Microengineered Platforms for Cell Mechanobiology

Deok-Ho Kim,[1] Pak Kin Wong,[2] Jungyul Park,[3] Andre Levchenko,[1] and Yu Sun[4]

[1]Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, Maryland 21218; email: dhkim@jhu.edu

[2]Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, Arizona 85721

[3]Department of Mechanical Engineering, Sogang University, Seoul 121-742, Korea

[4]Advanced Micro and Nanosystems Laboratory, University of Toronto, Canada M5S 3G8; email: sun@mie.utoronto.ca

Annu. Rev. Biomed. Eng. 2009. 11:203–33

First published online as a Review in Advance on April 13, 2009

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

This article's doi:
10.1146/annurev-bioeng-061008-124915

1523-9829/09/0815-0203$20.00

Key Words

cell mechanics, microfabrication, microelectromechanical systems (MEMS), extracellular matrix, mechanotransduction

Abstract

Mechanical forces play important roles in the regulation of various biological processes at the molecular and cellular level, such as gene expression, adhesion, migration, and cell fate, which are essential to the maintenance of tissue homeostasis. In this review, we discuss emerging bioengineered tools enabled by microscale technologies for studying the roles of mechanical forces in cell biology. In addition to traditional mechanobiology experimental techniques, we review recent advances of microelectromechanical systems (MEMS)-based approaches for cell mechanobiology and discuss how microengineered platforms can be used to generate in vivo–like micromechanical environment in in vitro settings for investigating cellular processes in normal and pathophysiological contexts. These capabilities also have significant implications for mechanical control of cell and tissue development and cell-based regenerative therapies.

Contents

1. INTRODUCTION

Living cells possess physical and biochemical modules that enable them to adapt to dynamic changes in the physiological environment and to maintain appropriate biological functions. For instance, cells actively sense and respond to biochemical cues, including diffusible factors (autocrine and paracrine), juxtacrine cell-cell signaling, and the active part of the extracellular matrix (ECM). Cells also face diverse biomechanical environments and respond to exernally applied or internally generated mechanical stress, such as changes in plasma membrane tension by the topography and rigidity of ECM, shear stress, hydrostatic pressure, and compression in a human body (see **Figure 1**). They are capable of sensing mechanical forces and converting them into biological signals via mechanotransduction mechanisms. Impairments of these cellular processes contribute to the underlying causes of many diseases and pathological conditions. Much research has been conducted to investigate cellular responses to soluble biochemical factors, such as growth factors, cytokines, and hormones. However, the effects of mechanical forces exerted on and by the cell are relatively less understood.

The term cell mechanobiology refers to the study of the role of mechanical forces in cell biology. Two aspects of cell mechanobiology include (*a*) the elucidation of the mechanisms by which cells sense, transduce, and respond to mechanical forces and modulate their functions and (*b*) the characterization of cellular mechanical properties. This area is largely underexplored. Few answers as to specific mechanisms have been given in an unambiguous manner, despite the importance of these mechanisms in fundamental biological processes and biomedical applications, such as cell-based therapy wherein mechanical effects on cell fate and growth can affect tissue remodeling and

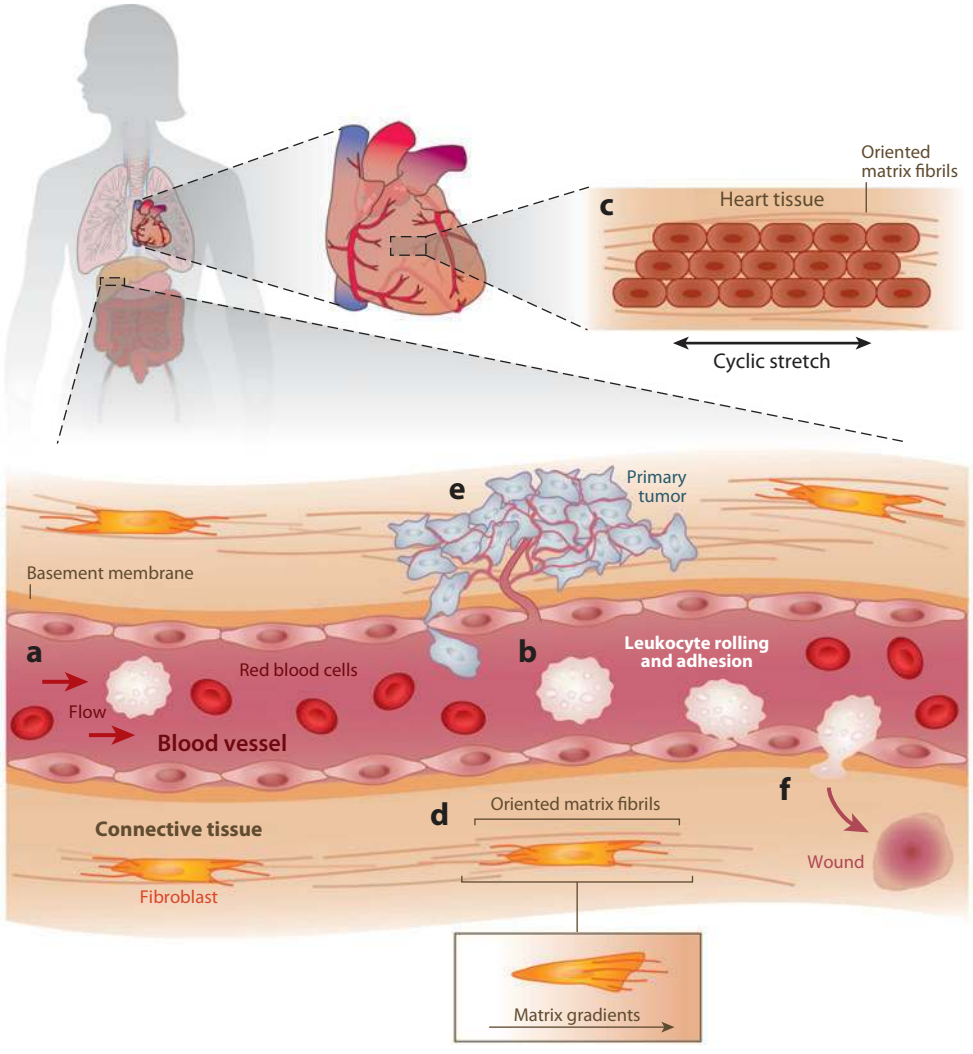


Figure 1

The biomechanical environment of cells and tissues in a human body. Cells experience various mechanical stimuli in a human body, particularly important in the cardiovascular and musculoskeletal systems. To permit human locomotion, tensile muscular forces and compressive loads act on cartilage and bones through tendons to move joints. In blood vessels, cells are continuously subjected to shear and hydrostatic stress from blood flow (*a*). For instance, shear stress in blood vessels modulates leukocyte rolling and adhesion (*b*). Similarly, tissues in lung and heart are stretched cyclically during breathing and heart beating (*c*). Cell motility and tissue organization are also affected by mechanical and structural properties of extracellular matrix (ECM) components. For instance, gradients and structural organization of matrix proteins appear to provide guidance cues to myocardial alignment during cardiovascular development (*c*) as well as directional organization of cells in connective tissue (*d*). Cancer cells spread from a primary tumor site (*e*) and invade other organs by stimulations from soluble factors and by rapidly tracking collagenous matrix fibers (141). Another example is a skin wound (*f*). In a connective tissue scar, the collagen matrix has been poorly reconstituted, in dense parallel bundles, unlike the mechanically efficient basket-weave meshwork of collagen in normal dermis (225).

regeneration. This may be primarily because, unlike biochemical stimuli, the spatial and temporal control of which can be relatively easily implemented, variation of external mechanical stimuli affecting a cell and precise manipulation of these stimuli are more challenging. Despite existing demonstrations that mechanical forces are an essential factor in determination of the homeostasis, differentiation, and function of many cells and tissues, the underlying fundamental conundrum of how mechanical forces exert their effects on the cell and tissue levels remains to be resolved.

As early as 1892, Julius Wolff described bone remodeling, in which bones changed shape, density, and stiffness when mechanical loading was altered (1). Loading conditions can also play important roles in tissue or organ pathology, such as the processes of osteoporosis, atherosclerosis, and fibrosis (2). Several cell mechanics models, including the liquid droplet model (3), the soft-glassy model (4), the elastic solid model (5), and the tensegrity model (6), have been developed to describe mechanical responses of living cells subjected to static and dynamic loads. Despite the knowledge acquired from these seminal works, our understanding of the complex and intricate relationship between applied loads and the resulting biological outcomes is, unfortunately, still far from complete.

There has also been mounting evidence of the correlation between changes in cell mechanical properties and progression in disease states, for example, in cancers (7, 8), malaria (9, 10), and sickle cell anemia (11) at the cellular level. The red blood cell (RBC) becomes stiff and cytoadherent when a malarial parasite invades and matures within a RBC. Similar changes occur when a RBC is affected by sickle cell anemia (11). Cell stiffness of metastatic cancer cells was more than 70% lower than that of the benign cells, according to studies of a number of patient samples (12). Further research at the cellular and molecular levels of human diseases can provide a possible avenue for disease diagnostics and prognostics through quantitative biomechanical analysis of healthy and diseased cells.

Mechanical forces can play a critical role in the regulation of cell signaling and function under normal physiological conditions on the microscale—from tethering junctions at cell-matrix and cell-cell contacts within a tissue to externally applied loads (e.g., cell stretch, shear stress, substrate rigidity, and topography) arising in the cellular microenvironment (see **Figure 2**). Mechanical forces also have been demonstrated to alter gene expression and to control differentiation in stem cells. For instance, soft matrices favored the differentiation of mesenchymal stem cells into neuronal-like cells, moderate stiffness promoted myogenic differentiation, and a rigid matrix stimulated osteogenic differentiation (13), suggesting that mechanical effects on cell function are greater than previously thought.

Mechanical studies of cells, subcellular components, and individual biological molecules have rapidly evolved in the past decade. The progress was facilitated by new capabilities of applying and measuring forces and displacements with piconewton and nanometer resolutions as well as by improvements in live-cell imaging techniques (14). Micromanipulation techniques based on magnetic, optical, and mechanical means have considerably expanded our capabilities to precisely probe cellular structures and to study their responses. Microelectromechanical systems (MEMS) have also emerged as an enabling platform technology for cell mechanobiology studies.

These microengineering technologies provide novel experimental capabilities to determine the type and magnitude of forces experienced at the cellular and subcellular levels and identify the force sensors/receptors that initiate the cascade of cellular and molecular events. The advent of microengineered platforms portends endless possibilities for new insights into interactions between cells and their microenvironment that underlie the physiology of human tissues. Microfabrication technology is increasingly used for controlling the architecture and adhesiveness of in vitro cellular microenvironment and for enabling accurate, quantitative measurements of cellular responses in high-throughput experiments. In the context of regulating cellular

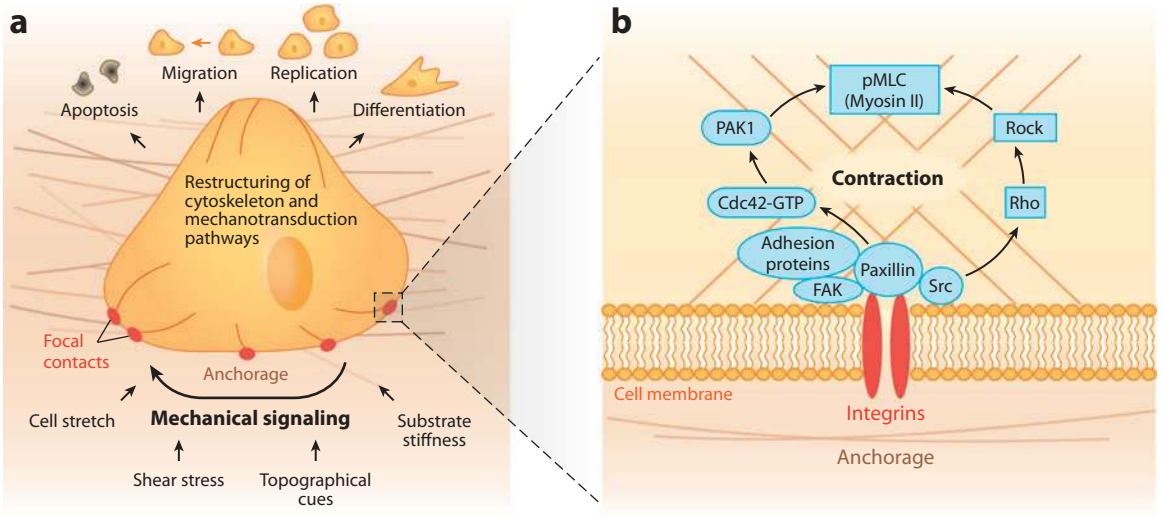


Figure 2

(*a*) A myriad of biomechanical cues (e.g., substrate stiffness, cell stretch, shear stress, topographic cues) in the microenvironment regulate cell signaling and function. Many cell activities that are critical for maintaining tissue homeostasis can be controlled by mechanical interactions between cells and their extracellular matrix (ECM) adhesions that alter the mechanical force balance in the ECM, cell, and cytoskeleton. Cells sense mechanical forces and convert such mechanical signals into biological responses by triggering a restructuring of the cytoskeleton and intracellular signaling pathways (e.g., FAK and Src) transmitted through transmembrane adhesion receptors (i.e., integrins) or mechanosensitive ion channels (17). The mechanotransduction pathways can be triggered to regulate gene expression, cell contractility, and ultimately cell apoptosis, migration, proliferation, differentiation, and growth. (*b*) For example, changing ECM mechanics or altering cytoskeletal tension generation through manipulating the small Rho GTPases can regulate stem cell differentiation (13, 15). Cells also dynamically remodel ECM through protein secretion and cytoskeletal reconfiguration, which in turn changes the set of cues cells receive from their environment (mechanical feedback) (213, 226).

microenvironments, directing cell fate, and elucidating the underlying biological mechanisms, techniques based on microfluidics, surface patterning, and MEMS tools have been under intensive development in recent years.

Several excellent reviews on mechanobiology need to be brought to the readers' attention before we move into further discussions (2, 7, 8, 16–19). In contrast to these reviews of the rapidly evolving field, our article focuses on bioengineered tools particularly enabled by microscale technologies for studying cell mechanobiology (see **Figure 3**). We first discuss traditional microengineered techniques, followed by a review of MEMS-based tools used to apply localized forces to single cells or to measure forces exerted by the cell. Then we discuss how microfabrication-based approaches can be used to create in vivo–like biomechanical stimuli in in vitro experimental settings. We also highlight emerging opportunities and challenges.

2. MICROMANIPULATION TECHNIQUES FOR CELL MECHANOBIOLOGY

Quantitative investigations of how cells sense and respond to mechanical stress depend on techniques that can apply controlled mechanical forces to living cells and simultaneously measure changes in cellular deformation and alterations of molecular events. Micromanipulation techniques using mechanical, optical, and magnetic means have been used to manipulate and measure the mechanical properties of cells, nucleus, cell membrane, and cytoskeleton via a combined use of

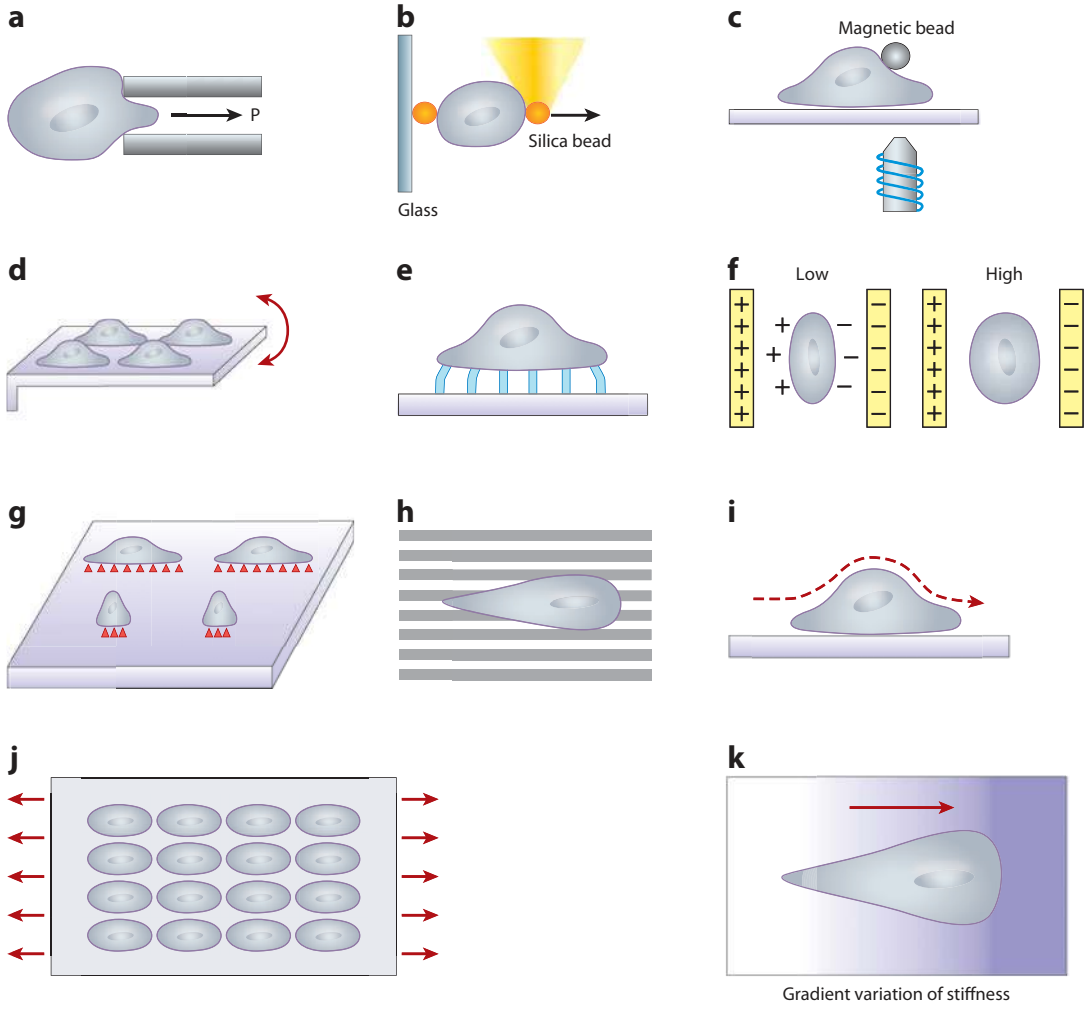


Figure 3

Schematic illustrations of microengineered techniques for characterizing and stimulating single cells for cell mechanobiology. (*a*) Micropipette aspiration. (*b*) Laser trapping. (*c*) Magnetic bead measurement. (*d*) Microcantilever sensor. (*e*) Micropost sensor array. (*f*) Electrodeformation. (*g*) ECM micropatterning. (*h*) Micro- and nanotopographic substrate. (*i*) Microfluidic shear device. (*j*) Micromechanical cell stretching. (*k*) Substrate stiffness. Reproduced with permission from Reference 14.

molecular cell biology techniques and microscopic analysis of intracellular signaling. A number of key traditional techniques use mechanical perturbations as a means to probe cellular components, such as cytoskeleton and plasma membrane.

2.1. Micropipette Aspiration

To measure the mechanical properties of a single cell, the cell must be deformed in some way by a known force or stress, and its deformations must be measured. Micropipette aspiration is a classical technique for quantifying the mechanical properties of individual cells, such as elastic

modulus and viscosity. The technique applies a low-magnitude, negative pressure to deform a cell, elongating a portion of the cell into the micropipette.

A glass micropipette with an internal diameter of 1–5 μm is typically used to deform a cell. The micropipette is moved by a micromanipulator to contact a cell. Vacuum is applied through the micropipette to the cell. The aspiration length varies with the applied pressure. An adjustable fluid reservoir is typically used to create fine pressure steps that are measured with a precision pressure sensor. Cell deformation images are recorded through a camera.

Continuum models have been used to treat the cell as either a homogeneous elastic solid [e.g., endothelial cells (20, 21)] or a liquid surrounded by an elastic cortical shell [e.g., neutrophils (22) and erythrocytes (23, 24)]. From experimental and modeling perspectives, cells are classified as solid or liquid cells according to the response to a threshold/critical pressure (5). For liquid-like cells (e.g., neutrophils), pressure above the threshold/critical value causes complete cell aspiration into the micropipette. However, a cell exhibiting behavioral characteristics of a solid [e.g., chondrocyte (25), endothelial cells, and fibroblasts (26)] enters into the pipette by only a finite distance, even when applied pressure exceeds the threshold/critical value. The application of a sufficiently high pressure lyses the cell.

To characterize both solid-like and liquid-like cells, the applied negative pressure ΔP and the resulting aspiration length L must be experimentally measured. Additionally, characterizing liquid-like cells also requires the radius of the cell contour outside the micropipette (R_c) to be measured. Note that the cell contour data can also be used to extract other parameters required as input by different cell mechanics models, for example, unit vectors normal to the cell contour (27).

Aspiration pressures are typically on the order of 1 pN $\mu m^{-2} = 1$ Pa for soft cells and 1 nN $\mu m^{-2} = 1$ kPa for stiff cells. Correspondingly, forces required to deform soft cells are on the order of 10–100 pN and several nanonewtons for stiff cells. Key experimental factors that determine the validity of mechanical characterization results include the accuracy of applied pressure, the accuracy of cellular geometrical parameter measurements, and the synchronization of applied pressure and resulting geometrical changes of the cell (particularly important for viscoelastic characterization) (28).

To assess experimental data and extract material parameters, the elastic model often used is (29, 30)

$$\Delta P = 2K\frac{\Delta A}{A_0}\left(\frac{1}{R_p} - \frac{1}{R_c}\right), \tag{1}$$

where ΔP is the applied sucking pressure, K is the area elastic modulus (or cortical tension) with a unit of pN μm^{-1}, A_0 is the original surface area of the entire membrane, and $\Delta A \approx 2\pi R_p L(1 - \frac{R_p}{R_c})$ is the outer surface area change in terms of the aspiration length L, the radius of pipette R_p, and the radius of cell contour R_c.

The analysis for an infinite, homogeneous half-space drawn into a micropipette gives (31)

$$\Delta P = \frac{2\pi}{3}E\frac{L}{R_p}\phi, \tag{2}$$

where E is the Young's modulus for the cell, and φ is a constant with a typical value of 2.1. Reported Young's modulus values are 0.66 kPa for chondrocytes (25), 0.96 kPa for fibroblasts (26), 1.14 kPa for endothelial cells (20), and 0.047 kPa for neutrophils (32). Note that the micropipette technique does not take into account local stiffness variations. It is believed that a distribution of the elastic modulus values exists across the cell.

Micropipette aspiration also permits the characterization of viscoelastic properties of liquid-like cells by measuring the rate at which a cell flows into a micropipette in response to a stepwise

sucking pressure. Cytoplasm viscosity is estimated by (22, 33)

$$\eta = \frac{R_p \Delta P}{\left(\frac{dL}{dt}\right) m \left(1 - \frac{R_p}{R_c}\right)}, \quad (3)$$

where m is a constant with a typical value of 6. Based on a linear viscoelastic model consisting of two springs and one damper, the viscoelastic parameters were quantified for porcine aortic endothelial cells (19, 34) and porcine aortic valve interstitial cells (28).

The micropipette aspiration technique can also be used for time-lapse studies to understand molecular functions. For instance, this technique has been used in combination with live-cell imaging to explore roles of contractile proteins in response to shape perturbations during cell division (35). Responding to aspiration, the cell late in cytokinesis recruited green fluorescent protein (GFP)-tagged myosin II to both the pipette and the furrow, providing evidence for a mechanosensory system that directs contractile proteins to regulate cell shape during mitosis (**Figure 4*a***).

The extension of the technique was shown to employ two micropipettes for testing the strength of specific ligand-receptor bindings. In Reference 36, a microbead coated with a specific antibody was immobilized by a micropipette and was placed in contact with a cell. The second micropipette was used to pull the cell from the coated microbead by increasing the applied pressure difference. Thus, yield strength of the ligand-receptor interaction was determined.

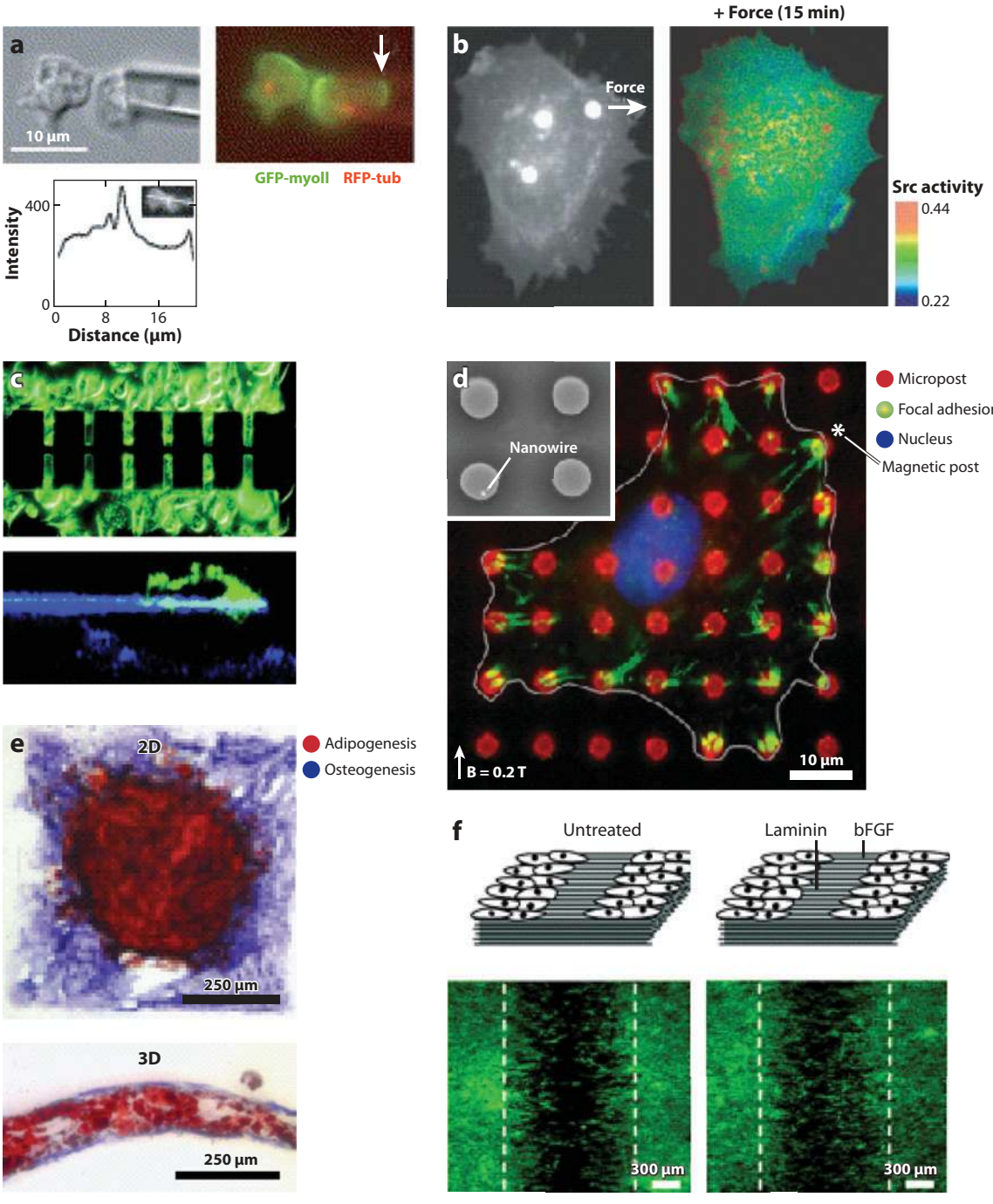
2.2. Laser Trapping

The instrument known as an optical trap or laser tweezer makes use of laser beams to create a potential well for trapping small objects within a defined region. Microparticles can be attached to a cell membrane for applying local stretching or bending forces. The laser power required to constrain the particle is proportional to the forces applied to the particle by the cell. Thus, the cell can be precisely manipulated, and the stiffness of the cell can be measured. The range of forces generated in laser traps is typically 0.1–1 nN.

Optical trapping is a popular technique for the manipulation and mechanical characterization of suspended cells. Various live entities, such as viruses and bacteria (37, 38), red blood cells (39, 40), natural killer cells (41), and outer hair cells (42), have been studied by laser tweezers. Typically, two microbeads are attached to a cell, with the use of adhesive ligands or antibodies to bind to

Figure 4

Applications of microengineered platforms in cell mechanobiology. (*a*) Micropipette aspiration of ameboid cells. A cell aspirated late in cytokinesis accumulated green fluorescent protein (GFP)-myosin II to both the pipette end and the furrow. Reproduced with permission from Reference 35. (*b*) Laser-tweezer traction on the bead at the upper right corner of the cell (*left*) caused FRET responses. White arrow represents force direction. Reproduced with permission from Reference 62. (*c*) Microcantilever array. Target cells in suspension were captured and immobilized on microcantilevers (*top panel*). The cells were then cultured, and the mass of a cell on a microcantilever was quantified via microcantilever resonance frequency shifts (*bottom panel*). Reproduced with permission from Reference 96. (*d*) Microfabricated arrays of magnetic and nonmagnetic posts for applying external forces to cells and measuring cell traction force response. Bending of an array of posts resolves forces generated at multiple locations across the cell body after magnetic force actuation. Reproduced with permission from Reference 107. (*e*) Micropatterned stem cell differentiation within multicellular structures. hMSC aggregates in a square shape (*top panel*) and a three-dimensional hMSC multicellular structure (*bottom panel*) were stained for oil droplets and alkaline phosphatase after 14 days. Reproduced with permission from Reference 140. (*f*) Wound-healing model based on nanofibrous polymers fabricated by electrospinning. Nanofibers were oriented perpendicular to the long edges of the wound. Chemically modified nanofibers with laminin and bFGF (*right*) enhanced cell migration compared with untreated group (*left*). Dotted white lines represent initial wound edges at 0 h. Reproduced with permission from Reference 156.



specific receptors, in diametric opposition to each other. The microbeads serve as handles or grips for displacing the cell membrane. One of the beads can be fixed to the surface of a glass slide, and the relative movement of the other bead generates steady or time-varying stretching forces to the cell. Because ligands or antibodies are coated on the surfaces of microbeads, stress applications can be highly selective and localized. Specific subcellular structures, such as lateral movements of membrane glycoproteins (43), neuronal growth cones (44), adhesiveness of chondrocytes (45), intracellular elasticity of neutrophils (46), and intracellular organelle transport in giant amoebas (47), have also been investigated using laser tweezers.

Although laser tweezers have proven effective in cell mechanobiology, long exposure of cells or using a high-powered laser could induce unwanted harmful effects to cells. For instance, morphology changes and decrease in deformability of cells after laser exposure have been reported (48, 49). These unwanted effects have been suggested to result from thermal and photochemical reactions (50, 51). Because there is a wavelength dependence of the absorption of laser, near-infrared radiation is commonly used for minimizing the degree of photodamage (37). However, high photon flux density can still cause cell damage via two-photon or multiphoton absorption mechanisms (52). Thus, care must be taken to minimize light-induced cell damage and to properly interpret experimental results.

There are several variations of laser tweezers. Using a weakly focused laser beam as an optical channel, guidance and deposition of living cells can be achieved with a high spatial resolution (53). If two nonfocused laser beams are directed opposite each other, a cell placed in between would experience surface forces stretching along the axis defined by the beams, and the net force on the cell would be zero. The stretching force depends on the size and type of the cell, the reflective index, and the laser power. A device based on this principle, termed an optical stretcher, has been used to measure the viscoelastic properties for several cell types (49). In a study reported in Reference 54, a whole cell was stretched by dual optical tweezers. By coupling the optical stretcher system with a microfluidic flow chamber, thousands of individual nonadherent cells were sequentially stretched and characterized, and cytoskeletal rigidity values were correlated with cell types. In optical stretchers, unfocused light beams are used, minimizing potential light-induced damage to the cells, and bead attachments are not required.

Multiple laser traps can also be generated simultaneously by physically splitting the original laser beam or by time-sharing the laser beam with a mechano-optical or acousto-optical mechanism to deflect the laser beam (55). This technique allows various modes of stress (e.g., tensile, biaxial, and bending) to be applied on the cell. Arrays of vertical cavity surface–emitting lasers have also been applied for optical trapping and active manipulation of multiple cells and microbeads simultaneously (56).

Innovation continues to refine and expand the capabilities of laser tweezers (57). For instance, optoelectronic tweezers utilizing direct optical images to create light-addressable electrokinetic forces were demonstrated for massively parallel manipulation of cells (58). Based on localized surface plasmon resonance excited by polarized light, researchers have demonstrated a way to manipulate and rotate biological cells (59). In addition, mechanical properties within live cells can be measured by laser-tracking microrheology (LTM) (60, 61). In LTM, a probe particle (e.g., a granule) is tracked by monitoring the forward-scattered light of a focused, low-power laser beam with a high spatiotemporal resolution. The Brownian motion of the particle reveals the mechanical properties of the subcellular domain or other complex viscoelastic materials to allow measurements of local changes in cell viscoelasticity. Laser-tweezer traction on fibronectin-coated beads tethered to a cell was used to generate a local mechanical stimulation to human umbilical vein endothelial cells (HUVECs) (62). Combined with fluorescent resonance energy transfer (FRET) imaging techniques, the mechano-activated signaling molecules, such as Src,

were visualized and quantified with high temporal and spatial resolutions (**Figure 4*b***). These developments are significantly broadening the capabilities for light-based cell manipulation and will continue to impact cell mechanobiology and mechanotransduction studies.

2.3. Magnetic Probes

Similar to microbeads in laser tweezers, magnetic microbeads can serve as the handle for a magnetic trap or tweezers (63). In the presence of a spatially varying magnetic field, the force F_{mag} experienced by a magnetic particle with a magnetic moment m is $F_{mag} = \nabla(m \cdot B)$. Assuming the induced moment is parallel to the magnetic field, and the field is large enough such that the magnetization of the particle saturates, the force acting on the magnetic particle can be approximated as

$$F_{mag} = MV \frac{dB}{dx}, \tag{4}$$

where V and M are the volume and magnetization of the particle. Thus, the magnetic force strongly depends on the material properties and the size of the particle. The magnetic force also depends on the spatial magnetic field gradient, which can be generated by various configurations.

Early applications of the magnetic bead technique mostly focused on endocytosed particles (64–66). Significant advance was made when ligand-coated (e.g., RGD) magnetic beads were used for specificity (67). The two working modes include magnetic gradient (68) and magnetic twisting cytometry (MTC) (69).

Magnetic fields are usually generated by movable permanent magnets (70) or by electromagnets (71). Electromagnets are more easily controlled and permit the generation of time-varying force fields. A strong electric field gradient can be obtained by using a single-pole electromagnet with a sharp tip, which generates large magnetic field gradients near the tip region. In such a configuration, the force applied to each magnetic bead is a function of the distance between the particle and the tip of the electromagnet. To obtain a homogeneous force field over a wide area, a pair of electromagnets can be used to produce a constant magnetic field gradient. In terms of the useful range of measurements, magnetic tweezers with pole pairs typically generate lower forces in the range of 0.1–10 pN than laser traps do (0.1–1 nN). Forces up to 10^4 pN on a 4.5 μm particle have also been reported in the region (10–100 μm) near the tip of a single-pole electromagnet (68). When there is a need to control multiple directions and rotations of particles at the same time, multiple pairs of electromagnetic poles are required.

As in laser trapping, probing specific cellular components can be achieved by using ligand-coated magnetic beads (67, 68, 71). In particular, MTC has been successfully applied in mechanotransduction studies to induce local stress on specific cellular receptors (69, 72, 73). Combined with FRET techniques, the MTC device can be further utilized to capture and quantify rapid mechanochemical signaling activities in living cells (74). The use of MTC has several advantages. First, magnetic tweezers can conveniently generate both linear forces and twisting torques on a particle. Note that torques can also be induced by electro arrays (75) and laser light (76). Second, magnetic manipulation avoids potential light-induced damage as in laser trapping. Another advantage of MTC is the wide frequency range (0.001–1000 Hz) that very few techniques (e.g., optical traps) have. Finally, magnetic tweezers also allow massive parallel simultaneous measurements of many biological events (77). Three-dimensional (3D)-MTC has been reported where torques can be applied in any direction for anisotropy quantification (78). Mannix et al. (79) recently demonstrated that non-mechanotransductive signaling can be activated by applying mechanical forces to receptor-bound magnetic nanoparticles to make the receptors cluster and crosslink.

3. MICROELECTROMECHANICAL SYSTEMS (MEMS) TOOLS

Besides the more traditional microengineered techniques discussed in previous sections, MEMS techniques have an increasingly strong impact on cell mechanobiology because the micrometer-scale sizes of most mammalian cells match the feature sizes of MEMS-based tools. The size matching permits accurate manipulation of cells and quantitative measurements of cellular responses with high spatial and temporal resolutions. Many of the cell characterization and sensing techniques are also capable of accurately applying minute forces to single cells, permitting the stimulation/probing of cells and the biological responses to be observed. A variety of cellular sensing mechanisms within a cell exist whereby forces can be transduced from mechanical to biochemical signals (i.e., mechanotransduction). We are only in the beginning of the journey to decipher some of these pathways, and MEMS-based mechanobiology approaches are significantly enhancing our capabilities in gaining new biological insights.

3.1. Microcantilever-Based Force Sensors

The use of atomic force microscopy (AFM) for probing living cells started in the early 1990s (80). In AFM, a vertical microcantilever is operated to deform a cell. Based on the deflection of the cantilever, the local stiffness of the cell is measured, and a map of cell stiffness across the cell surface can be generated. The measurements can provide valuable insights into the cytoskeletal structure and the effects of environmental parameters upon it. AFM is powerful for cellular and molecular mechanics studies. For example, spatiotemporal dynamics of the interaction between individual ligands and receptors either on isolated molecules or on cellular surfaces (81), such as VEGFR2 (i.e., a cell-surface receptor for a vascular endothelial growth factor) (82) and integrin $\alpha 2\beta 1$ (83), have been characterized. The use of AFM for cellular and molecular characterization has been reviewed in detail in References 84, 85.

The current major techniques, such as micropipette aspiration, optical trapping, and MTC, discussed in the previous sections, as well as AFM, normally induce small cell deformation ($\sim$1-2 μm) and measure their corresponding cell force response (relatively low) in the range of 1 pN–10 nN. However, cell deformations can be large (comparable to the undeformed size of a cell), inducing large force response in many physiological conditions [e.g., in muscle contraction and expansion or axonal injury (>50% strain)]. With recent advances in micro- and nanofabrication techniques, new types of microcantilevers or microcantilever-based MEMS devices have been developed to probe cell mechanical responses, such as cell stretch force response, cell indentation force response, and in situ observation of the cytoskeletal components during probing, under large deformations in the range of 1 nN to 1 μN, allowing wide applications in studying cell mechanobiology. Galbraith et al. (86) measured traction forces generated by fibroblasts using a microfabricated device capable of determining subcellular forces generated by individual adhesive contacts. The device can continuously monitor forces exerted on adhesive contacts. A cell can move over one or more of the 5904 pads, and each pad rests on a pedestal at the free end of one of the cantilevers of various lengths that are buried beneath the surface. Yang & Saif (87) developed a microfabricated force sensor allowing measurements of the responses of adherent fibroblasts to stretching forces. Force responses of single cells measured before and after Cytochalasin D (a cell permeable and potent inhibitor of actin polymerization) treatment suggest that actin filaments account for almost all of the cell internal forces due to stretch.

The same group (88) explored the role of mechanical tension in neurotransmission, using MEMS force sensors. They found that the density of vesicles that carry neurotransmitters dramatically increased at the neuromuscular synapse when mechanical tension was applied

to *Drosophila* embryo axon. Because neurons have irregular shapes and fragile membranes (10–1000 Pa), sensitive and quantitatively controlled devices are needed to measure the membrane properties and to reveal the cellular responses to mechanical stimulations. Gopal et al. (89) demonstrated the integration of nanoscale photonic gating with MEMS cantilever to probe neurons during growth to investigate neuronal cell mechanics. A series of MEMS force sensors converting microcantilever deflections into capacitance changes were developed for quantifying mechanical changes of the zona pellucida of mouse oocytes before and after fertilization (90) as well as for characterizing both elastic and viscoelastic properties of drug-delivery microcapsules (91, 92). Additionally, Polydimethylsiloxane (PDMS) microcantilevers have been developed to measure contractile forces of cardiomyocytes in real time (93, 94). Due to the low Young's modulus of PDMS, large deformations induced by the contractile forces of cardiomyocytes were optically measured.

A method for measuring force responses of a bovine endothelial cell attached to a substrate was enabled by a functionalized MEMS force sensor that applied local deformation to these cells (95). The sensor was a single-crystal silicon microcantilever beam coated with a thin layer of fibronectin. It was brought in contact with a cell to form adhesion and then moved by a piezoactuator to deform the cell locally. The force was transmitted from the cell adhesion sites on the substrate to the adhesion site of the cantilever through the cytoskeleton. The interaction force between the cell and the cantilever was optically measured from the deformation of the cantilever and its calibrated spring constant.

An array of functionalized silicon cantilevers overhanging microfluidic channels was devised to characterize mass of single live cells in fluids without detaching them from the surface (**Figure 4*c***) (96). Combined with microfluidic cell culture, this approach permits probing time-course force response of adherent live cells in its physiological condition in a noninvasive manner as well as provides optical observations of the same cell.

3.2. Micropost Arrays

Mechanical stress exerted at cell-substrate and cell-cell interfacial boundaries is involved in the regulation of a variety of physiological processes. Microfabricated silicone elastomeric post arrays have been used to measure forces exerted by single adhesion sites of a cell since at least 2001 (e.g., Reference 97). In this method, forces are calculated via visually measured micropost deflections, which have a well-defined relationship with local forces, given the Young's modulus of the polymer used for the post array fabrication. Unlike continuous cell adhesion substrates made of compliant materials to study traction forces generated by cells (99), isolated microposts permit researchers to engineer compliances relevant to cells without altering surface chemistry. Deflection of independent microposts quantitatively reports the location, direction, and magnitude of the cell-generated force (98). The disadvantage of this technique is that the substrate has a nontrivial topology that might affect cell adhesion and bias the measurements. For instance, it was found that for a height above 1 μm, focal adhesions were strongly affected by the pattern (N. Balaban, unpublished data and personal communication). The pattern was used for systematic tracking deformations to the substrate, similarly to what has been done previously with beads in (99).

In Reference 95, micropost array–based force measurements showed that a constant stress was applied by the cell (human foreskin fibroblast) at its various focal adhesions. Micropost arrays can also present a strategy to independently manipulate mechanical compliance and surface chemistry of underlying substrates to control the spatial presentation of these properties across a surface with a high spatial resolution and to measure traction forces generated by cells at multiple locations. These arrays can be designed to encourage cells to attach and spread across multiple posts and

to bend the posts as the cells probe the surface (98), revealing a coordination of biochemical and mechanical signals to regulate cell adhesion and mechanics.

Besides focal adhesions to extracellular matrix, the ability of cells to transduce mechanical signals is also governed by adherens junctions, which link adjacent cells through cadherins. Through the use of a dense array of vertical elatomer pillars, the tops of which were coated with N-cadherin–Fc chimaera and, for comparison, with fibronectin, forces exerted by cell-cell adhesion complexes were measured and mapped (100). The results show that cells transduce mechanical stress through cadherin contacts, producing forces similar in amplitude to those exerted at cell-matrix adhesions.

Image-processing techniques for improving the accuracy and speed of micropost force measurements were developed (101, 102). Direct and distinct relationships between cellular traction force and cell spreading area were demonstrated for fibroblasts, endothelial cells, epithelial cells, and smooth muscle cells (101). Additionally, PDMS micropost arrays were also used for measuring traction forces exerted by Madin-Darby canine kidney (MDCK) epithelial cells during migration (103) and for measuring contraction forces of myocytes (104, 105). To further scale down micropost arrays to alter stiffness and adhesive surface area, microfabrication techniques, such as high-resolution lithography and deep reactive ion etching, can be employed to reduce micropost spacing, to reduce the micropost diameter to the submicrometer level, and to increase the aspect of ratio of microposts (106).

In order to separately study the cellular response to external forces applied to a cell and the internal forces generated by the cell, magnetic microposts containing cobalt nanowires were recently described (**Figure 4*d***) (107). Resolving the bending motion of the magnetic micropost array measures forces generated at multiple locations across the cell body after magnetic force actuation. A step force applied by the magnetic micropost array led to an increase in local focal adhesion size at the site of application but not at adhesion sites to nearby nonmagnetic posts.

3.3. Microelectrode Arrays

The elastic and viscoelastic properties of cells can also be studied by cell deformation induced by external electric fields (108, 109). When a cell is subjected to an electric field, a dipole can be induced owing to interfacial polarization on the cell membrane. Stresses at the interfaces result in a deforming force, depending on the electric field strength and the effective polarization of the cells, a phenomenon called electrodeformation (110). Under small deformation, the elastic strain of the cell along the electric field direction is estimated as (111, 112)

$$\frac{\Delta L}{L_0} = K_S E^2 \,\mathrm{Re}(U(\omega)), \tag{5}$$

where ΔL is the deformation of the cell, L_0 is the original length of the cell, K_S is a constant representing the elastic properties of the cell, ω is the angular frequency of the AC electric field, and $U(\omega)$ is the complex Clausius-Mossotti factor that depends on the internal structures of the cell and is cell-type specific (110, 113).

To perform electrodeformation, an AC voltage is first applied to capture the suspended cells to the electrode edges through dielectrophoresis (DEP) (114, 115). After the cells are trapped, various voltages and frequencies can be applied to characterize the mechanical and electrical properties of the cells. Under small voltages, reversible (elastic) deformations of cells are usually observed. When a large electric field is applied, irreversible (plastic) deformation and cell membrane rupturing occur. To measure the relaxation of a deformed cell, the electric field can be suddenly removed.

Then cell relaxation is recorded and analyzed. Electrodeformation has been applied in mechanical characterization of several cell types (116, 117), with red blood cells the most extensively studied (108, 111, 112).

Traditionally, electrodeformation studies have been done using large metal electrodes and wires. With advancements in MEMS technology, microscale electrodes and arrays with precise dimensions can now be fabricated, which are more suitable for studying single-cell deformability (118). Biological objects in a large range of sizes can be directly manipulated with the electrodeformation technique by varying operation parameters (114). Because microfabrication techniques allow device multiplexing, simultaneous analysis of cells under different experimental conditions can be easily performed with microelectrode arrays. In addition to deformability and viscoelastic characterization, microelectrodes have also been applied in a variety of biomechanical studies at the cellular and molecular level.

4. MICROFABRICATION APPROACHES TO SIMULATE BIOMECHANICAL STIMULI IN THE CELL MICROENVIRONMENT

Advanced microfabrication techniques and surface chemistry provide highly useful tools for controlling the architecture and adhesiveness of a cell microenvironment and facilitating the study of cell-substrate, cell-cell, and cell-medium interactions (119, 120). Microscale technologies hold great promise for the creation of more in vivo–like biomechanical stimuli to live cells in in vitro settings, allowing us to exercise a high degree of control of the biomechanical environment on the micro and nano levels.

4.1. Extracellular Matrix Micropatterning

Cells are inherently sensitive to the local micro- and nanoscale and molecular adhesive patterns. Micropatterning techniques have been used to control cell-substrate and cell-cell interactions by patterning specific ECM proteins and producing functional model tissues in a 2D or a 3D context. Current techniques for patterning ECM proteins on a substrate include microcontact printing (120, 121), microfluidic patterning (122, 123), plasma etching-based patterning (124, 125), and stencil-based patterning (126). Micropatterned ECM islands can constrain cells to a certain shape by culturing cells in the corresponding, confined areas. Single cells can spread over multiple islands or can be confined to a single island, even in unnatural geometric shapes, such as squares and triangles (127, 128). Using this technique, it has been determined that cell death or proliferation can be controlled through constraints on the area of cell spreading, indicating that the resulting mechanical perturbations play an important role in determining cell fate (128). The shape of the islands can also determine planar cell polarity and directional motility in the absence of other polarity or spatial cues [e.g., teardrop-shaped islands (129, 130)]. The capability of patterning ECM islands on the size scale of individual focal adhesions ($\sim$1 μm) has enabled the investigation of specific roles of focal adhesion positioning in directional control of cell spreading and movement through Rac activation (131). The spatial distribution of subcellular components, such as actin, microtubules, centrisome, and Golgi apparatus, has been determined by establishing the orientation of cell polarity in respone to the micropatterned ECM geometry (132).

Inspired by the recent findings of distinctly different gene expression in 3D cell culture versus that in 2D culture systems (133, 134), efforts have been made in presenting extracellular cues to living cells in a 3D context to probe cell response in 3D patterned matrices. For instance, 3D micropatterning techniques were used to control the initial 3D structure of mammary epithelial tubules in culture for branching morphogenesis (135), to modulate cell-cell interactions

in 3D clusters regulating bovine articular chondrocyte biosynthesis (136), and to quantitatively investigate tumor cell migration in 3D matrices (137).

Cell adhesion substrates with complex features can be combined with microfluidic devices. Using a network of microchannels and laminar flows, for example, substrate-bound gradients of ECM proteins can be fabricated with complex shapes over a few hundred micrometers for studies on axonogenesis (122) and haptotaxis (i.e., directional cell movement toward regions of higher adhesion) (138, 139). The use of fluorescently labeled molecular probes with geometrically defined live cells could also open new vistas for probing cytoskeletal dynamics and could serve as potential platforms for high-throughput, high-content cell-based assays (127).

Micropatterning techniques are also enabling the investigation of the correlation between tissue geometry in 2D and 3D cultures and the functions of individual cells in a multicellular complex. For example, mesenchymal stem cells in patterned 2D and 3D cultures formed various geometries of tissue, including a rectangle, square, annulus or ring, ellipse, and sinusoidal bands (140). Using these patterned stem cell cultures, it was found that cells in the high-stress regions, for instance, at the corner of a square or at the outside of 3D blocks of cells, form osteocytes and that those in the low-stress regions form adipocytes (**Figure 4*e***).

4.2. Micro- and Nanotopographic Substrates

Living tissues are intricate ensembles of different cell types embedded in complex and well-defined ECM structures with micro- and nanoscale topographical features. For instance, cells at a wound site can come into contact with clusters of severed ECM fibrils that would be perceived by the cells in a manner similar to their perception of 2D surfaces covered with arrays of grooves, ridges, pores, wells, and pillars with variable features. Cancer cells have the ability to rapidly move along tracks of collagenous matrix fibers in vivo (141). It is therefore reasonable to expect that the functioning of many cell types can be significantly affected in vivo by topographical stimulation from the surrounding ECM. In the pursuit of understanding the interactions between cells and their underlying ECM topography (e.g., aligned fibrillar matrices, shape, texture), micro- and nanotopographic substrates are being incorporated to probe cellular processes, which in turn may enable researchers to ultimately modulate cell function with topography-controlled biomaterials for advanced tissue engineering (142).

Recent advancement of micro- and nanofabrication techniques has led to novel in vitro cell-culture models that mimic the in vivo cellular microenvironment with mechanical and structural similarity. Besides conventional photolithographic techniques, various methods have been employed for the fabrication of micro- and nanotopographic substrates, such as colloidal lithography (143), polymer demixing (144), electrospinning (145, 146), nanoimprinting (147), and capillary force lithography (148, 149). Previous studies with smooth muscle cells (147), keratocytes (150), epithelial cells (151), and neuronal cells (152) cultured on arrays of parallel groove or ridge patterns demonstrated that polarization of cell morphology and movement is regulated by topographic guidance (or contact guidance). The aligned fibers or grooves may promote the actin polymerization and protrusion in the parallel direction, which can result in aligned focal adhesions and traction force in the same direction (19). The mechanical signal induced by contact guidance may also cooperate with soluble factor-mediated signaling pathways to guide cellular function.

Topographic control of cell-substrate interactions has great potential in tissue engineering and cell-based regenerative therapies (142). For example, it was recently demonstrated that topographically treated human mesenchymal stem cells (hMSC) can produce bone mineral in vitro, despite the absence of osteogenic supplements (153). Nanofibrous scaffolds of biodegradable

poly(L-lactide) (PLLA) fabricated by electrospinning were employed to regulate cell and cytoskeleton alignment, myotube assembly, myotube striation, and myoblast proliferation (154). As a means to mimic the size and arrangement of the ECM fibers displayed in the heart tissue, well-defined, scalable (~3.5 cm^2), topographically nanopatterned arrays of polyethylene glycol (PEG) hydrogels were used to produce engineered cardiac tissue constructs with tunable structural and functional properties (D.-H. Kim and A. Levchenko, unpublished data). These results reveal that the naturally occurring nanoscale ECM can be a powerful guidance cue regulating not only cell alignment into anisotropic arrays but also the fine details of the tissue structure and function, a finding that has implications for mechanical control of cell and tissue development and cell-based regenerative therapies.

Current efforts in the area of biomimetic topographic definition are centered on rather simplistic patterns. Cell adhesion substrates are commonly patterned with micro- and nano-ridges of specified pitch, width, and height, whereas supporting ECM structures in living tissues and the scaffolds that might be used for tissue engineering and engraftment are generally inhomogeneous, with complex structures that may vary on the scale of a single cell. Because ECM forms a meshwork of interlinked fibers or bundles of fibrils of variable local density and complexity of organization in many tissues in vivo, microtextured substrates with variable local density and anisotropy were recently designed and fabricated to guide the organization and migration of cells in spatially desirable patterns (155). Additionally, in a study described in Reference 156, chemical modification of PLLA nanofibers with matrix-bound growth factors (e.g., bFGF) enhanced cell migration into a wound (**Figure 4*f***), suggesting synergistic effects of nanotopography and chemical signaling on cell guidance.

Despite considerable ongoing research, the mechanisms of transduction of extracellular topographic signals into eventual biochemical and molecular responses of cells remain unclear. Better elucidation of the underlying mechanisms through the micro- and nanoscale control of cell interaction with ECM topography could open up novel strategies to manipulate cell locomotion, matrix assembly, and cell interactions in tissue engineering applications.

4.3. Microfluidic Shear Devices

Fluid shear stress, which occurs naturally in a variety of physiological conditions, is one of the most important mechanostimuli. Numerous cellular functions are known to be regulated by shear stress, such as nitric oxide–dependent cell death of human neuroblastoma (157) and apoptosis of adherent neutrophils (158). Furthermore, shear stress strengthens bacterial attachment to their target cells (159). Bone cells, particularly osteocytes, are sensitive to shear stress (160, 161), which is believed to be related to bone mechanical adaptation. Fluid shear stress is also known to play an important role in the development and differentiation of various types of stem cells, including embryonic stem cells and endothelial progenitor cells into vascular endothelial cells (162–164).

Shear stress is particularly important in the cardiovascular systems. The fluid environment near arterial bends and branches is distinct from the laminar pulsatile environment present near the long, straight section of the vessel wall. Geometries that mimic the in vivo vessel architectures have been designed for investigating flow dynamics of cardiovascular systems. In general, studies suggest that atherogenesis is associated with both temporal and spatial shear stress patterns, which modulate gene expression in endothelial cells, growth of smooth muscle cells, as well as uptake of lipoproteins (165–169). For instance, the physicochemical environment during the recruitment and migration of leukocytes and monocytes can be simulated in well-controlled fluidic systems, which allow in-depth interrogation of cell-cell interaction events. The effects of fluid shear stress on vascular cells have been reviewed extensively (170–172).

Conventionally, there are two major apparatus designs for generating hydrodynamic shear stress: (*a*) cone-and-plate rotating chambers and (*b*) parallel-plate flow channels. With recent advances in microfabrication technologies, microfluidic flow chambers with various geometries, surface chemistries, and topographies can be fabricated. In microscale devices, the typical Reynolds number is much less than the critical value characterizing transition into the turbulence regime because of the small transverse length scale, which results in a high velocity gradient and thus high viscous force (173, 174). This guarantees laminar flows for a large range of shear stresses that are required in cell studies.

Microfluidic systems have been adopted to improve the resolution, throughput, and reliability of mechanobiology experiments. An important advantage of microchannels over conventional flow chambers is the ability to generate a wide range of shear stresses for the investigation of the cellular responses, such as adhesion and cell alignment (175–178). As an example, a microfluidic flow system can generate linear shear stress gradients with careful design of the channel geometries (179). The parallel processing nature of microfluidic systems also facilitates the interrogation of multiple physicochemical parameters concurrently (175, 180, 181). Both steady and time-varying flows, especially flow patterns that mimic in vivo situations, have been studied extensively using flow channels (182–184). Integrated with MEMS shear stress sensors, microfluidic devices are capable of resolving spatial variations in shear stress in a 3D blood vessel bifurcation model for small-scale hemodynamics (185, 186).

4.4. Micromechanical Stretching Devices

Cells are known to alter their physiology in response to mechanical strain generated by increased tension in the substrate to which the cells are attached. Several tools have been designed for mechanically stretching cells to create a variety of strain environments for cell culture. These tools are capable of inducing uniaxial stretching, substrate bending, or in-plane substrate distention (187). Uniaxial tension of deformable substrates (static loading or oscillatory loading) led to its widespread use. Static loading (188) and oscillatory loading systems (189) were shown to cause marked changes in protein and DNA synthesis by aortic smooth muscle cells. Studies that utilized uniaxial tension systems showed that stretching of cells induced various biological responses, including cell morphology (190), cell reorientation (191), actin cytoskeletal remodeling (192), altered cell proliferation (193), gene expression, and protein synthesis (189, 194).

Substrate bending provides an alternative means for creating uniaxial strain (195, 196). In addition to uniaxial strain, substrate flexure can stimulate cell cultures by uniform and cyclic biaxial strain of the cell-culture surface (197). Compared with uniaxial stretching, biaxial stretching has thus far been less studied, although biaxial loading conditions are common in cells within certain tissues (e.g., the pericardium).

There are also cell stretching systems operating on the basis of out-of-plane distention. Using static loading (198), vacuum (199), or pressure (200), a flexible substrate is deformed. The commercially available Flexcell system is of the out-of-plane distention type (199). These traditional stretching devices have been used to study the mechanical response of large numbers of cells and tissues and have been modified to include micropatterned membranes to generate desired strain profiles (201).

Employing precision actuators and controllers, researchers continue to develop tools to more realistically mimic tissue/cell deformation. Smith et al. (202) investigated the ability of the integrated central nervous system to grow over considerable distances in response to continuous mechanical tension. Using a motor system, they progressively separated two membranes and found that the large bundles of axons readily adapted to mechanical elongation and that these

bundles gradually consolidated into larger tracts. A uniaxial stretching device using voice coil actuators (203) demonstrated that prespecified displacement profiles can be realized precisely, and the stretching deformation generated by the device was uniaxial, uniform, and highly reproducible. In addition, Geddes-Klein et al. (204) reported two types of cell stretchers (uniaxial and biaxial) that can cause differences in the response to injury mechanisms and cell fate.

Advances in microfabrication techniques permit the application of mechanical stress to single cells or a small population of cells with enhanced control of forces and displacements. For example, a clamp-ratchet microstructure was designed to exert mechanical tension along radial glial processes between groups of neural stem cells to study the effect of tension on cerebral cortex neurogenesis (205). The stretching concept was similar to the typical uniaxial stretching method, but the MEMS device could better control tensile forces to selected groups of neurons within a developing cortex. One can combine microtopography and mechanical stretching. Using micropatterned elastomers, Wang et al. (206) demonstrated that cyclic stretching of HPTFs increased α-smooth muscle actin (α-SMA) protein expression and affected neuronal development, especially under physiological strain conditions. Gopalan et al. (207) found that transverse stretch produces more significant effects than longitudinal stretch in regulating sarcomere organization, hypertrophy, and cell-to-cell junctions. Selby & Shannon (208) demonstrated the use of a microfabricated circular diaphragm in stretch experiments not only to strain epithelial sheets in culture but also as a means for measuring the mechanical response of the epithelial sheet to the imposed strain.

Further development is needed in spite of preliminary progress in developing microfabricated cell-straining devices (e.g., References 208 and 209). Since microfabrication technologies permit enhanced control of forces and displacements as well as the construction of large arrays of devices in a batch mode, it is envisioned that microdevice arrays will be demonstrated in the near future for mechanically straining single cells or small cell populations in a high-throughput manner.

4.5. Substrate Stiffness

Living cells are exquisitely sensitive to cell adhesion substrate mechanics, requiring appropriate substrate stiffness to function properly. Adherent cells sense the local elasticity of their matrix by pulling on the substrate via cytoskeleton-based contraction. These contractile forces, generated by the cross-bridging interactions of actin and myosin filaments, are tuned by the cell to balance the resistance provided by the substrate (210).

Cellular responses to substrate stiffness have been investigated since the 1990s (211). Despite several open questions that require further investigations (e.g., molecular mechanisms involved in cellular responses to matrix stiffness, which are not yet clearly understood, and of the nature mechanochemical pathways, which is not yet completely elucidated), many interesting experimental findings have been made. Several cell types, such as epithelial cells, fibroblasts, endothelial cells, and smooth muscle cells, have been reported to sense and to respond distinctly to soft versus stiff substrates (210). It was also found that the behavior of some cells on a soft anchoring substrate can be used to identify important changes in more general phenotypic characteristics (e.g., the growth of cells on soft agar gels can be used to identify cancer cells) (212).

In spite of the many tools available to manipulate the biochemical adhesiveness of substrates, relatively few approaches have been developed to engineer substrate mechanics to investigate the effects of the mechanical forces of cell adhesion on the regulation of cell signaling and function (98). Experimentally, the elastic modulus of a substrate can be varied by orders of magnitude (e.g., 1 kPa–100 kPa), often via controlling the extent of polymer cross-linking in gels, such as polyacrylamide (PA) gels with a thin coating of covalently attached collagen or fibronectin

(211). These ECM-cross-linked PA gels have been successfully used to reveal new findings for appreciating the role of the ECM rigidity in differentiation (13) and tumorigenesis (213).

Leveraging microfabrication techniques, substrates exhibiting anisotropic stiffness were made to induce directional cell growth and guide cell migration along the direction of greatest stiffness (214). Substrates with well-controlled stiffness gradients of PA gels were also used for studying durotaxis (i.e., the directed movement of cell motility or outgrowth either up or down a rigidity gradient) (215). Cells exerted less tension on softer, collagen-coated gels, causing an accumulation of cells at the stiffer substrate area.

It is now known that different cell types can best function at different matrix rigidities (216). For instance, myotubes differentiate optimally on gels with stiffness comparable to that of normal muscle (217). However, cells on soft gels are generally less contractile than on stiff gels, and their adhesion strengths are not as strong. It appears as though a cell attempts to match its stiffness with that of the underlying substrate by altering the organization of its cytoskeleton and possibly through strain stiffening (210). Alteration of matrix mechanics activates integrins, which not only promotes mitogenic signaling through the mitogen-activated kinases (e.g., Erk) but also cell contractility through small G-proteins (e.g., Rho), can further increase matrix stiffness, suggesting a positive feedback loop (213). In short, varying the substrate stiffness can clearly produce profound effects on many cellular processes (e.g., adhesion, growth, movement, differentiation, and death) (13, 218) as well as the progression in tumorigensis (212, 213) and will continue to be an important piece in the tool set for cell mechanobiology.

5. TOWARD DYNAMICAL AND INTEGRATIVE BIOMECHANICAL MICROENVIRONMENT: EMERGING OPPORTUNITIES AND CHALLENGES

The biomechanical microenvironment of cells is indeed complex and dynamically changing. In most of the studies performed, however, cells were exposed to spatially homogenous, constant, predefined mechanical stimuli over time. Most of these studies also have not involved the integrated presentation of multivariant mechanical stimuli. Although it is known that biochemical and biomechanical cues are both important, the creation of varying external mechanical stimuli and the precise mechanical manipulation of single cells are still challenging tasks. Besides the many interesting results that were enabled by microengineered techniques, microengineering-based approaches promise even greater potential in advancing our understanding of cell mechanobiology by providing novel tools to explore cell responses to complex micromechanical environments.

First, microengineering-based approaches are providing novel capabilities for mechanically probing cells in response to spatially and temporally variable stimuli. Electroactive cell substrates were designed to turn on adhesion and migration of mammalian cells on demand, thus enabling temporal regulation (219). The technique to fabricate reconfigurable protein matrices that can span the 100–3000 nm scale has been recently introduced (220). Such reconfigurable ECM protein patterns are desired because the sizes of adhesion plaques fall within this spatial range, and the dynamics of these plaques are known to vary according to the state of the cell and its surrounding matrix. In situ multiphoton-based fabrication that allows definition of the microscopic topographies of neuronal environments can provide a means to guide the contact position of neuronal cells (221). The method is rapid and minimally invasive to cells, creating physically and chemically interactive microstructures within cell cultures, thus opening the possibility of creating well-defined sets of synaptic interactions in a temporally and spatially controlled manner.

Second, microengineering-based approaches are leading to novel in vitro culture systems in more biomimetic, multi-input contexts. Many of the experimental techniques discussed in this

review can be used in combination, enabling integrative cell stimulation by multiple mechanical guidance cues in a single experiment. For example, elastomeric membranes with parallel microgrooves were used to simulate the vascular cell alignment and investigate the anisotropic mechanical sensing by mesenchymal stem cells (222). The topographic micropattern together with cyclic uniaxial strain produced changes in global gene expression in mesenchymal stem cells and increased their proliferation, effects not observed when cells were perpendicularly aligned with respect to the axis of mechanical strain. A microfluidics-based assay system integrated with microengineered substrates will further enable the analysis of concerted cell responses to composite gradients of precisely generated and aligned surface-bound ECM molecules and diffusible guidance cues (223) or topographic guidance cues (D.-H. Kim and A. Levchenko, unpublished data), which better mimic the complex microenvironment in vivo, under physiological conditions.

Further development of microengineered platforms will enable researchers to collect large quantities of data for hypothesis generation, model prediction, and experimental validation by performing a limited number of high-throughput experiments. The measurements performed so far were commonly static, taking snapshots of bio-mechanically stimulated cells with inferences about the mechanisms regulating mechanobiology at the cellular and the molecular levels. Combining live-cell time-lapse imaging with fluorescence proteins or application of advanced technologies such as FRET (62, 73, 224) will further enhance our capabilities of quantifying the integrative response of cells to combinatorial biochemical and biomechanical cues by visualizing mechanotransduction events with high spatiotemporal resolutions in live cells.

6. CONCLUDING REMARKS

Cells live in a complex microenvironment and are inherently sensitive to a myriad of biomechanical cues as well as continuous stimulation by a temporally variable cocktail of soluble factors. Recent evidence suggests that mechanical forces have a far greater impact on cell functions than previously deemed. With this new dimension to cellular biology come a number of unique experimental challenges. Microengineered platforms enable single-cell stimulation and direct observation and quantitative analysis, capabilities that are critically important to help gain a more complete assessment of how cells function. Microengineering appears well poised to revolutionarily enhance the currently extant experimental capabilities, for example, to enable researchers to sweep through a wide range of parameters and collect large, statistically significant quantities of data, which has not been possible with traditional techniques.

Microfabricated platforms are ideally suited to tackle the challenges at the cellular and subcellular scales due to length-scale matching. In particular, in stem-cell mechanobiology, which has direct implications in tissue engineering and cell-based regenerative therapies, engineering approaches to control the stem cell microenvironment are employed to alter gene expression and to regulate stem cell differentiation. Toward this end, novel tools such as arrays of microdevices that allow analyzing responses to a wide spectrum of combinatorial stimuli (biochemical and mechanical) in a high-throughput manner for regulating stem cell responses and cell fate will appear in the near future.

In summary, the development of novel experimental techniques and microengineered platforms is crucial to gain new insights into pathways of mechanotransduction; to elucidate the relationship of mechanotransduction, cell mechanical property changes, and human disease states; and to design new modes of therapeutic intervention. These developments are still at an early stage of application. The next few years will witness even more intense development of innovative microengineered platforms for cell mechanobiology studies that will enable intriguing new findings in this relatively new area and will answer a multitude of questions that currently remain elusive.

The next generation of microengineerd experimental platforms will provide capabilities for creating complex, combinatorial cell microenvironments through the use of multivariant mechanical guidance cues in a single experiment in which single cells can experience a dynamically changing set of mechanical and biochemical conditions.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank Nathalie Balaban, Shu Chien, Gaudenz Danuser, Adam Hsieh, Sanjay Kumar, Scot Kuo, Benoit Ladoux, Song Li, Saurabh Paliwel, Beth Pruitt, Douglas Robinson, Taher Saif, David Serrell, Alexander Spector, Subra Suresh, Leslie Tung, Ning Wang, and Yingxiao Wang for critical reading and valuable comments. This work was supported by a grant from the American Heart Association 0815104E to D.-H.K., NIH 1U01AI082457-01 and a grant from the Prevent Cancer Foundation to P.W., NIH R21-EB008562 to A.L., and a Canada Research Chair and a discovery grant from the Natural Sciences Ontario Ministry of Research and Innovation to Y.S. We apologize to the many authors whose work is not cited owing to space limitations.

LITERATURE CITED

1. Wolff J. 1892. *Das Gasetz der Transformation der Knochen*. Berlin: A. Hirschwald
2. Wang JH, Thampatty BP. 2006. An introductory review of cell mechanobiology. *Biomech. Model. Mechanobiol.* 5:1–16
3. Rosenbluth MJ, Lam WA, Fletcher DA. 2006. Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. *Biophys. J.* 90:2994–3003
4. Sollich P. 1998. Rheological constitutive equation for a model of soft glassy materials. *Phys. Rev. E* 58:738–59
5. Hochmuth RM. 2000. Micropipette aspiration of living cells. *J. Biomech.* 33:15–22
6. Ingber DE. 1997. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* 59:575–99
7. Suresh S. 2007. Biomechanics and biophysics of cancer cells. *Acta Biomater.* 3:413–38
8. Makale M. 2007. Cellular mechanobiology and cancer metastasis. *Birth Defects Res. C Embryo. Today* 81:329–43
9. Park Y, Diez-Silva M, Popescu G, Lykotrafitis G, Choi W, et al. 2008. Refractive index maps and membrane dynamics of human red blood cells parasitized by *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA* 105:13730–35
10. Shelby J, White J, Ganesan K, Rathod P, Chiu D. 2003. A microfluidic model for single-cell capillary obstruction by *Plasmodium falciparum* infected erythrocytes. *Proc. Natl. Acad. Sci. USA* 100:14618–22
11. Lee GY, Lim CT. 2007. Biomechanics approaches to studying human diseases. *Trends Biotechnol.* 25:111–18
12. Cross SE, Jin YS, Rao J, Gimzewski JK. 2007. Nanomechanical analysis of cells from cancer patients. *Nat. Nanotechnol.* 2:780–83
13. Engler AJ, Sen S, Sweeney HL, Discher DE. 2006. Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–89
14. Bao G, Suresh S. 2003. Cell and molecular mechanics of biological materials. *Nat. Mater.* 2:715–25
15. McBeath R, Pirone D, Nelson CM, Bhadriraju K, Chen CS. 2004. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6:483–95

16. Ingber DE. 2003. Mechanobiology and diseases of mechanotransduction. *Ann. Med.* 35:564–77
17. Huang H, Kamm RD, Lee RT. 2004. Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am. J. Physiol. Cell Physiol.* 287:C1–11
18. Pedersen JA, Swartz MA. 2005. Mechanobiology in the third dimension. *Ann. Biomed. Eng.* 33:1469–90
19. Li S, Guan JL, Chien S. 2005. Biochemistry and biomechanics of cell motility. *Annu. Rev. Biomed. Eng.* 7:105–50
20. Sato M, Theret DP, Wheeler LT, Ohshima N, Nerem RM. 1990. Application of the micropipette technique to the measurement of cultured porcine aortic endothelial cell viscoelastic properties. *J. Biomech. Eng.* 112:263–68
21. Sato M, Levesque MJ, Nerem RM. 1987. Micropipette aspiration of cultured bovine aortic endothelial cells exposed to shear stress. *Arteriosclerosis* 7:276–86
22. Needham D, Hochmuth RM. 1990. Rapid flow of passive neutrophils into a 4 μm pipet and measurement of cytoplasmic viscosity. *J. Biomech. Eng.* 112:269–76
23. Discher DE, Boal DH, Boey SK. 1998. Simulations of the erythrocyte cytoskeleton at large deformation. II. Micropipette aspiration. *Biophys. J.* 75:1584–97
24. Waugh R, Evans E. 1979. Thermoelasticity of red blood cell membrane. *Biophys. J.* 26:115–32
25. Jones WR, Ting-Beall HP, Lee GM, Kelley SS, Hochmuth RM, Guilak F. 1999. Alterations in the young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage. *J. Biomech.* 32:119–27
26. Thoumine O, Ott A. 1997. Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation. *J. Cell Sci.* 110:2109–16
27. Herant M, Marganski WA, Dembo M. 2003. The mechanics of neutrophils: synthetic modeling of three experiments. *Biophys. J.* 84:3389–413
28. Liu XY, Wang YF, Sun Y. 2009. Cell contour tracking and data synchronization for real-time, high accuracy micropipette aspiration. *IEEE Trans. Autom. Sci. Eng.* In press
29. Evans E, Skalak R. 1980. *Mechanics and Thermodynamics of Biomembranes*. Boca Raton, FL: CRC
30. Needham D, Nunn RS. 1990. Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* 58:997–1009
31. Theret DP, Levesque MJ, Sato M, Nerem RM, Wheeler LT. 1988. The application of a homogeneous half-space model in the analysis of endothelial cell micropipette measurements. *J. Biomech. Eng.* 110:190–99
32. Chien S, Sung KP. 1984. Effect of colchicine on viscoelastic properties of neutrophils. *Biophys. J.* 46:383–86
33. Evans E, Yeung A. 1989. Apparent viscosity and cortical tension of blood granulocytes determined by micropipette aspiration. *Biophys. J.* 56:151–60
34. Sato M, Ohshima N, Nerem RM. 1996. Viscoelastic properties of cultured porcine aortic endothelial cells exposed to shear stress. *J. Biomech.* 29:461–67
35. Effler JC, Kee YS, Berk JM, Tran MN, Iglesias PA, Robinson DN. 2006. Mitosis-specific mechanosensing and contractile-protein redistribution control cell shape. *Curr. Biol.* 16:1962–67
36. Shao JY, Hochmuth RM. 1996. Micropipette suction for measuring piconewton forces of adhesion and tether formation from neutrophil membranes. *Biophys. J.* 71:2892–901
37. Ashkin A, Dziedzic J, Yamane T. 1987. Optical trapping and manipulation of single cells using infrared laser beams. *Nature* 330:769–71
38. Ashkin A, Dziedzic J. 1987. Optical trapping and manipulation of viruses and bacteria. *Science* 235:1517–20
39. Dao M, Lim C, Suresh S. 2003. Mechanics of the human red blood cell deformed by optical tweezers. *J. Mech. Phys. Solids* 51:2259–80
40. Henon S, Lenormand G, Richert A, Gallet F. 1999. A new determination of the shear modulus of the human erythrocyte membrane using optical tweezers. *Biophys. J.* 76:1145–51
41. Seeger S, Monajembashi S, Hutter K, Futterman G, Wolfrum J, Greulich K. 1991. Application of laser optical tweezers in immunology and molecular-genetics. *Cytometry* 12:497–504
42. Murdock D, Ermilov S, Spector A, Popel A, Brownell W, Anvari B. 2005. Effects of chlorpromazine on mechanical properties of the outer hair cell plasma membrane. *Biophys. J.* 89:4090–95

43. Edidin M, Kuo S, Sheetz M. 1991. Lateral movements of membrane-glycoproteins restricted by dynamic cytoplasmic barriers. *Science* 254:1379–82
44. Dai J, Sheetz M. 1995. Mechanical-properties of neuronal growth cone membranes studies by tether formation with laser optical tweezers. *Biophys. J.* 68:988–96
45. Huang W, Anvari B, Torres JH, LeBaron RG, Athanasiou KA. 2003. Temporal effects of cell adhesion on mechanical characteristics of the single chondrocyte. *J. Orthop. Res.* 21:88–95
46. Yanai M, Butler J, Suzuki T, Kanda A, Kurachi M, et al. 1999. Intracellular elasticity and viscosity in the body, leading, and trailing regions of locomoting neutrophils. *Am. J. Physiol. Cell Physiol.* 277:C432–40
47. Ashkin A, Schutze K, Dziedzic J, Euteneur U, Schliwa M. 1990. Force generation of organelle transport measured invivo by an infrared-laser trap. *Nature* 348:346–48
48. Bronkhorst P, Streekstra G, Grimbergen J, Nijhof E, Sixma J, Brakenhoff G. 1995. A new method to study shape recovery of red blood cells using multiple optical trapping. *Biophys. J.* 69:1666–73
49. Kaneta T, Makihara J, Imasaka T. 2001. An "Optical channel": A technique for the evaluation of biological cell elasticity. *Anal. Chem.* 73:5791–95
50. Peterman E, Gittes F, Schmidt C. 2003. Laser-induced heating in optical traps. *Biophys. J.* 84:1308–16
51. Xie C, Goodman C, Dinno M, Li Y. 2004. Real-time raman spectroscopy of optically trapped living cells and organelles. *Opt. Express.* 12:6208–14
52. Schneckenburger H, Hendinger A, Sailer R, Gschwend M, Strauss W, et al. 2000. Cell viability in optical tweezers: high power red laser diode versus nd : YAG laser. *J. Biomed. Opt.* 5:40–44
53. Nahmias Y, Schwartz R, Verfaillie C, Odde D. 2005. Laser-guided direct writing for three-dimensional tissue engineering. *Biotech. Bioeng.* 92:129–36
54. Guck J, Ananthakrishnan R, Mahmood H, Moon T, Cunningham C, Kas J. 2001. The optical stretcher: a novel laser tool to micromanipulate cells. *Biophys. J.* 81:767–84
55. Guilford W, Tournas J, Dascalu D, Watson D. 2004. Creating multiple time-shared laser traps with simultaneous displacement detection using digital signal processing hardware. *Anal. Biochem.* 326:153–66
56. Flynn R, Birkbeck A, Gross M, Ozkan M, Shao B, et al. 2002. Parallel transport of biological cells using individually addressable VCSEL arrays as optical tweezers. *Sens. Actuators. B Chem.* 87:239–43
57. Moffitt JR, Chemla YR, Smith SB, Bustamante C. 2008. Recent advances in optical tweezers. *Annu. Rev. Biochem.* 77:205–28
58. Chiou P, Ohta A,Wu M. 2005. Massively parallel manipulation of single cells and microparticles using optical images. *Nature* 436:370–72
59. Miao XY, Lin LY. 2007. Trapping and manipulation of biological particles through a plasmonic platform. *IEEE J. Sel. Top. Quantum Electron.* 13:1655–62
60. Kuo SC. 2001. Using optics to measure biological forces and mechanics. *Traffic* 2:757–63
61. Yamada S,Wirtz D, Kuo SC. 2000. Mechanics of living cells measured by laser tracking microrheology. *Biophys. J.* 78:1736–47
62. Wang Y, Botvinick EL, Zhao Y, Berns MW, Usami S, et al. 2005. Visualizing the mechanical activation of Src. *Nature* 434:1040–45
63. Gosse C, Croquette V. 2002. Magnetic tweezers: micromanipulation and force measurement at the molecular level. *Biophys. J.* 82:3314–29
64. Gehr P, Brain JD, Bloom SB, Valberg PA. 1983. Magnetic particles in the liver: a probe for intracellular movement. *Nature* 302:336–38
65. Valberg PA. 1984. Magnetometry of ingested particles in pulmonary macrophages. *Science* 224:513–16
66. Crick F, Hughes A. 1950. The physical properties of cytoplasm—a study by means of the magnetic particle method. 1. Experimental. *Exp. Cell Res.* 1:37–80
67. Wang N, Butler J, Ingber D. 1993. Mechanotransduction across the cell-surface and through the cytoskeleton. *Science* 260:1124–27
68. Bausch A, Ziemann F, Boulbitch A, Jacobson K, Sackmann E. 1998. Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophys. J.* 75:2038–49
69. Wang N, Ingber DE. 1995. Probing transmembrane mechanical coupling and cytomechanics using magnetic twisting cytometry. *Biochem. Cell Biol.* 73:327–35

70. Smith SB, Finzi L, Bustamante C. 1992. Direct mechanical measurements of the elasticity of single DNA molecules by using magnetic beads. *Science* 258:1122–26
71. Ziemann F, Radler J, Sackmann E. 1994. Local measurements of viscoelastic moduli of entangled actin networks using an oscillating magnetic bead microrheometer. *Biophys. J.* 66:2210–16
72. Chen J, Fabry B, Schiffrin E, Wang N. 2001. Twisting integrin receptors increases endothelin-1 gene expression in endothelial cells. *Am. J. Physiol. Cell Physiol.* 280:C1475–84
73. Goldschmidt M, McLeod K, Taylor W. 2001. Integrin-mediated mechanotransduction in vascular smooth muscle cells—frequency and force response characteristics. *Circulation Res.* 88:674–80
74. Na S, Collin O, Chowdhury F, Tay B, Ouyang M, et al. 2008. Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proc. Natl. Acad. Sci. USA* 105:6626–31
75. Gimsa J. 2001. A comprehensive approach to electro-orientation, electrodeformation, dielectrophoresis, and electrorotation of ellipsoidal particles and biological cells. *Bioelectrochemistry* 54:23–31
76. Miao X, Wilson BK, Pun SH, Lin LY. 2008. Optical manipulation of micro/submicron sized particles and biomolecules through plasmonics. *Opt. Express.* 16:13517–25
77. Danilowicz C, Greenfield D, Prentiss M. 2005. Dissociation of ligand-receptor complexes using magnetic tweezers. *Anal. Chem.* 77:3023–28
78. Hu S, Eberhard L, Chen J, Love JC, Butler JP, et al. 2004. Mechanical anisotropy of adherent cells probed by a three-dimensional magnetic twisting device. *Am. J. Physiol. Cell Physiol.* 287:C1184–91
79. Mannix RJ, Kumar S, Cassiola F, Montoya-Zavala M, Feinstein E, et al. 2008. Nanomagnetic actuation of receptor-mediated signal transduction. *Nat. Nanotechnol.* 3:36–40
80. Radmacher M, Tillamnn RW, Fritz M, Gaub HE. 1992. From molecules to cells: imaging soft samples with the atomic force microscope. *Science* 257:1900–5
81. Hinterdorfer P, Dufrene YF. 2006. Detection and localization of single molecular recognition events using atomic force microscopy. *Nat. Methods* 3:347–55
82. Lee S, Mandic J, Van Vliet KJ. 2007. Chemomechanical mapping of ligand-receptor binding kinetics on cells. *Proc. Natl. Acad. Sci. USA* 104:9609–14
83. Taubenberger A, Cisneros DA, Friedrichs J, Puech PH, Muller DJ, Franz CM. 2007. Revealing early steps of $\alpha_2\beta_1$ integrin-mediated adhesion to collagen type I by using single-cell force spectroscopy. *Mol. Biol. Cell* 18:1634–44
84. Radmacher M. 2002. Measuring the elastic properties of living cells by the atomic force microscope. *Methods Cell Biol.* 68:67–90
85. Kedrov A, Janovjak H, Sapra KT, Muller DJ. 2007. Deciphering molecular interactions of native membrane proteins by single-molecule force spectroscopy. *Annu. Rev. Biophys. Biomol. Struct.* 36:233–60
86. Galbraith CG, Sheetz MP. 1997. A micromachined device provides a new bend on fibroblast traction forces. *Proc. Natl. Acad. Sci. USA* 94:9114–18
87. Yang S, Saif T. 2005. Reversible and repeatable linear local cell force response under large stretches. *Exp. Cell Res.* 305:42–50
88. Yang S, Siechen S, Sung J, Chiba A, Saif T. 2008. *MEMS based sensors to explore the role of tension in axons for neuro-transmission*. Presented at 21st IEEE Int. Conf. MEMS, Tucson
89. Gopal A, Luo Z, Lee JY, Kumar K, Li B, et al. 2008. Nano-opto-mechanical characterization of neuron membrane mechanics under cellular growth and differentiation. *Biomed. Microdevices* 10:611–22
90. Sun Y, Wan K, Roberts K, Bischof J, Nelson B. 2003. Mechanical property characterization of mouse zona pellucida. *IEEE Trans. Nanobiosci.* 2:279–86
91. Kim KY, Cheng J, Liu Q,Wu XY, Sun Y. 2009. Investigation of mechanical properties of soft hydrogel microcapsules in relation to protein delivery using a MEMS force sensor. *J. Biomed. Mater. Res. A* In press
92. Kim KY, Liu XY, Zhang Y, Cheng J, Wu XY, Sun Y. 2009. Elastic and viscoelastic characterization of microcapsules for drug delivery using a force-feedback MEMS microgripper. *Biomed. Microdev.* 11:421–27
93. Park J, Kim J, Roh D, Park S, Kim B, Chun K. 2006. Fabrication of complex 3D polymer structures for cell-polymer hybrid systems. *J. Micromech. Microeng.* 16:1614–19
94. Park J, Ryu J, Choi SK, Seo E, Cha JM, et al. 2005. Real-time measurement of the contractile forces of self-organized cardiomyocytes on hybrid biopolymer microcantilevers. *Anal. Chem.* 77:6571–80

95. Saif MTA, Sager CR, Coyer S. 2003. Functionalized biomicroelectromechanical systems sensors for force response study at local adhesion sites of single living cells on substrates. *Ann. Biomed. Eng.* 31:950–61
96. Park K, Jang J, Irimia D, Sturgis J, Lee J, et al. 2008. 'Living cantilever array' for characterization of mass of single live cells in fluids. *Lab Chip* 8:1034–41
97. Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, et al. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3:466–72
98. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl. Acad. Sci. USA* 100:1484–89
99. Dembo M, Wang YL. 1999. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys. J.* 76:2307–16
100. Ganz A, Lambert M, Saez A, Silberzan P, Buguin A, et al. 2006. Traction forces exerted through N-cadherin contacts. *Biol. Cell* 98:721–30
101. Lemmon CA, Sniadecki NJ, Ruiz SA, Tan JL, Romer LH, Chen CS. 2005. Shear force at the cell-matrix interface: enhanced analysis for microfabricated post array detectors. *Mech. Chem. Biosyst.* 2:1–16
102. Li B, Xie L, Starr Z, Yang Z, Lin J, Wang J. 2007. Development of micropost force sensor array with culture experiments for determination of cell traction forces. *Cell Motil. Cytoskelet.* 64:509–18
103. du Roure O, Saez A, Buguin A, Austin R, Chavrier P, et al. 2005. Force mapping in epithelial cell migration. *Proc. Natl. Acad. Sci. USA* 102:2390–95
104. Kajzar A, Cesa CM, Kirchgessner N, Hoffmann B, Merkel R. 2008. Toward physiological conditions for cell analyses: forces of heart muscle cells suspended between elastic micropillars. *Biophys. J.* 94:1854–66
105. Zhao Y, Lim CC, Sawyer DB, Liao R, Zhang X. 2007. Simultaneous orientation and cellular force measurements in adult cardiac myocytes using three-dimensional polymeric microstructures. *Cell Motil. Cytoskeleton* 64:718–25
106. Yang MT, Sniadecki NJ, Chen CS. 2007. Geometric considerations of micro- to nanoscale elastomeric post arrays to study cellular traction forces. *Adv. Mater.* 19:3119–23
107. Sniadecki NJ, Anguelouch A, Yang MT, Lamb CM, Liu Z, et al. 2007. Magnetic microposts as an approach to apply forces to living cells. *Proc. Natl. Acad. Sci. USA* 104:14553–58
108. Engelhardt H, Gaub H, Sackmann E. 1984. Viscoelastic properties of erythrocyte-membranes in high-frequency electric fields. *Nature* 307:378–80
109. Zimmermann U, Friedrich U, Mussauer H, Gessner P, Hamel K, Sukhoruhov V. 2000. Electromanipulation of mammalian cells: fundamentals and application. *IEEE Trans. Plasma Sci.* 28:72–82
110. Jones TB. 1995. *Electromechanics of Particles*. New York: Cambridge Univ. Press. 287 pp.
111. Sukhorukov V, Mussauer H, Zimmermann U. 1998. The effect of electrical deformation forces on the electropermeabilization of erythrocyte membranes in low- and high-conductivity media. *J. Membr. Biol.* 163:235–45
112. Engelhardt H, Sackmann E. 1988. On the measurement of shear elastic moduli and viscosities of erythrocyte plasma membranes by transient deformation in high frequency electric fields. *Biophys. J.* 54:495–508
113. Pethig R, Markx G. 1997. Applications of dielectrophoresis in biotechnology. *Trends Biotech.* 15:426–32
114. Wong P, Wang T, Deval J, Ho C. 2004. Electrokinetics in micro devices for biotechnology applications. *IEEE/ASME Trans. Mechatron.* 9:366–76
115. Pohl HA. 1978. *Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields*. Cambridge, New York: Cambridge Univ. Press
116. Korlach J, Reichle C, Muller T, Schnelle T, Webb W. 2005. Trapping, deformation, and rotation of giant unilamellar vesicles in octode dielectrophoretic field cages. *Biophys. J.* 89:554–62
117. Wong P, Tan W, Ho C. 2005. Cell relaxation after electrodeformation: effect of latrunculin A on cytoskeletal actin. *J. Biomech.* 38:529–35
118. Voldman J. 2006. Electrical forces for microscale cell manipulation. *Annu. Rev. Biomed. Eng.* 8:425–54
119. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. 2006. Microscale technologies for tissue engineering and biology. *Proc. Natl. Acad. Sci. USA* 103:2480–87
120. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. 2001. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.* 3:335–73
121. Ruiz SA, Chen CS. 2007. Microcontact printing: a tool to pattern. *Soft Matter* 3:168–77

122. Dertinger SK, Jiang X, Li Z, Murthy VN,Whitesides GM. 2002. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl. Acad. Sci. USA* 99:12542–47
123. Vahidi B, Park JW, Kim HJ, Jeon NL. 2008. Microfluidic-based strip assay for testing the effects of various surface-bound inhibitors in spinal cord injury. *J. Neurosci. Methods* 170:188–96
124. Keyes J, Junkin M, Cappello J, Wu X, Wong PK. 2008. Evaporation-induced assembly of biomimetic polypeptides. *Appl. Phys. Lett.* 93:023120
125. Rhee SW, Taylor AM, Tu CH, Cribbs DH, Cotman CW, Jeon NL. 2005. Patterned cell culture inside microfluidic devices. *Lab. Chip* 5:102–7
126. Folch A, Jo BH, Hurtado O, Beebe DJ, Toner M. 2000. Microfabricated elastomeric stencils for micropatterning cell cultures. *J. Biomed. Mater Res.* 52:346–53
127. Kandere-Grzybowska K, Campbell C, Komarova Y, Grzybowski BA, Borisy GG. 2005. Molecular dynamics imaging in micropatterned living cells. *Nat. Methods* 2:739–41
128. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. 1997. Geometric control of cell life and death. *Science* 276:1425–28
129. Jiang X, Bruzewicz DA,Wong AP, Piel M, Whitesides GM. 2005. Directing cell migration with asymmetric micropatterns. *Proc. Natl. Acad. Sci. USA* 102:975–78
130. Kumar G, Ho CC, Co CC. 2007. Guiding cell migration using one-way micropattern arrays. *Adv. Mater.* 19:1084–90
131. Xia N, Thodeti CK, Hunt TP, Xu Q, Ho M, et al. 2008. Directional control of cell motility through focal adhesion positioning and spatial control of Rac activation. *FASEB J.* 22:1649–59
132. Thery M, Racine V, Piel M, Pepin A, Dimitrov A, et al. 2006. Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. USA* 103:19771–76
133. Grinnell F. 2003. Fibroblast biology in three-dimensional collagen matrices. *Trends Cell Biol.* 13:264–69
134. Cukierman E, Pankov R, Stevens DR, Yamada KM. 2001. Taking cell-matrix adhesions to the third dimension. *Science* 294:1708–12
135. Nelson CM, Vanduijn MM, Inman JL, Fletcher DA, Bissell MJ. 2006. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. *Science* 314:298–300
136. Albrecht DR, Underhill GH,Wassermann TB, Sah RL, Bhatia SN. 2006. Probing the role of multicellular organization in three-dimensional microenvironments. *Nat. Methods* 3:369–75
137. Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, et al. 2006. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. USA* 103:10889–94
138. Rhoads DS, Guan JL. 2007. Analysis of directional cell migration on defined FN gradients: role of intracellular signaling molecules. *Exp. Cell Res.* 313:3859–67
139. Gunawan RC, Silvestre J, Gaskins HR, Kenis PJA, Leckband DE. 2006. Cell migration and polarity on microfabricated gradients of extracellular matrix proteins. *Langmuir* 22:4250–58
140. Ruiz SA, Chen CS. 2008. Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells* 11:2921–27
141. Condeelis J, Segall JE. 2003. Intravital imaging of cell movement in tumours. *Nat. Rev. Cancer* 3:921–30
142. Kim DH, Levchenko A, Suh KY. 2008. Engineered surface nanotopography for controlling cell-substrate interactions. In *Micro- and Nanoengineering of the Cell Microenvironment: Technologies and Applications*, ed. A Khademhosseini, J Borenstein, S Takayama, M Toner, pp. 185–208. Boston: Artech House
143. Wood MA. 2007. Colloidal lithography and current fabrication techniques producing in-plane nanotopography for biological applications. *J. R. Soc. Interface* 4:1–17
144. Dalby MJ, Riehle MO, Johnstone HJ, Affrossman S, Curtis AS. 2002. Polymer-demixed nanotopography: control of fibroblast spreading and proliferation. *Tissue Eng.* 8:1099–108
145. Liang D, Hsiao BS, Chu B. 2007. Functional electrospun nanofibrous scaffolds for biomedical applications. *Adv. Drug. Deliv. Rev.* 59:1392–412
146. Sill TJ, von Recum HA. 2008. Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials* 29:1989–2006
147. Yim EK, Reano RM, Pang SW, Yee AF, Chen CS, Leong KW. 2005. Nanopattern-induced changes in morphology and motility of smooth muscle cells. *Biomaterials* 26:5405–13

148. Kim DH, Kim P, Song I, Cha JM, Lee SH, et al. 2006. Guided three-dimensional growth of functional cardiomyocytes on polyethylene glycol nanostructures. *Langmuir* 22:5419–26
149. Kim DH, Park J, Suh KY, Kim P, Choi SK, et al. 2006. Fabrication of patterned micromuscles with high activity for powering biohybrid microdevices. *Sens. Actuat. B Chem.* 117:391–400
150. Teixeira AI, Nealey PF, Murphy CJ. 2004. Responses of human keratocytes to micro- and nanostructured substrates. *J. Biomed. Mater. Res. A* 71:369–76
151. Teixeira AI, Abrams GA, Bertics PJ, Murphy CJ, Nealey PF. 2003. Epithelial contact guidance on well-defined micro- and nanostructured substrates. *J. Cell Sci.* 116:1881–92
152. Rajnicek A, Britland S, McCaig C. 1997. Contact guidance of CNS neurites on grooved quartz: influence of groove dimensions, neuronal age and cell type. *J. Cell Sci.* 110:2905–13
153. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, et al. 2007. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat. Mater.* 6:997–1003
154. Huang NF, Patel S, Thakar RG, Jun W, Hslao BS, et al. 2006. Myotube assembly on nanofibrous and micropatterned polymers. *Nano Lett.* 6:537–42
155. Kim DH, Seo CH, Han K, Kwon KW, Levchenko A, Suh KY. 2009. Guided cell migration on microtextured substrates with variable local density and anisotropy. *Adv. Funct. Mater.* In press
156. Patel S, Kurpinski K, Quigley R, Gao H, Hsiao BS, et al. 2007. Bioactive nanofibers: synergistic effects of nanotopography and chemical signaling on cell guidance. *Nano Lett.* 7:2122–28
157. Triyoso D, Good T. 1999. Pulsatile shear stress leads to DNA fragmentation in human SH-SU5Y neuroblastoma cell line. *J. Physiol.* 515:355–65
158. Shive M, Salloum M, Anderson J. 2000. Shear stress-induced apoptosis of adherent neutrophils: a mechanism for persistence of cardiovascular device infections. *Proc. Natl. Acad. Sci. USA* 97:6710–15
159. Thomas W, Trintchina E, Forero M, Vogel V, Sokurenko E. 2002. Bacterial adhesion to target cells enhanced by shear force. *Cell* 109:913–23
160. Jacobs C, Yellowley C, Davis B, Zhou Z, Cimbala J, Donahue H. 1998. Differential effect of steady versus oscillating flow on bone cells. *J. Biomech.* 31:969–76
161. Klein-Nulend J, Helfrich M, Sterck J, MacPherson H, Joldersma M, et al. 1998. Nitric oxide response to shear stress by human bone cell cultures is endothelial nitric oxide synthase dependent. *Biochem. Biophys. Res. Commun.* 250:108–14
162. Yamamoto K, Sokabe T, Watabe T, Miyazono K, Yamashita JK, et al. 2005. Fluid shear stress induces differentiation of Flk-1-positive embryonic stem cells into vascular endothelial cells in vitro. *Am. J. Physiol. Heart Circ. Physiol.* 288:H1915–24
163. Yamamoto K, Takahashi T, Asahara T, Ohura N, Sokabe T, et al. 2003. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *J. Appl. Physiol.* 95:2081–88
164. Metallo CM, Vodyanik MA, de Pablo JJ, Slukvin II, Palecek SP. 2008. The response of human embryonic stem cell-derived endothelial cells to shear stress. *Biotechnol. Bioeng.* 100:830–37
165. Traub O, Berk BC. 1998. Laminar shear stress—mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscl. Thromb. Vasc. Biol.* 18:677–85
166. Hsiai TK, Cho SK,Wong PK, Ing M, Salazar A, et al. 2003. Monocyte recruitment to endothelial cells in response to oscillatory shear stress. *FASEB J.* 17:1648–57
167. Papadaki M, Eskin SG. 1997. Effects of fluid shear stress on gene regulation of vascular cells. *Biotech. Prog.* 13:209–21
168. Alon R, Ley K. 2008. Cells on the run: shear-regulated integrin activation in leukocyte rolling and arrest on endothelial cells. *Curr. Opin. Cell Biol.* 20:525–32
169. Warabi E, Takabe W, Minami T, Inoue K, Itoh K, et al. 2007. Shear stress stabilizes NF-E2-related factor 2 and induces antioxidant genes in endothelial cells: role of reactive oxygen/nitrogen species. *Free Radic. Biol. Med.* 42:260–69
170. Chien S. 2008. Role of shear stress direction in endothelial mechanotransduction. *Mol. Cell Biomech.* 5:1–8
171. Davies P, Spaan J, Krams R. 2005. Shear stress biology of the endothelium. *Ann. Biomed. Eng.* 33:1714–18
172. Chiu JJ, Usami S, Chien S. 2008. Vascular endothelial responses to altered shear stress: pathologic implications for atherosclerosis. *Ann. Med.* 41:19–28

173. Ho C, Tai Y. 1998. Micro-electro-mechanical-systems (MEMS) and fluid flows. *Annu. Rev. Fluid Mech.* 30:579–612
174. Wong PK, Lee YK, Ho CM. 2003. Deformation of DNA molecules by hydrodynamic focusing. *J. Fluid Mech.* 497:55–65
175. Vartanian KB, Kirkpatrick SJ, Hanson SR, Hinds MT. 2008. Endothelial cell cytoskeletal alignment independent of fluid shear stress on micropatterned surfaces. *Biochem. Biophys. Res. Commun.* 371:787–92
176. Uttayarat P, Chen M, Li M, Allen FD, Composto RJ, Lelkes PI. 2008. Microtopography and flow modulate the direction of endothelial cell migration. *Am. J. Physiol. Heart Circ. Physiol.* 294:H1027–35
177. Gutierrez E, Groisman A. 2007. Quantitative measurements of the strength of adhesion of human neutrophils to a substratum in a microfluidic device. *Anal. Chem.* 79:2249–58
178. Lu H, Koo L, Wang W, Lauffenburger D, Griffith L, Jensen K. 2004. Microfluidic shear devices for quantitative analysis of cell adhesion. *Anal. Chem.* 76:5257–64
179. Tsou JK, Gower RM, Ting HJ, Schaff UY, Insana MF, et al. 2008. Spatial regulation of inflammation by human aortic endothelial cells in a linear gradient of shear stress. *Microcirculation* 15:311–23
180. Young EWK, Wheeler AR, Simmons CA. 2007. Matrix-dependent adhesion of vascular and valvular endothelial cells in microfluidic channels. *Lab Chip* 7:1759–66
181. Goldfinger LE, Tzima E, Stockton R, Kiosses WB, Kinbara K, et al. 2008. Localized α4 integrin phosphorylation directs shear stress-induced endothelial cell alignment. *Circ. Res.* 103:177–185
182. Balcells M, Suarez M, Vazquez M, Edelman E. 2005. Cells in fluidic environments are sensitive to flow frequency. *J. Cell Physiol.* 204:329–35
183. Bao X, Lu C, Frangos J. 1999. Temporal gradient in shear but not steady shear stress induces PDGF-A and MCP-1 expression in endothelial cells. *Arterioscl. Thromb. Vasc. Biol.* 19:996–1003
184. McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, et al. 2001. DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc. Natl. Acad. Sci. USA* 98:8955–60
185. Rouhanizadeh M, Soundararajan G, Lo R, Arcas D, Browand FK, Hsiai TK. 2006. MEMS sensors to resolve spatial variations in shear stress in a 3-D blood vessel bifurcation model. *IEEE Sensors J.* 6:78–88
186. Rouhanizadeh M, Lin TC, Arcas D, Hwang J, Hsiai TH. 2005. Spatial variations in shear stress in a 3-D bifurcation model at low Reynolds numbers. *Ann. Biomed. Eng.* 33:1360–74
187. Brown TD. 2000. Techniques for mechanical stimulation of cells in vitro: a review. *J. Biomech.* 33:3–14
188. Somjen D, Binderman I, Berger E, Harell A. 1980. Bone remodeling induced by physical stress is prostaglandin-E2 mediated. *Biochim. Biophys. Acta* 627:91–100
189. Leung DYM, Glagov S, Mathews MB. 1977. A new in vitro system for studying cell response to mechanical stimulation. Different effects of cyclic stretching and agitation on smooth-muscle cell biosynthesis. *Exp. Cell Res.* 109:285–98
190. Jones DB, Nolte H, Scholubbers JG, Turner E, Veltel D. 1991. Biochemical signal transduction of mechanical strain in osteoblast-like cells. *Biomaterials* 12:101–10
191. Wang HC, Ip W, Boissy R, Grood ES. 1995. Cell orientation response to cyclically deformed substrates: experimental validation of a cell model. *J. Biomech.* 28:1543–52
192. Wang JHC. 2000. Substrate deformation determines actin cytoskeleton reorganization: a mathematical modeling and experimental study. *J. Theor. Biol.* 202:33–41
193. Neidlinger-Wilke C, Grood ES, Wang JHC, Brand RA, Claes L. 2001. Cell alignment is induced by cyclic changes in cell length: studies of cells grown in cyclically stretched substrates. *J. Orthopaed. Res.* 19:286–93
194. Carver W, Nagpal ML, Nachtigal M, Borg TK, Terracio L. 1991. Collagen expression in mechanically stimulated cardiac fibroblasts. *Circ. Res.* 69:116–22
195. Neidlinger-Wilke C, Wilke HJ, Claes L. 1994. Cyclic stretching of human osteoblasts affects proliferation and metabolism: a new experimental method and its application. *J. Orthopaed. Res.* 12:70–78
196. Bottlang M, Simnacher M, Schmitt H, Brand RA, Claes L. 1997. A cell strain system for small homogeneous strain applications. *Biomedizinische Technik(Berl)* 42:305–9
197. Andersen KL, Norton LA. 1991. A device for the application of known simulated orthodontic forces to human cells in vitro. *J. Biomech.* 24:649–54

198. Hasegawa S, Sato S, Saito S, Suzuki Y, Brunette DM. 1985. Mechanical stretching increases the number of cultured bone cells synthesizing DNA and alters their pattern of protein synthesis. *Calcif. Tissue Int.* 37:431–36
199. Banes AJ, Gilbert J, Taylor D, Monbureau O. 1985. A new vacuum-operated stress-providing instrument that applies static or variable duration cyclic tension or compression to cells in vitro. *J. Cell Sci.* 75:35–42
200. Winston FK, Macarak EJ, Gorfien SF, Thibault LE. 1989. A system to reproduce and quantify the biomechanical environment of the cell. *J. Appl. Physiol.* 67:397–405
201. Tan W, Scott D, Belchenko D, Qi HJ, Xiao L. 2008. Development and evaluation of microdevices for studying anisotropic biaxial cyclic stretch on cells. *Biomed. Microdev.* 10:869–82
202. Smith DH, Wolf JA, Meaney DF. 2001. A new strategy to produce sustained growth of central nervous system axons: continuous mechanical tension. *Tissue Eng.* 7:131–39
203. Pfister BJ,Weihs TP, Betenbaugh M, Bao G. 2003. An in vitro uniaxial stretch model for axonal injury. *Ann. Biomed. Eng.* 31:589–98
204. Geddes-Klein D, Schiffman K, Meaney D. 2006. Mechanisms and consequences of neuronal stretch injury in vitro differ with the model of trauma. *J. Neurotraum.* 23:193–204
205. Wu VC, Law T, Hsu C-M, Lin G, Tang WC, Monuki ES. 2005. *MEMS platform for studying neurogenesis under controlled mechanical tension*. Presented at Proc. Annu. 3rd Int. IEEE EMBS Spec. Top. Conf. of Microtechnol. Med. and Biol., Kahuku, Oahu
206. Wang JHC, Yang GG, Li ZZ. 2005. Controlling cell responses to cyclic mechanical stretching. *Ann. Biomed. Eng.* 33:337–42
207. Gopalan SM, Flaim C, Bhatia SN, Hoshijima M, Knoell R, et al. 2003. Anisotropic stretch-induced hypertrophy in neonatal ventricular myocytes micropatterned on deformable elastomers. *Biotechnol. Bioeng.* 81:578–87
208. Selby JC, Shannon MA. 2007. Mechanical response of a living human epidermal keratinocyte sheet as measured in a composite diaphragm inflation experiment. *Biorheology* 44:319–48
209. Scuor N, Gallina P, Panchawagh HV, Mahajan RL, Sbaizero O, Sergo V. 2006. Design of a novel MEMS platform for the biaxial stimulation of living cells. *Biomed. Microdev.* 8:239–46
210. Discher DE, Janmey P, Wang YL. 2005. Tissue cells feel and respond to the stiffness of their substrate. *Science* 1 26:677–89
211. Pelham RJ Jr, Wang Y. 1997. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* 94:13661–65
212. Welch DR, Sakamaki T, Pioquinto R, Leonard TO, Goldberg SF, et al. 2000. Transfection of constitutively active mitogen-activated protein/extracellular signal-regulated kinase kinase confers tumorigenic and metastatic potentials to NIH3T3 cells. *Cancer Res.* 60:1552–56
213. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, et al. 2005. Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–54
214. Saez A, Ghibaudo M, Buguin A, Silberzan P, Ladoux B. 2007. Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. *Proc. Natl. Acad. Sci. USA* 104:8281–86
215. Zaari N, Rajagopalan P, Kim SK, Engler AJ, Wong JY. 2004. Photopolymerization in microfluidic gradient generators: microscale control of substrate compliance to manipulate cell response. *Adv.Mater.* 16:2133–37
216. Jiang G, Huang AH, Cai Y, Tanase M, Sheetz MP. 2006. Rigidity sensing at the leading edge through $\alpha_v\beta_3$ integrins and RPTPα. *Biophys. J.* 90:1804–9
217. Engler AJ, Griffin MA, Sen S, Bonnemann CG, Sweeney HL, Discher DE. 2004. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J. Cell Biol.* 166:877–87
218. Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, et al. 2005. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil. Cytoskelet.* 60:24–34
219. Yousaf MN, Houseman BT, Mrksich M. 2001. Turning on cell migration with electroactive substrates. *Angew Chem. Int. Ed. Engl.* 40:1093–96
220. Zhu X, Mills KL, Peters PR, Bahng JH, Liu EH, et al. 2005. Fabrication of reconfigurable protein matrices by cracking. *Nat. Mater.* 4:403–6

221. Kaehr B, Allen R, Javier DJ, Currie J, Shear JB. 2004. Guiding neuronal development with in situ microfabrication. *Proc. Natl. Acad. Sci. USA* 101:16104–8
222. Kurpinski K, Chu J, Hashi C, Li S. 2006. Anisotropic mechanosensing by mesenchymal stem cells. *Proc. Natl. Acad. Sci. USA* 103:16095–100
223. Joanne Wang C, Li X, Lin B, Shim S, Ming GL, Levchenko A. 2008. A microfluidics-based turning assay reveals complex growth cone responses to integrated gradients of substrate-bound ECM molecules and diffusible guidance cues. *Lab Chip* 8:227–37
224. Wang Y, Shyy JY, Chien S. 2008. Fluorescence proteins, live-cell imaging, and mechanobiology: seeing is believing. *Annu. Rev. Biomed. Eng.* 10:1–38
225. Hunt TK, Burke J, Barbul A, Gimbel ML. 1999. Wound healing. *Science* 284:1775
226. Meshel AS, Wei Q, Adelstein RS, Sheetz MP. 2005. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* 7:157–64

Annual Review of Biomedical Engineering
Volume 11, 2009

Contents