Annals of Biomedical Engineering (© 2005)
DOI: 10.1007/s10439-005-9006-3

Nanotechnology for Cell–Substrate Interactions

NATHAN J. SNIADECKI,[1] RAVI A. DESAI,[1] SAMI ALOM RUIZ,[1,2] and CHRISTOPHER S. CHEN[1]

[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and [2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland 21205.

(Received 13 July 2005; accepted 12 August 2005)

Abstract—In the pursuit to understand the interaction between cells and their underlying substrates, the life sciences are beginning to incorporate micro- and nanotechnology-based tools to probe and measure cells. The development of these tools portends endless possibilities for new insights into the fundamental relationships between cells and their surrounding microenvironment that underlie the physiology of human tissue. Here, we review techniques and tools that have been used to study how a cell responds to the physical factors in its environment. We also discuss unanswered questions that could be addressed by these approaches to better elucidate the molecular processes and mechanical forces that dominate the interactions between cells and their physical scaffolds.

Keywords—Cell mechanics, Cell shape, Extracellular Matrix, Focal Adhesions, Integrins, Mechanotransduction, Micropatterning, Nanotopology, Self-Assembled Monolayers (SAMs), Traction Forces.

INTRODUCTION

We are on the verge of a new revolution in life sciences. Discoveries arising from biology and the physical sciences are rapidly converging on the study of nanoscale phenomena. Already a collaboration between biologists, chemists, physicists, and engineers has resulted in the mapping of the human genome. While this blueprint provides the template for all life processes, it is the unique experiential history of interactions between each cell and its environment that dictates which genes are expressed, and hence, determines cell behavior. Importantly, it is not only the milieu of soluble, diffusible factors, but also the adhesive, mechanical interactions with physical scaffolds that drive the different states and functions of a cell, including gene expression, adhesion, migration, proliferation, and differentiation.[26] For the binding interactions between cells and surfaces, it has become increasingly evident that cells are influenced by spatial domains, structural compositions, and mechanical forces at the microscale and nanoscale. To begin to understand the sensory and regulatory mechanisms that are involved, micro- and nano-technologies are providing key advances.

Although nanobiotechnology is a recent partnership, the micro- and nano-technology fields have been fast-maturing areas, benefiting from advances made in the semiconductor industry and material sciences in the last century for the production of microprocessors and other devices. In placing and patterning materials at the micro- and nanoscale, the industry has relied upon chemical vapor deposition, physical vapor deposition, electrochemical deposition, photolithography, electron-beam lithography, ion milling, and reactive ion etching—tools that Richard Feynman would love to have had in his 'machine' shop.[47] These tools have enabled a menagerie of innovative nanostructures such as nanofilms, self-assembled monolayers, nanomechanical resonators, and lots of nanoconfetti–nanoparticles, nanoshells, quantum dots, buckyballs, nanotubes, nano-peapods, and nanowires. In the transition of these tools and structures for the life sciences, new twists have evolved including biocompatible and protein-coated materials, microfabricated soft polymers, and biological microelectromechanical systems (bioMEMS). Together, these tools have been adopted for the study of the micro- and nanoscale interactions between a cell and its surrounding microenvironment.

In vivo, cells are immobilized within tissue, bound to a diverse array of scaffoldings known as the extracellular matrix (ECM). The individual components of the ECM exist in the nanometer length scale and thus many tools from nanotechnology are appropriate to mimic their features. The ECM consists predominantly of interwoven protein fibers such as collagen or elastin that have 10–300 nm diameters.[4] These meshed fibers provide tensile strength to the ECM, while other proteins and proteoglycans form hydrated gels which function to resist compressive forces. Extracted basement membranes imaged with electron microscopy show that its three-dimensional architecture consists of nanopores, roughly 70 nm in diameter, and intertwined fibrils that form a felt-like landscape with peaks and valleys that are approximately 100 nm in height and depth.[2,3] The meshwork of ECM can be organized randomly or with semi-alignment, and the size of fibrils and

Address correspondence to Christopher S. Chen, Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104. Electronic mail: cschen2@seas.upenn.edu

pores differ, depending on the source tissue. Fibronectin, vitronectin, tenascin, thrombospondins, entactin, nidogen, and/or laminin are present to a lesser degree than collagen or elastin, but nevertheless play a substantial role in defining ECM function by acting as adhesive ligands. It is now clear that cells detect and respond to numerous features of the ECM, including the composition and availability of adhesive ligands, mechanical stiffness, and spatial and topological organization of these scaffolds, through surface receptors known as integrins.

Integrin receptors span the cell membrane and link the ECM to the cytoskeleton. They are approximately 10 nm wide and are 10–100 times more prevalent on the cell's surface than other receptors types.[4] These transmembrane receptors have extracellular domains that bind with low affinity to the ECM and intracellular domains that link to the cytoskeleton. In their inactive state, integrins are freely diffusive within the cell membrane until they encounter an available binding domain in the ECM. Upon ligand binding, integrins undergo a conformational change that leads to the recruitment of two groups of cytoplasmic proteins—those that biomechanically connect the integrins to the cytoskeleton and those that biochemically initiate or regulate intracellular signaling pathways. Through physical clustering of multiple integrins, more cytoplasmic proteins are recruited to the adhesion site to increase its size, adhesion strength, and biochemical signaling activity.[18,57] These larger, clustered structures of integrins and cytoplasmic proteins are commonly called focal adhesions. The dual nature of adhesions, i.e. their mechanical and signaling activity, has led us to believe that they act as sensors of the ECM environment (sensing both mechanical and biochemical changes in the ECM), regulators of the cytoskeleton, and centers of signal transduction.[18,57] These focal adhesions are scattered across the cell surface and are typically 0.25–0.50 μm wide and 2–10 μm long, though they arise from much smaller clusters. Their formation, development, and disassembly are not only key activities in cell spreading and migration (because they are the points of contact with the ECM), but also appear to be central modulators of many cellular functions such as cell proliferation and differentiation (owing to their ability to modulate intracellular signaling). There is strong evidence that the mechanism for focal adhesion assembly and disassembly is force-mediated due to the coupling between focal adhesions and actomyosin contractility machinery of the cytoskeleton.[53,113] This interaction between focal adhesions and contractile actomyosin microfilaments allows a cell to grip the ECM to contract or propel itself. However, the mechanism by which mechanical forces and focal adhesions are coupled remains poorly understood.

All living cells exert forces on their ECM through the actomyosin microfilament sliding mechanism similar to that used in muscle. In non-muscle cells, microfilaments are organized within a loose, mesh-like network rather than in the highly parallel alignment found in muscle and appropriate for contraction along one direction. The microfilaments terminating at focal adhesion, also known as stress fibers, are used to generate centripetal forces during normal cell spreading and traction forces during cell migration. Through actomyosin forces, cells are active participants in mechanically restructuring the ECM through tugging and stretching nearby ECM fibrils.[78,91,118] Such physical remodeling is essential to organize newly synthesized ECM proteins for the mechanical structure of new tissue or for repair of wounds.[4] The organization of the cytoskeleton and focal adhesions are different on soft or hard substrates, which suggests that a cell probes the stiffness of the ECM and regulates itself accordingly.[101] Cells not only apply forces to the ECM, but also sense and respond to them. Through linkages between cells and the ECM in tissue, cells are subjected to mechanical forces that are transmitted through the tissue during physiological activity. For example, hemodynamic forces act on the endothelium in the vascular system, compressive and tensile forces distort fibroblasts in the connective tissue of the musculoskeletal system, and transpulmonary pressure acts on the epithelium of the respiratory system. These mechanical stresses play a key role in the determination of the tissue's growth and form.[6,68] Unfortunately, we do not have the tools necessary to fully understand how individual cells perceive physical forces or how they regulate their intracellular signaling pathways in response.

Micro- and nano-engineered tools and techniques have played a critical role in discovering that binding interactions between cells and their supporting environment are important to many basic life processes—a remarkable paradigm shift from the focus on extracellular signaling molecules as the sole source of these changes. As a classic example, under internal reflection microscopy (IRM), fibroblasts were observed to have non-uniform adhesion with the glass substrate, which was later confirmed with electron microscopy.[1,32] Non-uniform dark spots underneath the cell indicated areas of close contact (less than 10 nm), while a larger percentage of the cell area had glass-membrane gaps (approximately 30 nm). These areas of close contact are now known to be focal adhesions, but were unidentified at the time because it was assumed that a cell had full intimate contact with its underlying substrate. A second example is micro-patterning of ECM proteins on a surface for tissue culture.[25] When cells adhere to the patterned ECM, they typically spread and flatten against the surface. With spatial control over where a cell can adhere and where it cannot, the investigators were able to separate changes in a cell's fate due to these global changes in cell shape from those of local integrin-ligand binding. They discovered that whether a cell proliferates or dies is determined not by the amount integrin-ligand interactions, but by the degree to which a cell physically extends. Lastly, optical tweezers, which can trap nanoscale objects with focused light, have been used to physically restrain ECM-coated microbeads on the surface

of cells.[28] The cell–bead adhesion strength was found to grow stronger as the external force applied by the optical tweezers was raised. Such active reinforcement of the adhesion site suggests that a cell can sense forces in the ECM and recruit adhesion-related proteins to strengthen its grip. These examples highlight that even basic function of cells are often elucidated only with the advent of new technology. For this reason, nanoscale techniques or nanotechnology-based tools are well poised to provide further insights into uncovering the inner workings of cells.

In incorporating nanotechnology with the biological sciences, there is one cautionary warning: cells are shrouded, complex systems. Although there have been significant advances in visualizing the structure and interactions inside of a cell using tools based on electron microscopy and immunofluorescence microscopy, the insulating cell membrane blocks many other attempts to monitor the function of most proteins and track the plethora of protein–protein interactions. That is, a cell is still a black box, wherein multi-physics activity such as protein conformation changes, membrane-bound proteins signaling, ion gradients, and active transport along the cytoskeleton form a network of interactions that define a cell's activity. Moreover, this black box has non-deterministic elements, so non-repeatable responses are often encountered. Specific to cell–microenvironment interactions, an additional hindrance is the reciprocal relationship between the cell and the ECM. The structure of the ECM can define a cell's adhesion, morphology, motility, and function. On the other hand, these same responses from the cell often feedback to cause structural remodeling of the ECM: new ECM protein synthesis, protease disassembly, and physical realignment of ECM fibrils through cellular forces. Therefore, these considerations should act as a simple warning that one must treat each new technique, device, and resulting data on a cell's inner workings with a healthy degree of guarded skepticism.

Nevertheless, it is precisely *because* of these active and highly complex responses that cells are attractive systems to investigate. A cell is a unique, self-regulating, and self-replicating micro-factory, wherein proteins are synthesized and then spontaneously or actively assembled to form complex multifunctional aggregates that compose the cell's structure and functionality; an intricate system that, once understood, offers the potential to mimic or reverse engineer these activities for bioinspired new technologies, medical implants, and therapies. Throughout this review, we will highlight how nanotechnology has enabled new discovery in the area of cell–ECM interactions, as well as how understanding these interactions has created a need for engineering new tools for better study. Specifically, we will examine accomplishments achieved by (1) tailoring the surface chemistry of the ECM with well-defined spatial and molecular composition, (2) fabricating synthetic ECM substrates with nanotopologies, (3) building microscale and nanoscale tools to measure cellular forces, and (4) applying forces to cells that mimic those that arise in cell's surroundings.

SURFACE CONTROL

Since the discovery of focal adhesions with IRM, biologists have accrued extensive knowledge regarding the biochemistry behind cell–substrate interactions.[134] Nonetheless, understanding the adhesion-mediated response of cells is limited to the extent that investigators are able to characterize and define the composition of the underlying substrate, often a polystyrene tissue-culture dish. It is known that cells bind to surfaces via ECM proteins that are adsorbed as a layer onto surfaces; to manipulate cell adhesion one can either alter physical surface properties to modulate the ability of the surface to capture proteins from solution, or alternatively, directly deposit proteins onto a surface.

Early work demonstrated the importance of surface wettability on cell adhesiveness, and the role of adhesiveness on cell function. By culturing cells on differentially wettable (and hence adhesive) surfaces, Carter[21] and Harris[60] each observed that spatial adhesive cues on the surface affected cell spreading and motility. They reported increased area of cell spreading on more adhesive surfaces and migration towards regions of higher adhesiveness, which Carter coined "haptotaxis." Additionally, Folkman and Moscona[51] found that both cell area and proliferation increased with the degree of adhesivity of the surface in the absence of influences from other cells. From such observations, these authors postulated that the environment's adhesiveness governs cell shape and movement in tissues, which in turn regulates tissue development and tumor vascularization at the macroscale. Importantly, the surface modifications in early work by Carter and Harris (metal deposition and oxidation), and Folkman and Moscona (polymer deposition) altered the ability of the surface to capture ECM proteins from solution. Through nonpolar interactions, proteins rapidly adsorb from solution to hydrophobic surfaces.[106] In the presence of cell–culture serum, such hydrophobic surfaces capture albumin most readily (perhaps because it is the most abundant protein in serum), effectively preventing adsorption of the adhesive ECM proteins. On the other hand, hydrophilic surfaces nonspecifically adsorb less protein in general. These findings provided an early framework to engineer model surfaces capable of controlling protein adsorption from solution and therefore cell adhesion, simply by altering surface wettability such as with plasma treatment of plastics.

Unfortunately, the ability of proteins to adsorb to surfaces is a complex multi-parametric problem not entirely explained by wettability, and has required the advent of approaches to provide more chemically-defined model surfaces. A key advance involved use of self-assembled monolayers (SAMs) of alkanethiols on gold to serve as model surfaces to study protein adsorption and cell adhe-

sion. A SAM surface is created by deposition of nanoscale monolayers of alkanethiols, which have the general structure $SH(CH_2)_nX$. The sulfur atom coordinates with the underlying metal, and X is the terminal group that is presented at the surface. On metallic surfaces, alkanethiols self-assemble into highly ordered, high density, stable, semi-crystalline monolayers approximately 2–3 nm thick, depending on the number of methylene groups that form the long chain of the molecule. The terminal X group can be tailored along with the length of the alkyl chain to achieve desired surface properties.[8–10] Hydrophobic terminal groups, such as methyl, readily adsorb many ECM proteins (and therefore support cell adhesion) from solution, whereas terminal groups such as poly(ethylene glycol) resist ECM protein adsorption[105,106] (and therefore block cell adhesion). Furthermore, the ability of a SAM surface to adsorb protein can be tuned by adjusting the hydrophilicity of the terminal group and by altering the ratio of alkanethiols on the surface that support or prevent protein adsorption. These model surfaces, while unlikely to be used in clinical settings owing to their long-term instability, are likely to provide the conduit for identifying novel chemistries that alter the ability of cells and proteins to interact with surfaces.

One very important feature about cellular interactions *in vivo* is that they are hardly ever homogeneous or isotropic. That is, cells are often exposed to gradients or patterns of adhesiveness, and it has long been postulated that such spatial cues are detectable by cells. That is, cells do not merely integrate or average the adhesiveness of the surface, but respond to subcellular domains of ECM. To study such questions, scientists have developed several approaches to generate surfaces engineered with gradients or patterns of adhesiveness. A gradient of alkanethiols, and therefore surface adhesiveness, can be achieved using a variety of methods.[12,84,95,104] As one example, cross-diffusion of different alkanethiols was used to generate a one-dimensional gradient of surface-immobilized fibronectin on a SAM surface.[117] Endothelial cells seeded on the gradient migrated towards regions of higher ECM density, and displayed an increase in directed, but not random, migration speed. Thus, S.B. Carter's initial demonstrations of haptotaxis on differential surfaces were replicated with further characterization and higher precision on a model SAM surface. Predictable, well-defined gradients are possible using microfluidic approaches to control the degree of mixing in laminar flow.[40,72,85] The small volumes flowed through these microchannels have produced gradients of ECM proteins to control cellular morphology.[41] These results raise intriguing yet unanswered questions on how cells respond to the ECM gradient. It is unclear if ECM gradients lead to differential integrin clustering and thus different traction forces for propulsion. Haptotaxis may vary on gradients of ECM proteins other than fibronectin or it may rely on ECM deposited by the cell or from the culture media. The signaling pathways that underlie haptotactic movement and how they compare with chemotaxis (movement up a gradient of soluble factors) remain unknown. Such questions could be addressed with finer control over ECM gradients on alkanethiols in order to limit the clustering of integrins, co-depositing two or more ECM protein gradients simultaneously to monitor different ligand-integrin signaling pathways, or combining this technology with various biochemical assays to identify downstream signaling pathways that may be affected.

In addition to gradients, it is also interesting to generate 'digital' patterns of adhesiveness, where defined regions on a surface contain one kind of chemistry and the remaining regions contain another. One such strategy uses SAMs to generate microscale patches of ECM on a surface that is otherwise non-adhesive; this approach is known as microcontact printing (Fig. 1A) (reviewed in Ref. 97 and 131). In this method, poly(dimethylsiloxane) (PDMS) is cast from a mold to generate a stamp with bas relief features, using conventional photo- or electron-beam lithography (Fig. 1Aa and Fig. 1b). The stamp is then loaded with an alkanethiol solution in ethanol and blown dry (Fig. 1Ac). The resulting 'inked' stamp is briefly placed into physical contact with a gold substrate to transfer alkanethiols to the metallic surface, which generates SAMs of the same pattern (Fig. 1Ad and Fig. 1Ae). Non-printed regions can be filled with a different SAM by immersing the substrate in a different alkanethiol solution (Fig. 1Af). Patterning hydrophobic SAM islands with ethylene-glycol-containing alkanethiol creates regions of defined size for ECM protein adsorption.[96] In contrast to modifying the surface's ability to capture proteins from solution, a technique that allows direct printing of ECM proteins onto standard tissue culture surfaces, such as glass, silicone rubber, or polystyrene also has been developed.[15,71,120] This approach obviates the need for metallic substrates or alkanethiol chemistry and instead uses a PDMS stamp 'inked' with adsorbed protein to directly transfer protein to a surface via physical contact. To render non-printed regions resistant to protein adsorption and cell adhesion, the substrate is then immersed in a commercially available surfactant.[120] These two techniques have afforded investigators a high degree of control over surface immobilized ECM localization and have resolutions that could potentially approach the single nanometer range.[66]

Seeding cells onto "island" patterns of different sizes, such that one cell attaches to each island and spreads to the islands' size and shape (Fig. 1B), has allowed carefully defined studies that collectively support and make precise Folkman and Moscona's observation that cell shape is a potent regulator of cell function.[25,51,67,90,116] For example, constraining liver cells to relatively small (1600 μm^2) islands promoted liver-specific function, as measured by albumin secretion rate, whereas allowing liver cells to spread freely abrogated albumin secretion and instead enhanced proliferation.[116] How cells respond to different degrees

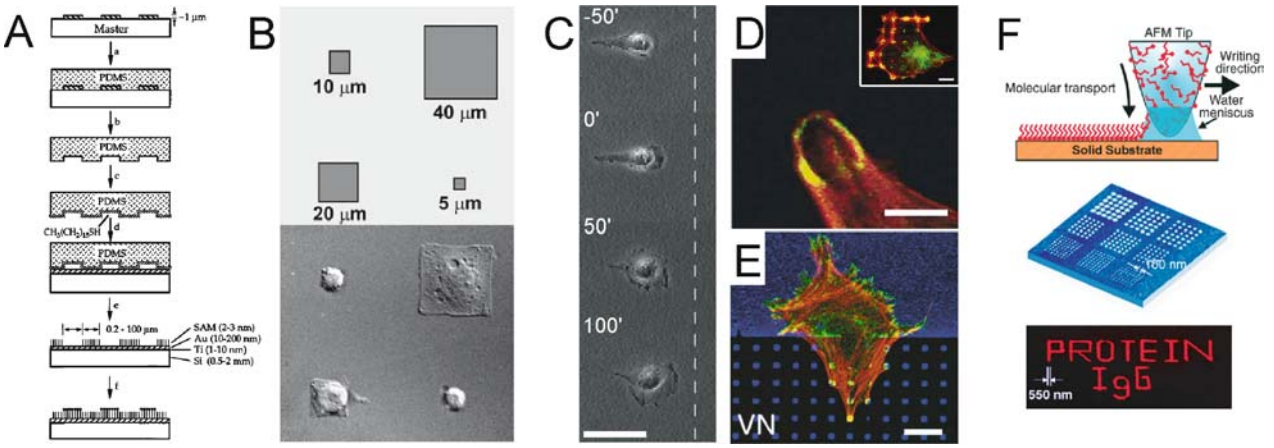

FIGURE 1. Surface control for the study of cell biology. (A) Schematic of microcontact printing to pattern two different alkanethiols. Details provided in text. Reproduced from Ref. 97. (B) Microcontact printing of SAMs controls cell spreading on ECM squares with different area (*top*). DIC image of cells is also shown (*bottom*). Reproduced from Ref. 23. (C) Fibroblast begins directed migration upon voltage application to release it from it micropatterned constraints. Time in each frame is relative to onset of voltage application. Dotted line given as reference point for clarity of migration. Bar: 10 μm. Reproduced from Ref. 74. (D) Immunofluorescent micrograph revealing asymmetric focal adhesion distribution on ECM microdots. *Inset*: Full cell body shown on an array of ECM microdots. *Red*: F-actin; *Green*: focal adhesion component vinculin. Bar: 3 μm. Inset bar: 10 μm. Reproduced from Ref. 23. (E) Melanoma cell expressing fluorescent β_3 integrin (*green*), labeled for actin (*red*) growing on vitronectin (*blue*) shows dramatic morphological differences at the border between uniform and micropatterned surfaces. Bar: 10 μm. Reproduced from Ref. 81. (F) Dip-Pen Nanolithography (DPN) uses a wetted AFM tip to deposit material onto a surface (*top*). Nanoarray of alkanethiols directly written on a gold surface using DPN (*middle*). Fluorescent immunoglobulin G protein directly "written" on a surface using DPN (*bottom*). Reproduced from Ref. 59.

of spreading depends on the cell type, as further experiments using capillary cells revealed.[25] These cells proliferated when allowed to spread on large islands (3000 μm^2) but underwent apoptosis when unspread on small islands (75 μm^2). Patterning cells on an array of subcellular adhesive islands allows independent control of cell–ECM contact and projected cell area. Applying this strategy allowed these investigators to find that degree of cell spreading rather than ECM contact regulated the switch between growth and apoptosis.[25] This finding could not have been demonstrated in the absence of these micropatterning tools, but also underscored a novel control pathway by which micropatterned surfaces can be used to control cell behavior.

Extending this initial work, investigators have since found that cell shape appears to regulate many behaviors, including cell differentiation,[42] stem cell differentiation,[90] and even organization of cell migration machinery.[16,100] Several indicators of cell migration, including lamellipodial ruffling, ECM deposition, and traction stresses occurred most often at the corners of cells cultured on squares and triangles.[100] However, because these micropatterns are static in nature, cells could not migrate from their spatial constraints, and so one could not prove that cell shape actually altered the direction of cell migration. In response, a new method was developed to apply a voltage pulse to the substrate to permanently switch non-adhesive SAMs to adhesive, allowing previously constrained cells to migrate.[74,133] Using asymmetrically patterned initial islands with this technique, the geometric constraints induced cell polarity and (following the electrical pulse) directed migration as a function of initial cell shape.[73] Cells did not migrate in the direction that earlier studies suggested (i.e., at pattern corners), but instead migrated toward the blunt end of the asymmetric initial pattern (Fig. 1C). The direction of migration after the adhesive switch suggests that cells can integrate global geometric polarity. How this is done mechanistically is far from clear, and so too is how long and to what extent the cell retains a memory of previous geometric polarity. Reversibly switchable substrates, where migration could be started and then stopped, would help answer these questions and clarify the role of cell shape in governing polarity and directed movement.

Patterning ECM islands at progressively smaller length scales provides numerous opportunities for future advancement in engineering, and hence, understanding cell–substrate interactions. Using conventional photolithography to fabricate stamps, ECM islands that were 3 μm in diameter (microdots) have been used to study the effect of isolated adhesion structures. Focal adhesions conformed asymmetrically on these ECM microdot in a tension-dependent manner as indicated by stress fibers and drug-induced inhibition of actomyosin contraction[23] (Fig. 1D). By using electron-beam lithography to generate stamp features with sub-micrometer resolution, Bastmeyer *et al.*[81] systematically reduced ECM island size to pursue the lower limits of cell–ECM contact necessary for cell spreading. Cell recognition of ECM nanodots (100 nm^2) was observed, as evidenced by enhanced intracellular signaling

and accumulation of focal adhesion components, but often times these small ECM dots were internalized by the cell. A possible explanation is that the ECM nanodots were not tightly bound to the substrate, and could be ripped from the surface by traction forces exerted by the cell. If true, this phenomenon may represent a limitation of the microcontact printing technique employed. Despite this, the study documented that cell morphology varies dramatically on different ECM island geometries (Fig. 1E). At these 100 nm length scales and smaller, we know little about how cells (and their receptors) probe their surroundings. Herein lies an opportunity for new nanotechnologies to deposit nanoscale ECM islands and detect adhesion dynamics such as integrin clustering and adhesive signaling to elucidate exactly how and to what degree cell spreading conforms to the ECM. Nanotechnologies to deposit ECM islands may include dip-pen nanotechnology[59,80,103] (DPN; Fig. 1F), which has the ability to directly write ECM molecules on a surface with sub-micron precision. Immensely promising, DPN could be used to shed light on cell adhesion.[80] Furthermore, new tools should be developed to study areas such as the size scale of ECM islands needed to elicit focal adhesion formation, intracellular signaling, and propagation of cell spreading; integrin clustering around an ECM molecule; and cell behavior in response to defined integrin clustering.

Defining surface chemistry has always led to new biological discoveries because it provides a means to control what cells sense in their local microenvironment. It is clear that microenvironmental constraints offered by such surface engineering will influence cells' ability to grow, survive, differentiate, migrate, and adhere. For example, certain studies suggest that when cells are tightly confined, they perform tissue-specific functions, but when given room to spread they will instead minimize function and proliferate.[25,116] On a two-dimensional surface, the limit of cells' sensing ability is unknown, and is likely far from achievable with any existing technologies. New technology that facilitates finer resolution will thus enable novel biological discoveries. *In vivo*, the cells' microenvironment is not planar, but consists of nanotextures and micro-features that influence cell shape. Concomitant with the drive toward finer two-dimensional studies, then, is the development of technologies to define topography of the *in vitro* cellular microenvironment.

NANOTOPOLOGY

While experiments done in typical culture dishes and other flat substrates are useful in understanding the cell–substrate interactions, they do not mimic the physical three-dimensional environment of ECM fibril ropes and meshes that support cells. It is now clear that the spatial presentation of ECM, exemplified by 2D micropatterning studies, has profound effects on cell adhesion and function. The structure and topology of that matrix also appears to encode important regulatory information for cells. The effects of topology on cell function have not been investigated as extensively as those of surface chemistry or cell shape, largely due to limitations and compatibility in fabrication techniques. However, such limitations are fast disappearing, and we are likely to see major advances in understanding how cells sense the micro- and nanotexture of their surroundings.

In the early 1960s, Curtis and Varde[31] investigated the effect that the topology of a surface has on cell behavior. They used silica fibers with diameters between 8 and 40 μm in between two chicken embryo heart explants. Fibroblasts migrated out of the explants onto the fibers to form sheets of cells between them (Fig. 2A). The explants further exhibited a topology preference by predominantly forming sheets at the acute angles of two intersecting fibers and in the concave bends of curved fibers. They also examined cells migrating onto substrates with grooves and ridges of microscale dimensions and observed that the migration was more extensive on the ridges than in the grooves. These early experiments indicated that topography of the substrate was relevant to the cell–substrate interactions. Improved fabrication methods have since made it possible to produce nanoscale features on substrates, which resemble the fibers, pores, peaks and depressions found in the ECM. In this pursuit, some approaches use random nanotopologies that closely match their *in vivo* counterparts in both texture and cellular response, while others employ nanofabrication techniques to understand how cells respond to uniform topographical presentations.

In mimicking the random structure of the extracellular matrix, multiwalled carbon nanofibers have been made by chemical vapor deposition to identify the range of cell functions that nanotopology can affect. Scanning electron microscope (SEM) images of these fibers show that their fibrillar nature and random orientation closely resemble the topology of matrices such as the corneal epithelial basement membrane[45] (Figs. 2B and C). On nanofibers (100 nm diameter), osteoblasts exhibited increased proliferation compared to flat glass surfaces. Alkaline phosphatase activity, an indicator of osteoblastic bone-formation, was also increased on these substrates indicating that specialized cellular function can be enhanced by closely matching the physical topology of the ECM. Nanofibers have also been made by electrospinning a polymer solution of polyamide onto a coverslip.[114] Breast epithelial cells seeded on these substrates formed multicellular spheroids similar to ones formed on three-dimensional collagen or matrigel substrates. Since these epithelial cells typically form a monolayer on glass (unlike what happens *in vivo*), nanofibers may provide advantageous substrate for investigating cell function and morphogenesis. Additionally, fibroblasts and kidney epithelial cells seeded onto the nanofibers had fewer stress fibers and smaller focal adhesions than on glass coverslips. Since neither cell type adhered to flat substrates

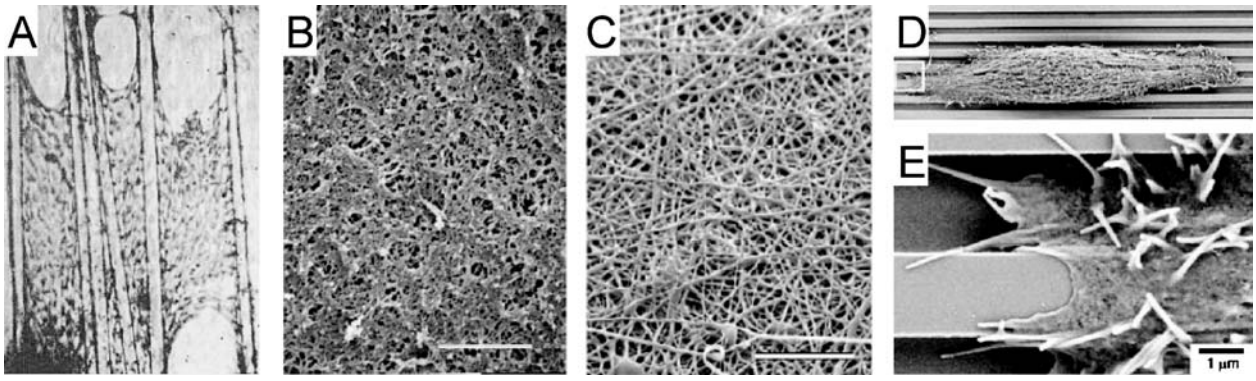

FIGURE 2. Nanofabricated substrates for mimicking the nanotoplogy of the ECM. (A) Sheets of fibroblasts suspended between silica fibers of approximately 30 μm diameter. Reproduced from Ref. 31. (B) Nanotopology of corneal epithelial basement membrane of a Macaque monkey shown by SEM. Bar: 1 μm. Reproduced from Ref. 49. (C) Nanofiber meshing, produced with chemical vapor deposition and shown by SEM, closely resembles the *in vivo* structure of the ECM. Bar: 5 μm. Reproduced from Ref. 114. (D) SEM micrograph shows microgrooves formed by reactive ion etching that are used for cell alignment and migration guidance. Reproduced from Ref. 122. (E) Lamellipodia and filopodia adhered to the floor of the grooves (image enlarged from D). Reproduced from Ref. 122.

of polyamide, it suggests that topography, not the surface chemistry of the polymer, can drive the improved cell adhesion, morphology, and cytoskeletal organization. Moreover, the predominated mechanosensors of the ECM environment are the cytoskeleton and focal adhesions and are likely to be involved in sensing topology. However, there may be other mechanisms by which the cells can respond to the texture of their surroundings.

Treated polymer membranes are another class of randomly generated substrates that elicit topology-dependent changes in cell behavior. Treating poly(lactide-co-glycolic acid) (PLGA) with NaOH, which is a biodegradable polymer used for biomedical implants, created surface roughness at the nanometer scale.[93,94] The etching of PLGA created pits of different height, width, and spacing, but also altered the surface chemistry of the polymer. To reproduce the topology without changing the surface chemistry, a negative cast was made in PDMS. This PDMS negative cast served as a mold for another PLGA structure displaying identical nanofeatures as the etched original but with native surface chemistry. Smooth muscle cells seeded onto the etched original and the molded PLGA substrates achieved greater adhesion than on the flat PLGA substrates, demonstrating that the effect of topology can be independent of surface chemistry.

To investigate what range in size of nanofeatures affect cell behavior, topographical islands, with random and controlled dimensions, have been used. Through varying the polymer blend and allowing spontaneous demixing, substrates with nanoscale islands with controllable heights of ten to hundreds of nanometers, but with large variation in diameter, were used for cell culture.[35] Tubulin and F-actin staining of fibroblasts on the substrates showed more defined cytoskeleton networks on both the flat surfaces and 13 nm islands as compared to taller islands, which suggests a lower threshold in topological sensitivity of the cytoskeleton. But, 13 nm high islands, when compared to flat surfaces, induced significantly larger cell spreading and proliferation rates, which showed that other mechanosensors may be involved. Moreover, when the island height was increased to 160 nm, with a different fabrication method, the fibroblast spread area was lower than that on flat surfaces. To understand the topology sensitivity, changes in gene expression of cells can be monitored using microarray analysis.[34,35] Combining this technique with nanotopology surfaces will help to determine which gene targets may be involved in the topographical-related responses.

Nanoscale grooves have been created in substrates as a means of studying the effects of spatial guidance on cellular shape and function. Nanogrooves present surfaces that resemble commonly encountered ECM structures such as topographical length of collagen-fiber bundles. Cells seeded onto nanogrooves aligned their shape and elongated in the direction of the nanogrooves, though the degree of morphology changes are cell-type dependent (Figs. 2D and E).[122,123,132] The topology-related alignment and guidance has proven to be useful *in vivo*, for implantable scaffolds have been molded with nanogrooves for improved tendon repair.[33] Additionally, adrenal gland cells were grown on nanogrooves,[50] which are commonly used cell models to study the repair of the nervous system because they exhibit neuronal characteristics in the presence of nerve growth factor (NGF). To investigate whether physical topology also affects neurite outgrowth, cells exposed to suboptimal concentrations of NGF extended more neurites on nanogrooves than on flat substrates. This study highlights that nanofeatures may also cooperate with existing signaling pathways initiated by soluble factors in order to guide cellular function.

While significant advances have been made since Curtis and Varde[31] hypothesized that topology was important in determining cell behavior, we are only beginning to understand their effects on cell function. Until recently, research in this area had been limited mainly to quantifying morphological parameters such as alignment, elongation, and area, perhaps because of the engineering background of the investigators. A major limitation has been a lack of control of the surfaces with defined chemistries, difficulties in characterization of the protein adsorption process, and understanding how cells bind to such nanotopographic surfaces. With the barrier between biologist and engineer disappearing however, these limitations will quickly be addressed, and future investigation will shift towards understanding changes in signaling pathways and gene expression. A key question is whether cells respond to topographic features using the same sensory apparatus that cells use to sense changes in surface adhesiveness, cell shape, and adhesion geometry. Understanding this effect will be critical as we begin to seek methods to engineer cellular environments with *in vivo*-like properties.

MEASURING CELLULAR FORCES ON THE ECM

It has become increasingly evident that a key component to understanding how cells sense and respond to the ECM involves their adhesion, but these structures are also involved in the generation of forces on the ECM. For this reason, cellular forces appear to be integral to how cells alter their adhesion, shape, and function in response to their surrounding. During spreading and migration, cell pull themselves through the ECM and tug at nearby ECM fibers. These forces are generated in cells by myosin moving in a step-wise linear ratchet mechanism along actin microfilaments. Each cycle advances the myosin head approximately 5 nm along the actin filament and produces an average force of 3–4 pN.[48] To understand how cells regulate and coordinate mechanical forces that they generate requires the engineering of micro- and nano-scale sensors that greatly differ from their macroscale load-cell counterparts. These tools culture cells on flexible substrates that can mechanically deform and thereby report the magnitude and direction of the force.

In the early 1980s, Albert Harris[62] pioneered the method of measuring cellular forces by using thin films of silicone that wrinkled upon force exertion of adherent cells. This technique has since evolved into devices that use microfabrication techniques and nanomaterials to obtain improved precision of force sensing. To form the wrinkling membranes, liquid silicone rubber was poured onto a glass coverslip and briefly exposed to an open flame to cross-link a thin skin of rubber (1 μm thickness) on top of the lubricating liquid silicone layer underneath.[19,20,61,62] Cells could be cultured on the silicone rubber and traction forces that they applied to the skin were strong enough to produce wrinkles and folds (Fig. 3A). Surprisingly, there was no observation of the cell pushing against the silicone membrane. This technique was a breakthrough in that cellular forces had not been experimentally observed before and that qualitative measurement of the different regions of compression and tension could be observed simultaneously. Wrinkling substrates still provide only a qualitative measure of force and do not have the resolution to measure the traction forces at individual focal adhesions. Despite its simplicity, wrinkling membranes were adopted as an assay to confirm that traction forces, through the small GTPase RhoA or Ca^{2+}/calmodulin pathways, are necessary for stress fiber formation and focal adhesion assembly, which has helped identify the molecular partners in force generation.[29,63]

To provide more quantitative analysis, traction force microscopy was developed, which is a technique employing

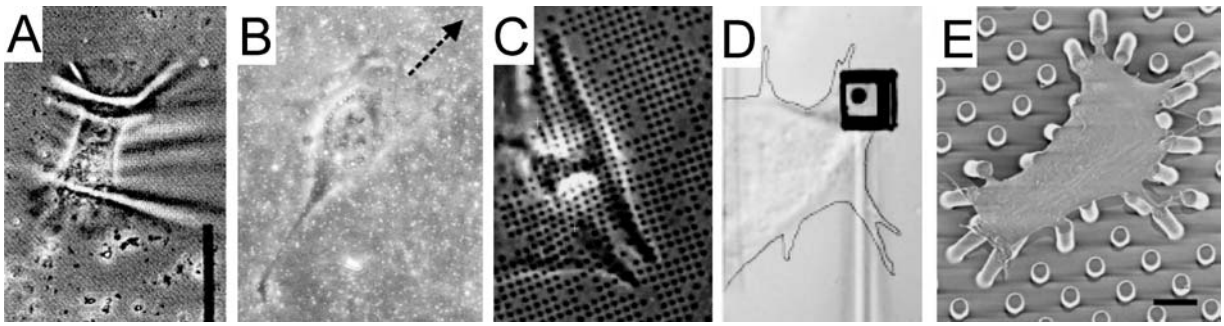

FIGURE 3. Micro- and nanoscale tools for the measurement of cellular forces. (A) Silicon rubber membrane wrinkles due to the traction forces from a fibroblast. Bar: 50 μm. Reproduced from Ref. 62. (B) Traction force microscopy reports the traction forces from a migrating fibroblast (*arrow* indicates direction) by measuring the displacement of fluorescent microbeads (0.2 μm) embedded in a gel substrate. Reproduced from Ref. 98. (C) Regular array of micropatterned fluorescent dots distorts underneath a contracting fibroblast. Reproduced from Ref. 11. (D) Bending of horizontal microcantilever locally reports the traction force during fibroblast migration. Reproduced from Ref. 52. (E) Array of vertical elastomeric microcantilevers bend and report the localized contractile forces of smooth muscle cell. Bar: 10 μm. Reproduced from Ref. 120.

elastic, but non-wrinkling, gels or membranes.[39,79,98] For force measurement, fluorescent nanobeads were embedded into the material during the fabrication to act as fiduciary markers which report the cellular traction forces or stresses (Fig. 3B). As the cell contracts, it deforms the membrane and displaces the beads from their original positions. The traction stress field can be found by inverting the strain field tensor in accordance with elasticity theory.[38] This inverse operation does not provide a unique solution and so assumptions about where and how forces are imposed in the solution are required. To improve upon the non-uniform, random seeding of markers, regular arrays of fluorescent beads, fabricated by electron-beam lithography, have been imprinted onto the elastomeric substrate for improved force tracking.[11] The deformation of the substrate is clearly observed in the deviation of the rows and columns of markers (Fig. 3C). The calculation for the force mapping in this case is similar to the random seeding method but with significant reduction in the number of possible solutions to the traction stress field due to the regular array of displacement markers.

Despite the uncertainty in measuring traction stress fields or total cell force using this approach, traction force microscopy has provided insights into the role of cellular forces, ECM adhesivity, and substrate stiffness in defining cell spreading or migration. Traction forces have been observed to change in magnitude depending on the adhesivity of the substrate.[56,109–111] Specifically, the degree of ligand density adsorbed onto the gels proportionally induced higher traction forces. Adjusting cross-linking in the gel to change its stiffness also elicited changes in the traction forces exerted by cells.[86,129] In these studies it was observed that cells exert higher traction forces on stiffer gels and migrated towards stiffer regions when available.

Measuring cell forces has also revealed that there is a positive correlation between local force and focal adhesion size, at least in stationary fibroblasts.[11] Since focal adhesion size varies on different ECM stiffnesses[101] or ECM ligand densities,[111] this study implies that the degree of ECM stiffness or amount of ECM ligands can lead to increased focal adhesion size through increasing cell contraction, and not solely by bringing integrins into close juxtaposition. Interestingly, the relationship between focal adhesions and traction forces may depend on other factors. Supporting this caveat, migrating cells exhibit an inverse relationship between traction force and nascent focal adhesion size at the lamellipodia.[14] The discrepancy between the studies[11,14] may be that nascent focal adhesions in migrating cells produce initially strong traction forces for propulsion, but diminish with adhesion turnover or increase at a lower rate with adhesion maturation. Lastly, the relationship between substrate adhesivity, stiffness, cell forces, and focal adhesion formation may involve changes in cell shape. For example, when ECM ligand density is increased, cells exhibit greater spreading across the substrate and so cell area and traction force also appear to be directly related.[56,109–111,125,130] The increase in cell spreading appears to directly alter focal adhesion assembly[23] and may be involved in the increase in traction forces. Together, these studies highlight the complex interplay between substrate adhesiveness, stiffness, cell shape, focal adhesions, and cell forces. It is evident that tools need to be developed that modulate each of these parameters independently in order to delineate the scope of their influence on the force-related cellular responses; a task that remains hardly complete.

The use of a wrinkling membranes or traction force microscopy has an inherent disadvantage that discrete forces applied at the focal adhesions are convoluted with the displacement of the entire membrane or gel. The lack of a direct, linear technique to measure traction forces at individual adhesions necessitated the use of microfabricated microcantilevers as force transducers.[44,52,54,121] The first demonstration of these sensors was a horizontal cantilever fabricated on a silicon wafer, which is bent in the plane of the substrate surface by the cell[52,54] (Fig. 3D). Since the sensor is mechanically decoupled from the substrate, the deflection of the cantilever directly reports only the local force, proportional to the measured spring constant for the cantilever. Interestingly, the traction forces observed were not steady, but oscillated as the cell migrated across the cantilever showing that the cell cycles its force generation mechanism. Even though this technique was simple in practice, the horizontal design of the cantilever restricts measurements along one axis and only at a single location underneath the cell. Modifying the design to a high-density array of vertical cantilevers improved both the spatial resolution of the force sensor and the scope of possible experiments.[44,121] With each cantilever placed perpendicular to the plane of traction forces, the spacing between each sensor is significantly reduced (Fig. 3E). These devices are made from silicone rubber with cylindrical cantilevers formed from a microfabricated mold. The cantilevers are not limited to force measurement along one axis and have high force measurement sensitivity[44] (50 pN). With the close proximity between the cantilevers and measurement independence between them, the device can examine cells at a higher population density than previous methods, such as the individual traction forces within a group of cells.[44] Using vertical cantilevers, several observations made with previous "flat" systems were confirmed; total force depends on the RhoA signaling pathway, magnitude of contractile force scales with adhesion size, and average force per post increased with cell area.[121] Previous force measuring techniques have been limited in patterning cell area[130] and so incorporating the surface chemistry technique of microcontact printing on PDMS cantilevers marks a significant breakthrough in the causative role of cell spreading on contractile forces.

The progress into understanding how contractile forces are regulated has produced unique micro-based tools, show-

ing improvement with each new design, but a greater jump forward with nano-engineered tools can expand upon how cellular forces influence the cell, the ECM, and the cell–ECM reciprocal interactions. The mechanosensitivity to adhesiveness, texture, and/or stiffness of the ECM may be force-related through the contractile probing of cells. For example, connective tissue (kPa) is often softer than the fibers (MPa) or molecular subunits (GPa) that compose it,[119] and so cells may identify the tissues and where they are within them through mechanosensation of local ECM stiffness. To explore this conjecture, we need to better understand on how cellular forces are generated and regulated. A wide gap in our knowledge exists between what we know about individual myosin motors and what we do not know about how power strokes from many myosin proteins on actin microfilaments result in traction forces at focal adhesion. In looking at minimal adhesion structures, the forces generated at individual (or trimeric[30]) integrin receptors remain difficult to measure, as well as the amount of actomyosin contractility machinery connected to them. In scaling up, it is not known what molecular or mechanical mechanisms cluster integrins receptors together. Identifying them may show that clustering is central to the cell–ECM interactions for it requires ligand availability in order to increase the adhesion strength of the cell, initiate adhesion-related signaling, and generate contractile forces. Moreover, the nature of force generation—the dynamics, the integrating signals, the feedback loops, the spatial regulation and localization, etc.—all remain elusive. Improvements in force measurements may come from optical techniques for sensitivity at the molecular level[76] or from construction of three-dimensional devices[13] for *in vivo*-related[127] force measurements. Lastly, it has been shown that mechanical forces can cause rearrangement of ECM proteins and exposure of cryptic sites.[99] In this light, ECM remodeling could also be a partner in behaviors attributed solely to traction forces and so simultaneous measurements of cellular forces and resulting deformations in the ECM molecules would help obviate such questions. Clearly, forces play a vital role in the function of cells and so developing new tools that sense forces and anatomical changes at the nanoscale is essential in explaining the mechanical sensitivity.

APPLYING FORCES AT INTEGRINS

As mentioned before, a cell not only exerts forces, but also actively senses and responds to them, and so, how a cell interprets mechanical forces is pertinent to understanding how a cell interrelates with its surroundings. A growing body of evidence has shown that cellular response to physical factors is important in the regulation of tissue physiology,[36,69,70] but the mechanisms by which cells transduce these mechanical stimuli into biochemical signals, also known as mechanotransduction, remain elusive. There appears to be multiple signaling pathways and interconnected cellular structures that may mediate this mechanotransduction. Despite the difficulties, micro- and nano-technology has provided novel materials and improved techniques with which basic understanding into mechanotransduction is being uncovered.

On the macro-scale, the investigations into the mechanisms through which cells sense and respond to applied mechanical stimuli have relied upon techniques in which the whole cell or a substantial portion of it was subjected to a stress or strain. Monolayers of endothelial cells presented with laminar flow were observed to elongate and reorient themselves in the direction of the shear stress.[37] Under no flow, the focal adhesions underwent normal remodeling with no preferred orientation, but at the onset of shear stress, they realigned in the direction of flow and coalesced into larger, but fewer adhesions. Similarly, when cells were stretched on a deformable membrane, they reoriented perpendicular to the direction of stretching, exhibited increased stress fiber formation, and produced more ECM proteins.[82,115] In both of these examples, the entire cell undergoes distortion and so changes in biochemical activities that could potentially be associated with mechanical stimuli are indistinguishable from changes associated with cell shape or membrane deformation. To reduce membrane distortion, external forces applied to the cell membrane with a micropipette, coated with ECM proteins, caused a local increase in focal adhesion size, which depended on the actin cytoskeleton remaining intact[112] (Fig. 4A). A similar technique revealed that external forces on the cell surface can distort the shape of the nucleus and reorient the nucleoli toward the direction of the applied force.[88] These works demonstrate the interplay between adhesions and the cytoskeleton in mechanotransduction, but other effects could be eliciting the response such as local but large deformations of the cell membrane, force application at a multitude of adhesion sites along the micropipette, or force transmission through the cytoskeleton to the underlying focal adhesions on the culture substrate. Through micro- and nanotechnology-based techniques, accurate control over the magnitude, direction, and position of the applied force can mitigate these concerns.

Magnetic twisting cytometry (MTC) was one of the first microscale tools to be used to study the role of mechanical forces at the cell–ECM adhesion.[128] Magnetic beads (5 μm diameter) were coated with ECM proteins and then randomly seeded onto the surface of the cell (Fig. 4B). A strong magnetic field was quickly pulsed in one direction to magnetize the magnetic moment of the beads in the same orientation. A second field was then applied at a lower strength, but perpendicular to the magnetic moments, to induce the rotation of the beads (Fig. 4C). This rotation creates a twisting stress at the integrin-ligand bond ranging up to hundreds of piconewtons per square micrometer. The investigators found that the strength of the adhesion

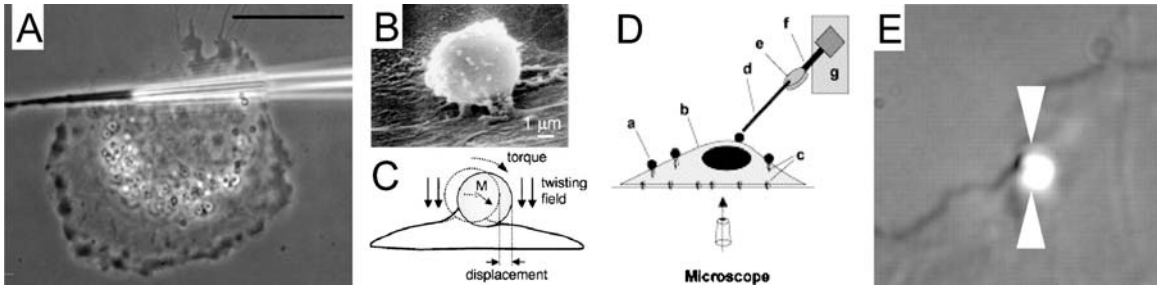

FIGURE 4. Micro- and nanotechnologies for the application of forces to cells. (A) Micropipette tip is dragged across the surface of a cell to apply an external force. Bar: 20 μm. Reproduced from Ref. 112. (B) Ferrimagnetic bead bound to the cell surface shown by SEM. Reproduced from Ref. 46. (C) Magnetic twisting cytometry uses a magnetic field to create a torque that causes the bead to rotate and apply a twisting stress to the cell. *M* indicates the direction of the bead's magnetic moment. Reproduced from Ref. 46. (D) Magnetic tweezers use (a) magnetic beads that are bound to the surface of the (b) cell through (c) integrin receptors. When the (d) magnetic tip is brought into close proximity to a bead with the (e–g) micro-manipulator set-up, the magnetic field pulls the bead towards the tip and imparts an external force on the cell. Reproduced from Ref. 89. (E) Optical tweezers constrain a 1 μm polystyrene bead at the lamellipodium of a cell. As the cell pulls the bead out of the center of the trap (*arrowheads*), the optical tweezers applies a resisting force to the cell. Reproduced from Ref. 55.

provided resistance to the twisting motion of the beads, depending on whether or not the integrins were connected in the intracellular actin cytoskeleton.[46,128] Adhesion strength depended on the type of ECM protein coating on the beads and decreased with drug-induced loss in cytoskeleton integrity or tension.[43,107] Moreover, there is gene expression associated with mechanical forces for MTC has been shown to induce recruitment of ribosomes and mRNA to the spot of force application and nuclear upregulation of gene transcription.[24,27,92] One criticism of MTC is that twisting stresses may not physiologically mimic the native mechanical forces that come from within the cell or from the ECM. However, the precision afforded by MTC, where forces can be directly applied to cellular adhesions without significant cell shape distortions, was a breakthrough in comparison to other techniques. It has played an important role in conceptualizing the specific effects of locally applied forces on cell structure, gene expression, and adhesion strength.

To mimic the larger, directed forces that a cell exerts on the ECM during endogenous contraction or migration, magnetic tweezers have been used to apply linear forces on a magnetic bead. As before, the beads are coated with ECM protein prior to seeding randomly on the cells. A magnetic tip is placed within a few micrometers of the target beads and when the magnetic field is turned on, the tip pulls the beads towards it with forces in the range of nanonewtons. With these larger forces, similar behaviors have been observed as with MTC. The adhesion strength was increased if both vinculin, a structural focal adhesion protein, and actin were able to accumulate at the bead.[5,89] Moreover, basal focal adhesions were displaced when nanonewton forces applied to the dorsal surface adhesions, suggesting that forces were transmitted through the cell to translocate the underlying adhesions.[87] However, for both MTC and magnetic tweezers, it should be pointed out that the magnetic beads are partially embedded into the dorsal surface or occasionally internalized completely, which may activate phagocytotic signaling pathways that misconstrue the results. Additionally, due to the random seeding, neither technique can *a priori* place the beads at a location of interest on the cell before applying forces.

In addressing these concerns, optical tweezers have provided considerable insights into the mechanical interaction between the cell's adhesions and the ECM due to its ability to apply piconewton forces with nanometer position control. This technique uses focused laser beams and photonic forces to manipulate objects. The laser spot creates an "optical trap" that is able to hold particles in its center as small as molecular particles,[17] viruses,[7] cellular organelles,[126] and strands of DNA.[102] For studying cellular forces, these instruments have been used on latex beads (0.5–10 μm diameters) coated with ECM proteins to initiate cell adhesion. Under specific experimental conditions, the trap acts like a spring; the force required to move the bead out of the trap is linearly proportional to the distance between the bead and the center of the trap. When beads were placed at the lamellipodia of migrating cells, there was rearward transport of the beads toward the nucleus due to retrograde actin flow.[28,55,58,75] When the trap was turned on, some beads failed to be pulled into the center of the trap and continued their retrograde flow because the adhesion linkage strengths were higher than the 10–20 pN tweezer force.[28] As with MTC and magnetic tweezers, the adhesion strength of a bead was determined to be dependent on the ECM protein coating. Additionally, if the bead was held in the center of the trap, the restraining force of the optical tweezers induced the cell to reinforce the adhesion strength in order to break free of the trap. The experiments with optical tweezers have also observed force-induced recruitment of focal adhesion proteins to the intracellular attachment with the bead.[55] Taken together, these findings suggest that when cells generate forces on the ECM, they respond by

recruiting focal adhesion proteins to help strengthen the connections between the integrin-ligand complex and the force-generating cytoskeleton. Studies with optical tweezers to identify the role of individual adhesion-related proteins in mechanotransduction look promising because talin has been identified as critical to the recruitment process and adhesion strength.[58,74] With the ability to trap nanoscale objects, optical tweezers could potentially probe the mechanotransduction at finer cellular structures such as filopodia or growth cones, instead of at lamellipodia in these studies. Demonstrations of controlling multiple beads with optical traps[17,124] are intriguing for these set-ups could be used to address other interesting questions such as how much cross-talk in protein recruitment is there between adjacent adhesions, how adhesion recruitment and strength are coordinated between different regions during cell migration, and to determine if active, external positioning of individual integrin-ligand bound receptors can elicit a similar response to that of native integrin clustering.

The micro- and nanotechnology-based techniques presented have shown that adhesion strength depends upon both the type of integrin-ligand bond and on the presence of cytoskeletal tension. Without either of these conditions, cells cannot transduce the applied forces. In light of these findings, it is still uncertain how a cell senses such physical signals, and so, scaling down tools to probe cellular adhesion will reveal more about the unknown phenomena and interactions. Greater control over the applied external force, better matching of the length scales between size of the probe and the cellular structures, and finer targeting of individual receptors or cellular structures underscores that nanotechnology is essential for these mechanotransduction investigations. In fact, many nanotechnology-based tools are available, but have not been used to address these questions. For example, atomic force microscopy is a powerful technique to measure the mechanical structure of DNA, proteins, and cells[22,77,83,99,108,135] and is just beginning to be used for adhesion strength[83] and mechanotransduction.[22] To illuminate the mechanical-to-biochemical and biochemical-to-mechanical interactions, tools used to measure cellular forces should be combined with force application techniques.[64,65] With integration of the tools, we can ask how are locally applied forces transmitted to the underlying focal adhesions as measured by traction forces, if force transmission can be predicted a priori by the structure of the cytoskeleton and activity of tension-related signaling pathways, and, in response to nearby probing, if unperturbed focal adhesions also recruit actomyosin microfilaments to cascade more cellular contraction. Additionally, by incorporating stress and strain feedback into the tool, it would be possible to simultaneously apply a stress while measuring the change in strain, or vice-versa. This is similar to mechanical testing of materials and would help delineate between stretch-activated and force-loading changes such as mechanical conformational changes of proteins[99] or distortion of the cell membrane and cytoskeleton. Unmistakably, we are just beginning to use the appropriate tools or techniques to answer these questions, but with the potentials in nanotechnology, the future holds promise for major answers to come.

CONCLUSIONS

The cell and its underlying ECM have many levels of interaction—chemical and geometrical presentation of ligands, topology of the microenvironment, and mechanical stiffness of the ECM—that guide the function and activity of a cell. The cell can integrate these mechanical cues through clustering of integrin receptors, recruiting focal adhesions and/or cytoskeleton proteins to adhesion sites, and regulating cytoskeleton tension in order to appropriately respond to its microenviroment. A key to better understanding these processes is to examine the cell–ECM interrelationship with the fine spatial and mechanical control offered by nanotechnology. Thus far, micro- and nano-tools have predominantly looked at only static interactions between cells and the ECM. Controlling dynamic aspects of the environment of the cell is just beginning to be addressed. Additionally, many of these studies are performed on a single cell basis. With more sophisticated tools that incorporate arrays of the same sensor, high throughput of the information will provide dramatically improved confidence in the results. Even though such tools have only recently been used to address these questions, micro- and nanotechnology-based tools have already provided significant insight into how the cell responds to changes in its local environment. By scaling down, we can peer into molecular mechanisms that dictate the collection of interactions that we have identified at the microscale.

With increased interaction between the fields of the biological and physical sciences, the future holds the potential of precision nanoinstruments through which well-defined interactions can be independently controlled to study the inner workings of the cell. As we bridge these fields, we must appreciate potential changes in signaling cascades and gene expression due to our physical probing, as well as important mechanical changes in cells resulting from biochemical manipulations. This includes an interdisciplinary understanding of the manipulations, the cellular response, and the indirect effects that arise from making the measurements. With both biomechanical and biochemical manipulations, we can put together a systems-based model of the cell in the hopes of providing new avenues for therapeutic opportunities.

ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Health (grants EB00262 and HL073305), the Department of Defense Multidisciplinary University Research Initiative, and DARPA. NS was supported by the National

Institutes of Health Ruth Kirschtein National Research Service Award Postdoctoral Fellowship, and RD acknowledges support from the National Science Foundation Graduate Research Fellowship Program.

REFERENCES

[1] Abercrombie, M., J. E. Heaysman, and S. M. Pegrum. The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. *Exp. Cell Res.* 67:359–367, 1971.

[2] Abrams, G. A., S. L. Goodman, P. F. Nealey, M. Franco, and C. J. Murphy. Nanoscale topography of the basement membrane underlying the corneal epithelium of the rhesus macaque. *Cell Tissue Res.* 299:39–46, 2000.

[3] Abrams, G. A., C. J. Murphy, Z. Y. Wang, P. F. Nealey, and D. E. Bjorling. Ultrastructural basement membrane topography of the bladder epithelium. *Urol. Res.* 31:341–346, 2003.

[4] Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. Molecular Biology of the Cell. New York, NY: Garland Science, 2002, 1616 pp.

[5] Alenghat, F. J., B. Fabry, K. Y. Tsai, W. H. Goldmann, and D. E. Ingber. Analysis of cell mechanics in single vinculin-deficient cells using a magnetic tweezer. *Biochem. Biophys. Res. Commun.* 277:93–99, 2000.

[6] Alenghat, F. J., and D. E. Ingber. Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci. STKE*, 2002:PE6, 2002.

[7] Ashkin, A., and J. M. Dziedzic. Optical trapping and manipulation of viruses and bacteria. *Science* 235:1517–1520, 1987.

[8] Bain, C. D., J. Evall, and G. M. Whitesides. Formation of monolayers by the coadsorption of thiols on gold: Variation in the head group, tail group, and solvent. *J. Am. Chem. Soc.* 111:7155–7164, 1989.

[9] Bain, C. D., and G. M. Whitesides. Correlations between wettability and structure in monolayers of alkanethiols adsorbed on gold. *J. Am. Chem. Soc.* 110:3665–3666, 1988.

[10] Bain, C. D., and G. M. Whitesides. Formation of monolayers by the coadsoption of thiols on gold: Variation in the length of the alkyl chain. *J. Am. Chem. Soc.* 111:7164–7175, 1989.

[11] Balaban, N. Q., U. S. Schwarz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, and B. Geiger. Force and focal adhesion assembly: A close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3:466–472, 2001.

[12] Balss, K. M., B. D. Coleman, C. H. Lansford, R. T. Haasch, and P. W. Bohn. Active spatiotemporal control of electrochemical reactions by coupling to in-plane potential gradients. *J. Phys. Chem.* 105:8970–8978, 2001.

[13] Beningo, K. A., M. Dembo, I. Kaverina, J. V. Small, and Y. L. Wang. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J. Cell Biol.* 153:881–888, 2001.

[14] Beningo, K. A., M. Dembo, and Y. L. Wang. Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. *Proc. Natl. Acad. Sci. USA* 101:18024–18029, 2004.

[15] Bernard, A., E. Delamarche, H. Schmid, B. Michel, H. R. Bosshard, and H. Biebuyck. Printing patterns of proteins. *Langmuir* 14:2225–2229, 1998.

[16] Brock, A., E. Chang, C. C. Ho, P. LeDuc, X. Jiang, G. M. Whitesides, and D. E. Ingber. Geometric determinants of directional cell motility revealed using microcontact printing. *Langmuir* 19:1611–1617, 2003.

[17] Burns, M. M., J. M. Fournier, and J. A. Golovchenko. Optical matter—Crystallization and binding in intense optical-fields. *Science* 249:749–754, 1990.

[18] Burridge, K., and M. Chrzanowska-Wodnicka. Focal adhesions, contractility, and signaling. *Annu. Rev. Cell Dev. Biol.* 12:463–518, 1996.

[19] Burton, K., J. H. Park, and D. L. Taylor. Keratocytes generate traction forces in two phases. *Mol. Biol. Cell* 10:3745–3769, 1999.

[20] Burton, K., and D. L. Taylor. Traction forces of cytokinesis measured with optically modified elastic substrata. *Nature* 385:450–454, 1997.

[21] Carter, S. B. Haptotaxis and the mechanism of cell motility. *Nature* 213:256–260, 1967.

[22] Charras, G. T., and M. A. Horton. Single cell mechanotransduction and its modulation analyzed by atomic force microscope indentation. *Biophys. J.* 82:2970–2981, 2002.

[23] Chen, C. S., J. L. Alonso, E. Ostuni, G. M. Whitesides, and D. E. Ingber. Cell shape provides global control of focal adhesion assembly. *Biochem. Biophys. Res. Commun.* 307:355–361, 2003.

[24] Chen, J., B. Fabry, E. L. Schiffrin, and N. Wang. Twisting integrin receptors increases endothelin-1 gene expression in endothelial cells. *Am. J. Physiol. Cell Physiol.* 280:C1475–C1484, 2001.

[25] Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber. Geometric control of cell life and death. *Science* 276:1425–1428, 1997.

[26] Chen, C. S., J. Tan, and J. Tien. Mechanotransduction at cell-matrix and cell-cell contacts. *Annu. Rev. Biomed. Eng.* 6:275–302, 2004.

[27] Chicurel, M. E., R. H. Singer, C. J. Meyer, and D. E. Ingber. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. *Nature* 392:730–733, 1998.

[28] Choquet, D., D. P. Felsenfeld, and M. P. Sheetz. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell* 88:39–48, 1997.

[29] Chrzanowska-Wodnicka, M., and K. Burridge. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* 133:1403–1415, 1996.

[30] Coussen, F., D. Choquet, M. P. Sheetz, and H. P. Erickson. Trimers of the fibronectin cell adhesion domain localize to actin filament bundles and undergo rearward translocation. *J. Cell Sci.* 115:2581–2590, 2002.

[31] Curtis, A. S. The mechanism of adhesion of cells to glass. A study by interference reflection microscopy. *J. Cell Biol.* 20:199–215, 1964.

[32] Curtis, A. S., and M. Varde. Control of cell behavior: Topological factors. *J. Natl. Cancer Inst.* 33:15–26, 1964.

[33] Curtis, A. S., C. D. Wilkinson, J. Crossan, C. Broadley, H. Darmani, K. K. Johal, H. Jorgensen, and W. Monaghan. An in vivo microfabricated scaffold for tendon repair. *Eur. Cell Mater.* 9:50–57, 2005.

[34] Dalby, M. J., M. O. Riehle, D. S. Sutherland, H. Agheli, and A. S. Curtis. Morphological and microarray analysis of human fibroblasts cultured on nanocolumns produced by colloidal lithography. *Eur. Cell Mater.* 9:1–8, 2005.

[35] Dalby, M. J., S. J. Yarwood, M. O. Riehle, H. J. Johnstone, S. Affrossman, and A. S. Curtis. Increasing fibroblast response to materials using nanotopography: Morphological and genetic measurements of cell response to 13-nm-high polymer demixed islands. *Exp. Cell Res.* 276:1–9, 2002.

[36] Davies, P. F. Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* 75:519–560, 1995.

[37]Davies, P. F., A. Robotewskyj, and M. L. Griem. Quantitative studies of endothelial cell adhesion. Directional remodeling of focal adhesion sites in response to flow forces. *J. Clin. Invest.* 93:2031–2038, 1994.

[38]Dembo, M., T. Oliver, A. Ishihara, and K. Jacobson. Imaging the traction stresses exerted by locomoting cells with the elastic substratum method. *Biophys. J.* 70:2008–2022, 1996.

[39]Dembo, M., and Y. L. Wang. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys. J.* 76:2307–2316, 1999.

[40]Dertinger, S. K., D. T. Chiu, N. L. Jeon, and G. M. Whitesides. Generation of gradients having complex shapes using microfluidics networks. *Anal. Chem.* 73:1240–1246, 2001.

[41]Dertinger, S. K., X. Jiang, Z. Li, V. N. Murthy, and G. M. Whitesides. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl. Acad. Sci. USA* 99:12542–12547, 2002.

[42]Dike, L. E., C. S. Chen, M. Mrksich, J. Tien, G. M. Whitesides, and D. E. Ingber. Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. *In Vitro Cell Dev. Biol. Anim.* 35:441–448, 1999.

[43]Doornaert, B., V. Leblond, E. Planus, S. Galiacy, V. M. Laurent, G. Gras, D. Isabey, and C. Lafuma. Time course of actin cytoskeleton stiffness and matrix adhesion molecules in human bronchial epithelial cell cultures. *Exp. Cell Res.* 287:199–208, 2003.

[44]du Roure, O., A. Saez, A. Buguin, R. H. Austin, P. Chavrier, P. Siberzan, and B. Ladoux. Force mapping in epithelial cell migration. *Proc. Natl. Acad. Sci. USA* 102:2390–2395, 2005.

[45]Elias, K. L., R. L. Price, and T. J. Webster. Enhanced functions of osteoblasts on nanometer diameter carbon fibers. *Biomaterials* 23:3279–3287, 2002.

[46]Fabry, B., G. N. Maksym, J. P. Butler, M. Glogauer, D. Navajas, and J. J. Fredberg. Scaling the microrheology of living cells. *Phys. Rev. Lett.* 87:148102, 2001.

[47]Feynman, R. P. There's plenty of room at the bottom. American Physical Society. Pasadena, CA: California Institute of Technology, 1959.

[48]Finer, J. T., R. M. Simmons, and J. A. Spudich. Single myosin molecule mechanics: Piconewton forces and nanometre steps. *Nature* 368:113–119, 1994.

[49]Flemming, R. G., C. J. Murphy, G. A. Abrams, S. L. Goodman, and P. F. Nealey. Effects of synthetic micro- and nano-structured surfaces on cell behavior. *Biomaterials* 20:573–588, 1999.

[50]Foley, J. D., E. W. Grunwald, P. F. Nealey, and C. J. Murphy. Cooperative modulation of neuritogenesis by PC12 cells by topography and nerve growth factor. *Biomaterials* 26:3639–3644, 2005.

[51]Folkman, J., and A. Moscona. Role of cell shape in growth control. *Nature* 273:345–349, 1978.

[52]Galbraith, C. G., and M. P. Sheetz. A micromachined device provides a new bend on fibroblast traction forces. *Proc. Natl. Acad. Sci. USA* 94:9114–9118, 1997.

[53]Galbraith, C. G., and M. P. Sheetz. Forces on adhesive contacts affect cell function. *Curr. Opin. Cell Biol.* 10:566–571, 1998.

[54]Galbraith, C. G., and M. P. Sheetz. Keratocytes pull with similar forces on their dorsal and ventral surfaces. *J. Cell Biol.* 147:1313–1324, 1999.

[55]Galbraith, C. G., K. M. Yamada, and M. P. Sheetz. The relationship between force and focal complex development. *J. Cell Biol.* 159:695–705, 2002.

[56]Gaudet, C., W. A. Marganski, S. Kim, C. T. Brown, V. Gunderia, M. Dembo, and J. Y. Wong. Influence of type I collagen surface density on fibroblast spreading, motility, and contractility. *Biophys. J.* 85:3329–3335, 2003.

[57]Geiger, B., A. Bershadsky, R. Pankov, and K. M. Yamada. Transmembrane extracellular matrix–cytoskeleton crosstalk. *Nat. Rev. Mol. Cell Biol.* 2:793–805, 2001.

[58]Giannone, G., G. Jiang, D. H. Sutton, D. R. Critchley, and M. P. Sheetz. Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation. *J. Cell Biol.* 163:409–419, 2003.

[59]Ginger, D. S., H. Zhang, and C. A. Mirkin. The evolution of dip-pen nanolithography. *Angew Chem. Int. Ed. Engl.* 43:30–45, 2004.

[60]Harris, A. Behavior of cultured cells on substrata of variable adhesiveness. *Exp. Cell Res.* 77:285–297, 1973.

[61]Harris, A. K., Jr. Tissue culture cells on deformable substrata: Biomechanical implications. *J. Biomech. Eng.* 106:19–24, 1984.

[62]Harris, A. K., P. Wild, and D. Stopak. Silicone rubber substrata: A new wrinkle in the study of cell locomotion. *Science* 208:177–179, 1980.

[63]Helfman, D. M., E. T. Levy, C. Berthier, M. Shtutman, D. Riveline, I. Grosheva, A. Lachish-Zalait, M. Elbaum, and A. D. Bershadsky. Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. *Mol. Biol. Cell* 10:3097–3112, 1999.

[64]Hu, S., J. Chen, B. Fabry, Y. Numaguchi, A. Gouldstone, D. E. Ingber, J. J. Fredberg, J. P. Butler, and N. Wang. Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. *Am. J. Physiol. Cell Physiol.* 285:C1082–C1090, 2003.

[65]Hu, S., L. Eberhard, J. Chen, J. C. Love, J. P. Butler, J. J. Fredberg, G. M. Whitesides, and N. Wang. Mechanical anisotropy of adherent cells probed by a three-dimensional magnetic twisting device. *Am. J. Physiol. Cell Physiol.* 287:C1184–C1191, 2004.

[66]Hua, F., Y. G. Sun, A. Gaur, M. A. Meitl, L. Bilhaut, L. Rotkina, J. F. Wang, P. Geil, M. Shim, J. A. Rogers, and A. Shim. Polymer imprint lithography with molecular-scale resolution. *Nano Lett.* 4:2467–2471, 2004.

[67]Huang, S., C. S. Chen, and D. E. Ingber. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol. Biol. Cell* 9:3179–3193, 1998.

[68]Ingber, D. E. Mechanobiology and diseases of mechanotransduction. *Ann. Med.* 35:564–577, 2003.

[69]Ingber, D. E. Tensegrity I. Cell structure and hierarchical systems biology. *J.Cell Sci.* 116:1157–1173, 2003.

[70]Ingber, D. E. Tensegrity II. How structural networks influence cellular information processing networks. *J. Cell Sci.* 116:1397–1408, 2003.

[71]James, C. D., R. C. Davis, L. Kam, H. G. Craighead, M. Isaacson, J. N. Turner, and W. Shain. Patterned protein layers on solid substrates by thin stamp microcontact printing. *Langmuir* 14:741–744, 1998.

[72]Jeon, N. L., H. Baskaran, S. K. Dertinger, G. M. Whitesides, L. Van De Water, and M. Toner. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat. Biotech.* 20:826–830, 2002.

[73]Jiang, X., D. A. Bruzewicz, A. P. Wong, M. Piel, and G. M. Whitesides. Directing cell migration with asymmetric micropatterns. *Proc. Natl. Acad. Sci. USA* 102:975–978, 2005.

[74]Jiang, X., R. Ferrigno, M. Mrksich, and G. M. Whitesides. Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements. *J. Am. Chem. Soc.* 125:2366–2367, 2003.

[75]Jiang, G. Y., G. Giannone, D. R. Critchley, E. Fukumoto, and M. P. Sheetz. Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. *Nature* 424:334–337, 2003.

[76]Kong, H. J., T. R. Polte, E. Alsberg, and D. J. Mooney. FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness. *Proc. Natl. Acad. Sci. USA* 102:4300–4305, 2005.

[77]Laurent, V. M., S. Kasas, A. Yersin, T. E. Schaffer, S. Catsicas, G. Dietler, A. B. Verkhovsky, and J. J. Meister. Gradient of rigidity in the lamellipodia of migrating cells revealed by atomic force microscopy. *Biophys. J.* 89:667–675, 2005.

[78]Lee, G. M., and R. F. Loeser. Cell surface receptors transmit sufficient force to bend collagen fibrils. *Exp. Cell Res.* 248:294–305, 1999.

[79]Lee, J., M. Leonard, T. Oliver, A. Ishihara, and K. Jacobson. Traction forces generated by locomoting keratocytes. *J. Cell Biol.* 127:1957–1964, 1994.

[80]Lee, K. B., S. J. Park, C. A. Mirkin, J. C. Smith, and M. Mrksich. Protein nanoarrays generated by dip-pen nanolithography. *Science* 295:1702–1705, 2002.

[81]Lehnert, D., B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof, and M. Bastmeyer. Cell behaviour on micropatterned substrata: Limits of extracellular matrix geometry for spreading and adhesion. *J. Cell Sci.* 117:41–52, 2004.

[82]Leung, D. Y., S. Glagov, and M. B. Mathews. Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. *Science* 191:475–477, 1976.

[83]Li, F., S. D. Redick, H. P. Erickson, and V. T. Moy. Force measurements of the alpha5beta1 integrin-fibronectin interaction. *Biophys. J.* 84:1252–1262, 2003.

[84]Liedberg, B., and P. Tengvall. Molecular gradients of omega-substituted alkanethiols on gold: Preparation and characterization. *Langmuir* 11:3821–3827, 1995.

[85]Lin, F., C. M. Nguyen, S. J. Wang, W. Saadi, S. P. Gross, and N. L. Jeon. Neutrophil migration in opposing chemoattractant gradients using microfluidic chemotaxis devices. *Ann. Biomed. Eng.* 33:475–482, 2005.

[86]Lo, C. M., H. B. Wang, M. Dembo, and Y. L. Wang. Cell movement is guided by the rigidity of the substrate. *Biophys. J.* 79:144–152, 2000.

[87]Mack, P. J., M. R. Kaazempur-Mofrad, H. Karcher, R. T. Lee, and R. D. Kamm. Force-induced focal adhesion translocation: Effects of force amplitude and frequency. *Am. J. Physiol. Cell Physiol.* 287:C954–C962, 2004.

[88]Maniotis, A. J., C. S. Chen, and D. E. Ingber. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA* 94:849–854, 1997.

[89]Matthews, B. D., D. R. Overby, F. J. Alenghat, J. Karavitis, Y. Numaguchi, P. G. Allen, and D. E. Ingber. Mechanical properties of individual focal adhesions probed with a magnetic microneedle. *Biochem. Biophys. Res. Commun.* 313:758–764, 2004.

[90]McBeath, R., D. M. Pirone, C. M. Nelson, K. Bhadriraju, and C. S. Chen. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6:483–495, 2004.

[91]Meshel, A. S., Q. Wei, R. S. Adelstein, and M. P. Sheetz. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* 7:157–164, 2005.

[92]Meyer, C. J., F. J. Alenghat, P. Rim, J. H. Fong, B. Fabry, and D. E. Ingber. Mechanical control of cyclic AMP signalling and gene transcription through integrins. *Nat. Cell Biol.* 2:666–668, 2000.

[93]Miller, D. C., K. M. Haberstroh, and T. J. Webster. Mechanism(s) of increased vascular cell adhesion on nanostructured poly(lactic-co-glycolic acid) films. *J. Biomed. Mater. Res. A* 73:476–484, 2005.

[94]Miller, D. C., A. Thapa, K. M. Haberstroh, and T. J. Webster. Endothelial and vascular smooth muscle cell function on poly(lactic-co-glycolic acid) with nano-structured surface features. *Biomaterials* 25:53–61, 2004.

[95]Morgenthaler, S., S. Lee, S. Zurcher, and N. D. Spencer. A simple, reproducible approach to the preparation of surface-chemical gradients. *Langmuir* 19:10459–10462, 2003.

[96]Mrksich, M., L. E. Dike, J. Tien, D. E. Ingber, and G. M. Whitesides. 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.* 235:305–313, 1997.

[97]Mrksich, M., and G. M. Whitesides. Patterning self-assembled monolayers using microcontact printing: A new technology for biosensors? *Trends Biotech.* 13:228–235, 1995.

[98]Munevar, S., Y. Wang, and M. Dembo. Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. *Biophys. J.* 80:1744–1757, 2001.

[99]Oberhauser, A. F., C. Badilla-Fernandez, M. Carrion-Vazquez, and J. M. Fernandez. The mechanical hierarchies of fibronectin observed with single-molecule AFM. *J. Mol. Biol.* 319:433–447, 2002.

[100]Parker, K. K., A. L. Brock, C. Brangwynne, R. J. Mannix, N. Wang, E. Ostuni, N. A. Geisse, J. C. Adams, G. M. Whitesides, and D. E. Ingber. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *Faseb. J.* 16:1195–1204, 2002.

[101]Pelham, R. J., Jr., and Y. Wang. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* 94:13661–13665, 1997.

[102]Perkins, T. T., S. R. Quake, D. E. Smith, and S. Chu. Relaxation of a single DNA molecule observed by optical microscopy. *Science* 264:822–826, 1994.

[103]Piner, R. D., J. Zhu, F. Xu, S. Hong, and C. A. Mirkin. "Dip-Pen" nanolithography. *Science* 283:661–663, 1999.

[104]Plummer, S. T., Q. Wang, and P. W. Bohn. Electrochemically derived gradients of the extracellular matrix protein fibronectin on gold. *Langmuir* 19:7528–7536, 2003.

[105]Prime, K. L., and G. M. Whitesides. Self-assembled organic monolayers: Model systems for studying adsorption of proteins at surfaces. *Science* 252:1164–1167, 1991.

[106]Prime, K. L., and G. M. Whitesides. Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): A model system usings self-assembled monolayers. *J. Am. Chem. Soc.* 115:10714–10721, 1993.

[107]Puig-de-Morales, M., E. Millet, B. Fabry, D. Navajas, N. Wang, J. P. Butler, and J. J. Fredberg. Cytoskeletal mechanics in adherent human airway smooth muscle cells: probe specificity and scaling of protein-protein dynamics. *Am. J. Physiol. Cell Physiol.* 287:C643–C654, 2004.

[108]Radmacher, M. Measuring the elastic properties of biological samples with the AFM. *IEEE Eng. Med. Biol. Mag.* 16:47–57, 1997.

[109]Rajagopalan, P., W. A. Marganski, X. Q. Brown, and J. Y. Wong. Direct comparison of the spread area, contractility, and migration of balb/c 3T3 fibroblasts adhered to fibronectin- and RGD-modified substrata. *Biophys. J.* 87:2818–2827, 2004.

[110]Reinhart-King, C. A., M. Dembo, and D. A. Hammer. Endothelial cell traction forces on RGD-derivatized polyacrylamide substrata. *Langmuir* 19:1573–1579, 2003.

[111]Reinhart-King, C. A., M. Dembo, and D. A. Hammer. The dynamics and mechanics of endothelial cell spreading. *Biophys. J*. 89:676–689, 2005.

[112]Riveline, D., E. Zamir, N. Q. Balaban, U. S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, and A. D. Bershadsky. 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*. 153:1175–1186, 2001.

[113]Sastry, S. K., and K. Burridge. Focal adhesions: A nexus for intracellular signaling and cytoskeletal dynamics. *Exp. Cell Res*. 261:25–36, 2000.

[114]Schindler, M., I. Ahmed, J. Kamal, A. Nur-E-Kamal, T. H. Grafe, H. Young Chung, and S. Meiners. A synthetic nanofibrillar matrix promotes in vivo-like organization and morphogenesis for cells in culture. *Biomaterials* 26:5624–5631, 2005.

[115]Shirinsky, V. P., A. S. Antonov, K. G. Birukov, A. V. Sobolevsky, Y. A. Romanov, N. V. Kabaeva, G. N. Antonova, and V. N. Smirnov. Mechano-chemical control of human endothelium orientation and size. *J. Cell Biol*. 109:331–339, 1989.

[116]Singhvi, R., A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides, and D. E. Ingber. Engineering cell shape and function. *Science* 264:696–698, 1994.

[117]Smith, J. T., J. K. Tomfohr, M. C. Wells, T. P. Beebe, Jr., T. B. Kepler, and W. M. Reichert. Measurement of cell migration on surface-bound fibronectin gradients. *Langmuir* 20:8279–8286, 2004.

[118]Stopak, D., N. K. Wessells, and A. K. Harris. Morphogenetic rearrangement of injected collagen in developing chicken limb buds. *Proc. Natl. Acad. Sci. USA* 82:2804–2808, 1985.

[119]Suki, B., S. Ito, D. Stamenovic, K. R. Lutchen, and E. P. Ingenito. Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces. *J. Appl. Physiol*. 98:1892–1899, 2005.

[120]Tan, J. L., W. Liu, C. M. Nelson, S. Raghavan, and C. S. Chen. Simple approach to micropattern cells on common culture substrates by tuning substrate wettability. *Tissue Eng*. 10:865–872, 2004.

[121]Tan, J. L., J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, and C. S. Chen. Cells lying on a bed of microneedles: An approach to isolate mechanical force. *Proc. Natl. Acad. Sci. USA* 100:1484–1489, 2003.

[122]Teixeira, A. I., G. A. Abrams, P. J. Bertics, C. J. Murphy, and P. F. Nealey. Epithelial contact guidance on well-defined micro- and nanostructured substrates. *J. Cell Sci*. 116:1881–1892, 2003.

[123]Teixeira, A. I., P. F. Nealey, and C. J. Murphy. Responses of human keratocytes to micro- and nanostructured substrates. *J. Biomed. Mater. Res. A* 71:3153–3164, 2004.

[124]Terray, A., J. Oakey, and D. W. M. Marr. Microfluidic control using colloidal devices. *Science* 296:1841–1844, 2002.

[125]Tolic-Norrelykke, I. M., and N. Wang. Traction in smooth muscle cells varies with cell spreading. *J. Biomech*. 38:1405–1412, 2005.

[126]Upadhyaya, A., and M. P. Sheetz. Tension in tubulovesicular networks of Golgi and endoplasmic reticulum membranes. *Biophys. J*. 86:2923–2928, 2004.

[127]Walpita, D., and E. Hay. Studying actin-dependent processes in tissue culture. *Nat. Rev. Mol. Cell Biol*. 3:137–141, 2002.

[128]Wang, N., J. P. Butler, and D. E. Ingber. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260:1124–1127, 1993.

[129]Wang, H. B., M. Dembo, S. K. Hanks, and Y. Wang. Focal adhesion kinase is involved in mechanosensing during fibroblast migration. *Proc. Natl. Acad. Sci. USA* 98:11295–11300, 2001.

[130]Wang, N., E. Ostuni, G. M. Whitesides, and D. E. Ingber. Micropatterning tractional forces in living cells. *Cell Motil. Cytoskeleton* 52:97–106, 2002.

[131]Xia, Y., and G. M. Whitesides. Soft lithography. *Annu. Rev. Mater. Sci*. 28:153–184, 1998.

[132]Yim, E. K., R. M. Reano, S. W. Pang, A. F. Yee, C. S. Chen, and K. W. Leong. Nanopattern-induced changes in morphology and motility of smooth muscle cells. *Biomaterials* 26:5405–5413, 2005.

[133]Yousaf, M. N., B. T. Houseman, and M. Mrksich. Using electroactive substrates to pattern the attachment of two different cell populations. *Proc. Natl. Acad. Sci. USA* 98:5992–5996, 2001.

[134]Zamir, E., and B. Geiger. Molecular complexity and dynamics of cell-matrix adhesions. *J. Cell Sci*. 114:3583–3590, 2001.

[135]Zlatanova, J., S. M. Lindsay, and S. H. Leuba. Single molecule force spectroscopy in biology using the atomic force microscope. *Prog. Biophys. Mol. Biol*. 74:37–61, 2000.