** Microcontact printing can be used to confine cell spreading. **b** and **c** reproduced from [97]. *Scales bars* 10 μm. **d** Platelets in a clot (*green*) deflect the microposts (*red*). Reproduced from [65]. **e** Two cells patterned in a bow-tie shape apply a tugging force against each other. *Green β*-catenin, *red* DAPI, *blue* DiI. Reproduced from [68]. *Bar* 10 μm. **f** Cell on nanopost arrays showing force vectors (*red*). Reproduced from [108]. *Bar* 20 μm. Refer to the electronic version of this chapter, DOI: 10.1007/8415_2010_26, for viewing color version of this figure

environment [27, 41, 88]. It is worth noting that in 3D, TFM has shown that the magnitude of bead displacements were constant for different gel stiffness [72]. It may be likely that a cell has an integrin-associated mechanosensory feedback system that regulates the overall strain that actin and myosin produce within a cell.

One advantage of using silicone rubber for the posts is its availability to be functionalized by microcontact printing [98]. Cells can be patterned on the posts with different sized square patterns to pattern single cells, pairs of cells, or monolayers (Fig. 10c) [68, 78, 87, 97]. It has been seen that cells with more spreading exerted higher traction forces than those that are less spread, which agrees with findings from TFM studies. Confinement of spread area on a force sensor can allow one to rule out the effect of spread area and focus on the influences of other factors on traction forces. For example, cells have been observed to exert more force on stiffer substrates, but at the same time, they also spread more on stiffer environments so the effect on traction forces may be confounded by cell spreading [29, 69, 84, 85, 88]. Techniques to isolate the roles of cell spreading and stiffness would be beneficial to understand how cells regulate their adhesion strength and traction forces. Microcontact printing can also be used to examine intercellular forces of cells in a monolayer or adjacent to one another (Fig. 10e) [68, 78]. It has been seen that cells transmit tugging forces through their adherens junctions and the length of the junctions can determine the strength of force that can be withstood.

As the contractile and adhesion biomechanics of different cell types become of wider interest, post arrays with micrometer dimensions may not provide enough spatial resolution. In particular, platelets are amongst the smallest cells in the human body and have adhesion and contractile properties that are essential for clotting and vascular healing. The contractility of aggregated platelets on different ligands have been tested with the microscale post arrays (Fig. 10d) [65]. Moving forward, measuring the contractility of individual platelets would be valuable for evaluating the strength of platelet–platelet versus platelet–ECM interactions. One approach that could be helpful is nanopost arrays. Nanoposts have sub-micrometer dimensions and can be fabricated using projection lithography and reactive ion etching [108]. Cells plated on these posts showed similar spreading area and morphology as those found on continuous substrates (Fig. 10f). Further development of nanoposts might also elucidate the contractile mechanics of cells during migration and spreading. In particular, the lamellipodium and lamellum regions at the leading edge of a cell have different molecular players involved in their extension and contraction [102]. It would be beneficial to use the improved spatial resolution of nanoposts to examine the different strengths of traction forces at each of these regions.

4 Conclusions

Cells respond to many aspects of their surrounding ECM—from chemical presentations such as adhesivity, ligand density, and nanoscale ligand spacing to mechanical presentations such as stiffness and spreadable area. Surface

characterization techniques have provided a platform to find that cell adhesion requires integrin clustering, focal adhesion strengthening, and stress fiber formation. At the same time, studies with force measurement tools have revealed that myosin-based traction forces are necessary for the stability of cell adhesions. As new nanotechnology-based tools are developed, however, it is worth noting that a cell contains a complex network of interactions that combine receptor signaling, protein conformational changes, ion gradients, and transport along the cytoskeleton, which are all integrated to determine the activity of a cell. Particularly for the cell–ECM interactions, there is a reciprocal relationship between the cell and the ECM that involves mechanical forces. Specifically, the structure of the ECM can affect the adhesion, morphology, contractility and motility of cells, and at the same time, those cellular activities can also cause the restructuring of the surrounding ECM. When developing and applying new technology, one should consider secondary causes and maintain a degree of guarded skepticism when interpreting the results from the data. Moreover, it would be a vast improvement if there were approaches to study the different chemical and mechanical factors independently and in combination with each other.

We have highlighted the tools for studying the adhesions and traction forces of cells in response to changes in the microenvironment. It is worth noting that there are tools that can be used to applying nanoscale forces to a cell, which help understand the sensation and response involved in mechanotransduction [93]. These tools use magnetic forces, optical forces, or atomic force microscopy to test the local adhesion strengthening and cytoskeletal connection to focal adhesion [19, 37, 70, 89, 94, 103]. One common finding from these tools was that in response to external force, cells strengthen the connections between their adhesions and the ECM by recruiting additional focal adhesion proteins. Interestingly the same adhesion strengthening occurred in response to internally generated traction forces, which implicates that cells respond to forces acting internally or externally. A number of questions on the molecular nature of mechanosensing, focal adhesion maturation, cytoskeletal connectivity, membrane extension, and release of adhesions remain unaddressed. The tools to address these questions are emerging and with them will likely be the answers to important questions in stem cell and cancer biology.

Acknowledgments The authors are grateful for support in part from grants from the National Institutes of Health (HL097284) and the National Science Foundation's CAREER Award.

References

1. Abercrombie, M., Heaysman, J.E., Pegrum, S.M.: The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. Exp. Cell Res. **67**(2), 359–367 (1971)
2. Alberts, B.: Molecular Biology of the Cell, 4th ed. Garland Science, New York (2002)
3. Alenghat, F.J., Ingber, D.E.: Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. Sci STKE **2002**(119), pe6 (2002)
4. Alexandrova, A.Y., Arnold, K., Schaub, S., Vasiliev, J.M., Meister, J.J., Bershadsky, A.D., Verkhovsky, A.B.: Comparative dynamics of retrograde actin flow and focal adhesions:

formation of nascent adhesions triggers transition from fast to slow flow. PLoS One **3**(9), e3234 (2008)
5. Arnold, M., Cavalcanti-Adam, E.A., Glass, R., Blummel, J., Eck, W., Kantlehner, M., Kessler, H., Spatz, J.P.: Activation of integrin function by nanopatterned adhesive interfaces. Chemphyschem **5**(3), 383–388 (2004)
6. Arnold, M., Hirschfeld-Warneken, V.C., Lohmuller, T., Heil, P., Blummel, J., Cavalcanti-Adam, E.A., Lopez-Garcia, M., Walther, P., Kessler, H., Geiger, B., Spatz, J.P.: Induction of cell polarization and migration by a gradient of nanoscale variations in adhesive ligand spacing. Nano Lett **8**(7), 2063–2069 (2008)
7. Bain, C.D., Whitesides, G.M.: Formation of 2-component surfaces by the spontaneous assembly of monolayers on gold from solutions containing mixtures of organic thiols. J. Am. Chem. Society **110**(19), 6560–6561 (1988)
8. Balaban, N.Q., Schwarz, U.S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L., Geiger, B.: Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat. Cell Biol. **3**(5), 466–472 (2001)
9. Beningo, K.A., Dembo, M., Wang, Y.L.: Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. Proc. Natl. Acad. Sci. U.S.A. **101**(52), 18024–18029 (2004)
10. Bernardo, J.M., Smith, A.F.M.: Bayesian Theory. Wiley, Chichester (2000)
11. Brock, A., Chang, E., Ho, C.C., LeDuc, P., Jiang, X.Y., Whitesides, G.M., Ingber, D.E.: Geometric determinants of directional cell motility revealed using microcontact printing. Langmuir **19**(5), 1611–1617 (2003)
12. Burton, K., Taylor, D.L.: Traction forces of cytokinesis measured with optically modified elastic substrata. Nature **385**(6615), 450–454 (1997)
13. Burton, K., Park, J.H., Taylor, D.L.: Keratocytes generate traction forces in two phases. Mol. Biol. Cell **10**(11), 3745–3769 (1999)
14. Carter, S.B.: Principles of cell motility: the direction of cell movement and cancer invasion. Nature **208**(5016), 1183–1187 (1965)
15. Carter, S.B.: Haptotaxis and the mechanism of cell motility. Nature **213**(5073), 256–260 (1967)
16. Cavalcanti-Adam, E.A., Volberg, T., Micoulet, A., Kessler, H., Geiger, B., Spatz, J.P.: Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. Biophys. J. **92**(8):2964–2974 (2007)
17. Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., Ingber, D.E.: Geometric control of cell life and death. Science **276**(5317), 1425–1428 (1997)
18. Choi, C.K., Vicente-Manzanares, M., Zareno, J., Whitmore, L.A., Mogilner, A., Horwitz, A.R.: Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. Nat. Cell Biol. **10**(9), 1039–1050 (2008)
19. Choquet, D., Felsenfeld, D.P., Sheetz, M.P.: Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell **88**(1), 39–48 (1997)
20. Chrzanowska-Wodnicka, M., Burridge, K.: Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol. **133**(6), 1403–1415 (1996)
21. Cukierman, E., Pankov, R., Stevens, D.R., Yamada, K.M.: Taking cell-matrix adhesions to the third dimension. Science **294**(5547), 1708–1712 (2001)
22. Curtis, A.S.: The mechanism of adhesion of cells to glass. A study by interference reflection microscopy. J. Cell Biol. **20**, 199–215 (1964)
23. Curtis, A.S., Forrester, J.V., McInnes, C., Lawrie, F.: Adhesion of cells to polystyrene surfaces. J. Cell Biol. **97**(5 Pt 1), 1500–1506 (1983).
24. Dembo, M., Oliver, T., Ishihara, A., Jacobson, K.: Imaging the traction stresses exerted by locomoting cells with the elastic substratum method. Biophys. J. **70**(4), 2008–2022 (1996)
25. Dembo, M., Wang, Y.L.: Stresses at the cell-to-substrate interface during locomotion of fibroblasts. Biophys. J. **76**(4), 2307–2316 (1999)

26. Discher, D., Dong, C., Fredberg, J.J., Guilak, F., Ingber, D., Janmey, P., Kamm, R.D., Schmid-Schonbein, G.W., Weinbaum, S.: Biomechanics: cell research and applications for the next decade. Ann. Biomed. Eng. **37**(5), 847–859 (2009)
27. Discher, D.E., Janmey, P., Wang, Y.L.: Tissue cells feel and respond to the stiffness of their substrate. Science **310**(5751), 1139–1143 (2005)
28. du Roure, O., Saez, A., Buguin, A., Austin, R.H., Chavrier, P., Silberzan, P., Ladoux, B.: Force mapping in epithelial cell migration. Proc. Natl. Acad. Sci. U.S.A. **102**(7), 2390–2395 (2005)
29. Engler, A., Bacakova, L., Newman, C., Hategan, A., Griffin, M., Discher, D.: Substrate compliance versus ligand density in cell on gel responses. Biophys. J. **86**(1), 617–628 (2004)
30. Engler, A.J., Sen, S., Sweeney, H.L., Discher, D.E.: Matrix elasticity directs stem cell lineage specification. Cell **126**(4), 677–689 (2006)
31. Engler, A.J., Carag-Krieger, C., Johnson, C.P., Raab, M., Tang, H.Y., Speicher, D.W., Sanger, J.W., Sanger, J.M., Discher, D.E.: Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating. J. Cell Sci. **121**(Pt 22), 3794–3802 (2008)
32. Even-Ram, S., Yamada, K.M.: Cell migration in 3D matrix. Curr. Opin. Cell Biol. **17**(5), 524–532 (2005)
33. Even-Ram, S., Doyle, A.D., Conti, M.A., Matsumoto, K., Adelstein, R.S., Yamada, K.M.: Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. Nat. Cell Biol. **9**(3), 299–309 (2007)
34. Folkman, J., Moscona, A.: Role of cell shape in growth control. Nature **273**(5661), 345–349 (1978)
35. Galbraith, C.G., Sheetz, M.P.: A micromachined device provides a new bend on fibroblast traction forces. Proc. Natl. Acad. Sci. U.S.A. **94**(17), 9114–9118 (1997)
36. Galbraith, C.G., Sheetz, M.P.: Forces on adhesive contacts affect cell function. Curr. Opin. Cell Biol. **10**(5), 566–571 (1998)
37. Galbraith, C.G., Yamada, K.M., Sheetz, M.P.: The relationship between force and focal complex development. J. Cell Biol. **159**(4), 695–705 (2002)
38. Gaudet, C., Marganski, W.A., Kim, S., Brown, C.T., Gunderia, V., Dembo, M., Wong, J.Y.: Influence of type I collagen surface density on fibroblast spreading, motility, and contractility. Biophys. J. **85**(5), 3329–3335 (2003)
39. Geiger, B., Bershadsky, A., Pankov, R., Yamada, K.M.: Transmembrane crosstalk between the extracellular matrix—cytoskeleton crosstalk. Nat. Rev. Mol. Cell Biol. **2**(11), 793–805 (2001)
40. Geiger, B., Spatz, J.P., Bershadsky, A.D.: Environmental sensing through focal adhesions. Nat. Rev. Mol. Cell Biol. **10**(1), 21–33 (2009)
41. Ghibaudo, M., Saez, A., Trichet, L., Xayaphoummine, A., Browaeys, J., Silberzan, P., Buguin, A., Ladoux, B.: Traction forces and rigidity sensing regulate cell functions. Soft Matter **4**(9), 1836–1843 (2008)
42. Goffin, J.M., Pittet, P., Csucs, G., Lussi, J.W., Meister, J.J., Hinz, B.: Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. J. Cell Biol. **172**(2), 259–268 (2006)
43. Grinnell, F.: Cellular adhesiveness and extracellular substrata. Int. Rev. Cytol. **53**, 65–144 (1978)
44. Harris, A.: Behavior of cultured cells on substrata of variable adhesiveness. Exp. Cell Res. **77**(1), 285–297 (1973)
45. Harris, A.K., Wild, P., Stopak, D.: Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science **208**(4440), 177–179 (1980)
46. Helfman, D.M., Levy, E.T., Berthier, C., Shtutman, M., Riveline, D., Grosheva, I., Lachish-Zalait, A., Elbaum, M., Bershadsky, A.D.: Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. Mol. Biol. Cell **10**(10), 3097–3112 (1999)

47. Hoover, D.K., Chan, E.W., Yousaf, M.N.: Asymmetric peptide nanoarray surfaces for studies of single cell polarization. J. Am. Chem. Soc. **130**(11), 3280–3281 (2008)
48. Hynes, R.O.: Integrins: versatility, modulation, and signaling in cell adhesion. Cell **69**(1), 11–25 (1992)
49. Ingber, D.E.: Mechanobiology and diseases of mechanotransduction. Ann. Med. **35**(8), 564–577 (2003)
50. Inoue, S., Iida, Y., Otani, Y., Hirano, Y., Tabata, Y.: Adhesion behavior of human adipo-stromal cells on self-assembled monolayers with different surface densities or gradients of RGD peptide. J. Biomater. Sci. Polym. Ed. **20**(4), 495–510 (2009)
51. Irvine, D.J., Mayes, A.M., Griffith, L.G.: Nanoscale clustering of RGD peptides at surfaces using Comb polymers. 1. Synthesis and characterization of Comb thin films. Biomacromolecules **2**(1), 85–94 (2001)
52. Isenberg, B.C., Dimilla, P.A., Walker, M., Kim, S., Wong, J.Y.: Vascular smooth muscle cell durotaxis depends on substrate stiffness gradient strength. Biophys. J. **97**(5), 1313–1322 (2009)
53. Jiang, X., Ferrigno, R., Mrksich, M., Whitesides, G.M.: Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements. J. Am. Chem. Soc. **125**(9), 2366–2367 (2003)
54. Jiang, X., Bruzewicz, D.A., Wong, A.P., Piel, M., Whitesides, G.M.: Directing cell migration with asymmetric micropatterns. Proc. Natl. Acad. Sci. U.S.A. **102**(4), 975–978 (2005)
55. Kilian, K.A., Bugarija, B., Lahn, B.T., Mrksich, M.: Geometric cues for directing the differentiation of mesenchymal stem cells. Proc. Natl. Acad. Sci. U.S.A. **107**(11), 4872–4877 (2010)
56. Koo, L.Y., Irvine, D.J., Mayes, A.M., Lauffenburger, D.A., Griffith, L.G.: Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. J. Cell Sci. **115**(Pt 7), 1423–1433 (2002)
57. Kumar, G., Ho, C.C., Co, C.C.: Guiding cell migration using one-way micropattern arrays. Adv. Mater. **19**(8), 1084–1090 (2007)
58. Landau, L.D., Lifshits, E.M., Kosevich, A.d.M., Pitaevskii, L.P.: Theory of Elasticity, 3rd English ed. Pergamon Press, Oxford (1986)
59. Lee, G.M., Loeser, R.F.: Cell surface receptors transmit sufficient force to bend collagen fibrils. Exp. Cell Res. **248**(1), 294–305 (1999)
60. Lee, J., Leonard, M., Oliver, T., Ishihara, A., Jacobson, K.: Traction forces generated by locomoting keratocytes. J. Cell Biol. **127**(6 Pt 2), 1957–1964 (1994)
61. Lee, K.B., Park, S.J., Mirkin, C.A., Smith, J.C., Mrksich, M.: Protein nanoarrays generated by dip-pen nanolithography. Science **295**(5560), 1702–1705 (2002)
62. Lele, T.P., Pendse, J., Kumar, S., Salanga, M., Karavitis, J., Ingber, D.E.: Mechanical forces alter zyxin unbinding kinetics within focal adhesions of living cells. J. Cell. Physiol. **207**(1), 187–194 (2006)
63. Lemmon, C.A., Sniadecki, N.J., Ruiz, S.A., Tan, J.L., Romer, L.H., Chen, C.S.: Shear force at the cell–matrix interface: enhanced analysis for microfabricated post array detectors. Mech. Chem. Biosyst. **2**(1), 1–16 (2005)
64. Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F., Csiszar, K., Giaccia, A., Weninger, W., Yamauchi, M., Gasser, D.L., Weaver, V.M.: Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell **139**(5), 891–906 (2009)
65. Liang, X.M., Han, S.J., Reems, J.A., Gao, D., Sniadecki, N.J.: Platelet retraction force measurements using flexible post force sensors. Lab Chip **10**(8), 991–998 (2010)
66. Liedberg, B., Tengvall, P.: Molecular gradients of omega-substituted alkanethiols on gold—preparation and characterization. Langmuir **11**(10), 3821–3827 (1995)
67. Liu, L., Ratner, B.D., Sage, E.H., Jiang, S.: Endothelial cell migration on surface-density gradients of fibronectin, VEGF, or both proteins. Langmuir **23**(22), 11168–11173 (2007)

68. Liu, Z., Tan, J.L., Cohen, D.M., Yang, M.T., Sniadecki, N.J., Ruiz, S.A., Nelson, C.M., Chen, C.S.: Mechanical tugging force regulates the size of cell–cell junctions. Proc. Natl. Acad. Sci. U.S.A. **107**(22), 9944–9949 (2010)
69. Lo, C.M., Wang, H.B., Dembo, M., Wang, Y.L.: Cell movement is guided by the rigidity of the substrate. Biophys. J. **79**(1), 144–152 (2000)
70. Mack, P.J., Kaazempur-Mofrad, M.R., Karcher, H., Lee, R.T., Kamm, R.D.: Force-induced focal adhesion translocation: effects of force amplitude and frequency. Am. J. Physiol. Cell Physiol. **287**(4), C954–C962 (2004)
71. Maheshwari, G., Brown, G., Lauffenburger, D.A., Wells, A., Griffith, L.G.: Cell adhesion and motility depend on nanoscale RGD clustering. J. Cell Sci. **113**(Pt 10), 1677–1686 (2000)
72. Maskarinec, S.A., Franck, C., Tirrell, D.A., Ravichandran, G.: Quantifying cellular traction forces in three dimensions. Proc. Natl. Acad. Sci. U.S.A. **106**(52), 22108–22113 (2009)
73. McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., Chen, C.S.: Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev. Cell **6**(4), 483–495 (2004)
74. Meshel, A.S., Wei, Q., Adelstein, R.S., Sheetz, M.P.: Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. Nat. Cell Biol. **7**(2), 157–164 (2005)
75. Montell, D.J.: Morphogenetic cell movements: diversity from modular mechanical properties. Science **322**(5907), 1502–1505 (2008)
76. Mrksich, M., Dike, L.E., Tien, J., Ingber, D.E., Whitesides, G.M.: Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. Exp. Cell Res. **235**(2), 305–313 (1997)
77. Munevar, S., Wang, Y., Dembo, M.: Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. Biophys. J. **80**(4), 1744–1757 (2001)
78. Nelson, C.M., Jean, R.P., Tan, J.L., Liu, W.F., Sniadecki, N.J., Spector, A.A., Chen, C.S.: Emergent patterns of growth controlled by multicellular form and mechanics. Proc. Natl. Acad. Sci. U.S.A. **102**(33), 11594–11599 (2005)
79. Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., Hammer, D.A., Weaver, V.M.: Tensional homeostasis and the malignant phenotype. Cancer Cell **8**(3), 241–254 (2005)
80. Pelham, R.J., Jr., Wang, Y.: Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc. Natl. Acad. Sci. U.S.A. **94**(25), 13661–13665 (1997)
81. Pellegrin, S., Mellor, H.: Actin stress fibres. J. Cell Sci. **120**(Pt 20), 3491–3499 (2007)
82. Petty, R.T., Li, H.W., Maduram, J.H., Ismagilov, R., Mrksich, M.: Attachment of cells to islands presenting gradients of adhesion ligands. J. Am. Chem. Soc. **129**(29), 8966–8967 (2007)
83. Prime, K.L., Whitesides, G.M.: Self-assembled organic monolayers: model systems for studying adsorption of proteins at surfaces. Science **252**(5010), 1164–1167 (1991)
84. Rajagopalan, P., Marganski, W.A., Brown, X.Q., Wong, J.Y.: Direct comparison of the spread area, contractility, and migration of balb/c 3T3 fibroblasts adhered to fibronectin- and RGD-modified substrata. Biophys. J. **87**(4), 2818–2827 (2004)
85. Reinhart-King, C.A., Dembo, M., Hammer, D.A.: The dynamics and mechanics of endothelial cell spreading. Biophys. J. **89**(1), 676–689 (2005)
86. Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., Bershadsky, A.D.: Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J. Cell Biol. **153**(6), 1175–1186 (2001)
87. Ruiz, S.A., Chen, C.S.: Emergence of patterned stem cell differentiation within multicellular structures. Stem Cells **26**(11), 2921–2927 (2008)
88. Saez, A., Buguin, A., Silberzan, P., Ladoux, B.: Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophys. J. **89**(6), L52–L54 (2005)

89. Selhuber-Unkel, C., Erdmann, T., Lopez-Garcia, M., Kessler, H., Schwarz, U.S., Spatz, J.P.: Cell adhesion strength is controlled by intermolecular spacing of adhesion receptors. Biophys. J. **98**(4), 543–551 (2010)
90. Sheetz, M.P., Felsenfeld, D.P., Galbraith, C.G.: Cell migration: regulation of force on extracellular-matrix-integrin complexes. Trends Cell Biol. **8**(2), 51–54 (1998)
91. Smith, J.T., Tomfohr, J.K., Wells, M.C., Beebe, T.P., Jr., Kepler, T.B., Reichert, W.M.: Measurement of cell migration on surface-bound fibronectin gradients. Langmuir **20**(19), 8279–8286 (2004)
92. Smith, J.T., Elkin, J.T., Reichert, W.M.: Directed cell migration on fibronectin gradients: effect of gradient slope. Exp. Cell Res. **312**(13), 2424–2432 (2006)
93. Sniadecki, N.J., Desai, R.A., Ruiz, S.A., Chen, C.S.: Nanotechnology for cell–substrate interactions. Ann. Biomed. Eng. **34**(1), 59–74 (2006)
94. Sniadecki, N.J., Anguelouch, A., Yang, M.T., Lamb, C.M., Liu, Z., Kirschner, S.B., Liu, Y., Reich, D.H., Chen, C.S.: Magnetic microposts as an approach to apply forces to living cells. Proc. Natl. Acad. Sci. U.S.A. **104**(37), 14553–14558 (2007)
95. Spatz, J.P., Mossmer, S., Hartmann, C., Moller, M., Herzog, T., Krieger, M., Boyen, H.G., Ziemann, P., Kabius, B.: Ordered deposition of inorganic clusters from micellar block copolymer films. Langmuir **16**(2), 407–415 (2000)
96. Stopak, D., Wessells, N.K., Harris, A.K.: Morphogenetic rearrangement of injected collagen in developing chicken limb buds. Proc. Natl. Acad. Sci. U.S.A. **82**(9), 2804–2808 (1985)
97. Tan, J.L., Tien, J., Pirone, D.M., Gray, D.S., Bhadriraju, K., Chen, C.S.: Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc. Natl. Acad. Sci. U.S.A. **100**(4), 1484–1489 (2003)
98. Tan, J.L., Liu, W., Nelson, C.M., Raghavan, S., Chen, C.S.: Simple approach to micropattern cells on common culture substrates by tuning substrate wettability. Tissue Eng. **10**(5–6), 865–872 (2004)
99. Thery, M., Racine, V., Piel, M., Pepin, A., Dimitrov, A., Chen, Y., Sibarita, J.B., Bornens, M.: Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. Proc. Natl. Acad. Sci. U.S.A. **103**(52), 19771–19776 (2006)
100. Verschueren, H.: Interference reflection microscopy in cell biology: methodology and applications. J. Cell Sci. **75**, 279–301 (1985)
101. Vicente-Manzanares, M., Zareno, J., Whitmore, L., Choi, C.K., Horwitz, A.F.: Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. J. Cell Biol. **176**(5), 573–580 (2007)
102. Vicente-Manzanares, M., Ma, X., Adelstein, R.S., Horwitz, A.R.: Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat. Rev. Mol. Cell Biol. **10**(11), 778–790 (2009)
103. Wang, N., Butler, J.P., Ingber, D.E.: Mechanotransduction across the cell surface and through the cytoskeleton. Science **260**(5111), 1124–1127 (1993)
104. Wang, Y.L., Pelham, R.J., Jr.: Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells. Methods Enzymol. **298**, 489–496 (1998)
105. Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X.Y., Ingber, D.E.: Soft lithography in biology and biochemistry. Ann. Rev. Biomed. Eng. **3**, 335–373 (2001)
106. Xia, Y., Whitesides, G.M.: Soft lithography. Annu. Rev. Mater. Sci. **28**, 153–184 (1998)
107. Xiong, J.P., Stehle, T., Diefenbach, B., Zhang, R.G., Dunker, R., Scott, D.L., Joachimiak, A., Goodman, S.L., Arnaout, M.A.: Crystal structure of the extracellular segment of integrin alpha V beta 3. Science **294**(5541), 339–345 (2001)
108. Yang, M.T., Sniadecki, N.J., Chen, C.S.: Geometric considerations of micro- to nanoscale elastomeric post arrays to study cellular traction forces. Adv. Mater. **19**(20), 3119–3123 (2007)
109. Yousaf, M.N., Houseman, B.T., Mrksich, M.: Using electroactive substrates to pattern the attachment of two different cell populations. Proc. Natl. Acad. Sci. U.S.A. **98**(11), 5992–5996 (2001)

110. Zaman, M.H., Trapani, L.M., Sieminski, A.L., Mackellar, D., Gong, H., Kamm, R.D., Wells, A., Lauffenburger, D.A., Matsudaira, P.: Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell–matrix adhesion and proteolysis. Proc. Natl. Acad. Sci. U.S.A. **103**(29), 10889–10894 (2006)
111. Zamir, E., Geiger, B.: Molecular complexity and dynamics of cell-matrix adhesions. J. Cell Sci. **114**(Pt 20), 3583–3590 (2001)