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MEMS sensors and microsystems for cell mechanobiology

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Abstract

Forces generated by cells play a vital role in many cellular processes such as cell spreading, motility, differentiation and apoptosis. Understanding the mechanics of single cells is essential to delineate the link between cellular force generation/sensing and function. MEMS sensors, because of their small size and fine force/displacement resolution, are ideal for force and displacement sensing at the single-cell level. In addition, the amenability of MEMS sensors to batch fabrication methods allows the study of large cell populations simultaneously, leading to robust statistical studies. In this paper, we discuss various microsystems used for studying cell mechanics and the insights on cell mechanical behavior that have resulted from their use. The advantages and limitations of these microsystems for biological studies are also outlined.

1. Introduction

It is now well accepted that mechanical forces play an important role in many cellular processes. *In vitro* cell behavior is highly sensitive to the mechanical properties of the substrates on which the cells are grown. Cell differentiation, locomotion and growth and development are all influenced by the mechanical properties of the microenvironment [1–3]. Similarly, externally applied forces alter many aspects of *in vitro* cell behavior across a variety of cell types [4–6]. The influence of forces on cellular level processes *in vivo* has also been widely recognized. Cells sense and exert forces on their *in vivo* environment, which comprises the extra cellular matrix (ECM) and basement membranes, as well as each other through cell–cell contacts. These forces and the mechanical microenvironment play an important role in widely disparate phenomena such as fibroblast migration during wound healing [7], regulation of synaptic plasticity of neurons [8] and regulation of tumor cell response [9].

Understanding how cells generate and sense forces and how the forces are transduced into biochemical signals is vital to address fundamental questions about cell behavior in both normal and pathological states [10, 11]. Accurate measurement of forces and displacements exerted by cells both *in vivo* and *in vitro* is an essential step in this endeavor [12]. Micromechanical sensors are especially suited for these studies because of their small size, which allows for easy interfacing with individual cells, and fine force/displacement resolution that makes them capable of measuring very small forces/displacements [13–15]. In addition, micromechanical platforms can be batch fabricated cheaply using either integrated circuit (IC) or soft lithography techniques. This allows hundreds of devices to be deployed in a single platform to monitor the response of large cell populations, leading to robust statistical studies.

Studies on mechanobiology range from the tissue level all the way down to individual proteins and DNA, involving a wide range of approaches and instrumentation size scales. The commonly used tools for probing cells and biomolecules, such as AFM, optical and magnetic tweezers etc, have already been the subject of many excellent reviews [10, 12]. Therefore, in this paper we restrict ourselves to a survey of micromechanical systems developed for cell mechanics research and the biological insights that have resulted from these studies. Distinct from other reviews on microengineered systems [13, 16], we highlight two recent developments in this area: (1) micromechanical devices for *in vivo* and small animal studies and (2) microsystems to manipulate the physiological behavior of cellular organisms through controlled application of forces.

The paper is organized as follows. First, we briefly outline the major classes of micromechanical systems used for cell mechanics research. We then describe results obtained using microsystems that cover various aspects of cell mechanics

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such as cytoskeletal properties and cell traction and contractile forces. Finally, we discuss some of the recent advances in *in vivo* measurements as well as mechanical manipulation of cellular organisms. Because of limited space and the emphasis on the mechanics of biological systems, we have not covered microfluidics-based approaches, which are more suited for combined biochemical and mechanical stimulation, in this paper.

2. Micromechanical systems for cell mechanics

Micromechanical systems used for cell mechanics studies broadly fall into two major categories. The first category comprises hard silicon-based devices that are fabricated using standard IC manufacturing techniques, whereas the second category comprises systems made of soft polymers and gels.

2.1. Silicon-based microsystems

Silicon-based devices, in general, comprise two parts—mechanical parts that move or deform when forces are applied on them, and electrical circuits that transduce this motion/deformation into currents/voltages. The mechanical parts are often of the same scale as an individual cell and undergo deformation/displacement of the order of a few micrometers in response to forces applied by cells. Typical silicon-based microsystems can have hundreds of individual devices, sometimes with integrated electronics for recording forces and displacement. These platforms are often used for large statistical studies, where the behavior of many cells needs to be monitored simultaneously [16]. However, individual silicon-based force sensors are also used to apply controlled forces/deformations on cells to precisely measure their mechanical properties such as stiffness [17]. More recently, silicon force sensors have been used for *in vivo* studies that require measurement of very small forces [18].

While silicon-based microplatforms are well suited for assaying large cell populations, they suffer from two main drawbacks. The first is the lack of optical transparency which makes it difficult to image sub-cellular structures when cells exert or respond to forces. Hence, it is difficult to uncover the molecular basis of cellular force generation/sensing. Secondly, the mechanical properties of silicon (Young's modulus, for example) are substantially different from the mechanical microenvironment encountered by cells *in vivo*. Therefore, it is challenging to infer how cells behave *in vivo* based on *in silico* studies.

2.2. Polymer-/gel-based microsystems

Polymer-/gel-based microsystems are fabricated by soft lithography [19]. A master pattern is created in silicon using standard IC techniques and the pattern is transferred to create the polymer-/gel-based device. The most common materials for these devices are polymers such as poly dimethyl siloxane (PDMS) and polyacrylamide (PA) [20], but biological gels such as matrigel and fibrin are also being increasingly used. Polymer-based devices offer several advantages compared to silicon-based devices. From a biological perspective, the mechanical properties of polymers are closer to the *in vivo* environment of cells. Furthermore, compared to silicon, both the mechanical properties and surface chemistry of polymers can be easily tuned to better mimic the *in vivo* environment. Polymers such as PDMS and PA are also optically transparent and therefore force measurement and immunofluorescence imaging of specific biological markers can be made simultaneously under light microscopes. Thus, one can correlate cell force generation/sensing with molecular processes and biochemical pathways.

Polymer-based devices, however, have some disadvantages compared to silicon-based devices. For example, it is not easy to integrate electronics into these devices, which means that displacements induced by cell generated forces have to be optically measured, usually by post-processing of images acquired during the experiments. It is also difficult to apply external forces on cells using these devices, though recent advances [21] have provided means to circumvent this problem. Overall, the better biocompatibility and optical transparency of polymer-based micromechanical systems, along with the ease of fabrication, have made them increasingly popular for cell mechanics studies [22].

3. Measurement of cell traction and focal adhesion forces

Traction forces exerted by migrating fibroblasts were first studied using MEMS devices by Galbraith and Sheetz [23, 24]. Their platform (figure 1) consisted of micro pads ranging from 4 to 25 μm^2 in area that rested on the ends of micro cantilevers with stiffness of approximately 75 nN μm^{-1}. As the cells migrated over the platform they applied traction forces on the micro pads that were transmitted to the cantilevers. The force exerted by the cells was deduced by optically measuring the deflection of the cantilevers. Their observations showed that the maximum force (100 nN) was exerted at the tail region of the fibroblast which was approximately ten times larger than the force at the lamella region. The experiments also showed that the direction of force changed across the fibroblasts—the force at the lamella was opposite to the direction of migration, whereas the force at the tail region was in the same direction. Furthermore, immunofluorescence imaging of $\beta 1$-integrin showed that the forces were generated at a small number of adhesive contacts, with a force of 3 nN at each contact. While this technique illustrated several key features of the force generation in migrating cells, it suffered from two limitations. Firstly, the cantilevers were sensitive to forces in only one direction. Secondly, at any time, only the force generated by the cell region in contact with the pad could be measured. Therefore, it was not possible to measure the traction distribution across the entire cell simultaneously.

These constraints were overcome by the development of traction force microscopy (TFM) [25, 26]. In this technique, fluorescent beads were embedded into soft polymer substrates on which cells were grown. When the cells applied traction forces, the substrate deformation was visualized by the movement of the fluorescent beads. To map the substrate

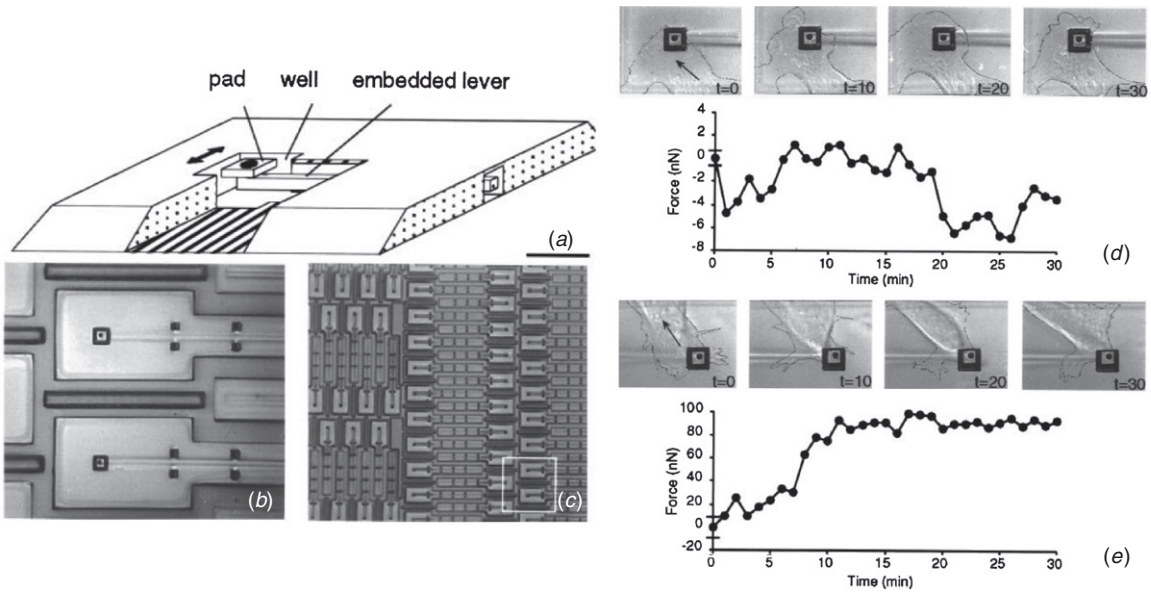


Figure 1. (*a*)–(*c*) Schematic and scanning electron microscope (SEM) images of micromachined cantilevers and pads used for the measurement of cell traction forces. Cells attach to the pads and exert forces which deflect the cantilevers. (*d*) A series of images of the front leading edge and lamella of a fibroblast moving across a pad. The plot shows the force exerted by the front edge over time. (*e*) Micrographs and traction force generated by the tail region of a fibroblast. Note that the force exerted by the tail region is opposite to the front region and is about ten times larger. Images reprinted from [23]. Copyright © 1997 National Academy of Sciences, USA, with permission.

deformation field, the reference position of the fluorescent beads has to be known, which was obtained by trypsinizing the cells after the experiments to relax the substrate. Once the substrate deformation field is known, the traction forces can be back-calculated from elasticity theory using computational algorithms. The main advantage over the cantilever-based approach of Galbraith and Sheetz was that in-plane traction forces, in all directions, over the entire cell basal surface could be mapped simultaneously. However, the determination of the forces from the substrate deformation is substantially more complex compared to obtaining forces from cantilever deflections [27].

An improved version of TFM was introduced by Balaban *et al* [28] to study the force exerted by cells at single focal adhesions (figure 2). They created regular patterns of markers on the surface of PDMS sheets, instead of randomly distributing fluorescent beads in the bulk of the substrate. The markers were either pits on the PDMS surface or fluorescent photoresist dots embedded on the surface. The advantage here was that any force exerted by the cell can be easily detected by the deviation of the markers from the regular pattern, without trypsinizing the cells to obtain the reference position of the markers. Using this technique, Balaban *et al* found that average force exerted by a fibroblast at a focal adhesion was about 10 nN. Of interest, they found that the stress at a single focal adhesion was constant (5.5 nN μm^{-2}) even though the area of the adhesion, and concomitantly the force, was variable.

As outlined above, cantilever-based approaches offer a simple method to calculate forces whereas TFM can map the full traction field of the cell. Combining the advantages of both these methods Tan *et al* [29] introduced a new class of devices, commonly referred to as microfabricated post array detectors (mPADs). mPADs comprise arrays of PDMS microposts, created using the soft lithography technique, that act as cantilevers. Adhesion molecules are coated on top of the posts by contacting the mPAD with a flat PDMS sheet coated with those molecules. When cells are cultured on the mPAD, they preferentially attach to the top of the microposts. Once the attachments are formed, the cells exert forces on the posts and deform them. Because the micro posts are isolated from each other, the force exerted by a cell on a post can be analyzed independently of both nearby cells and posts. This dramatically simplifies the calculation of force at each attachment point compared to TFM, which requires complex computational algorithms to localize the forces. Force calculation is further simplified by the fact that the post deflection, measured optically, has a linear relationship with applied force for small deflections.

mPADs have been used to study a variety of cells such as fibroblasts, epithelial cells, cardiac cells and smooth muscle cells since they offer several features for linking traction generation with the biochemistry of the cells [29–31]. Because the microposts serve as discrete attachment points, the nature and expression level of proteins at each post can be directly correlated to the force exerted at that location [32]. In addition, by appropriately selecting the adhesion molecules, one can mimic cell–extra cellular matrix interactions [30] or cell–cell interactions [33]. More recently, mPADs have been used to isolate the effect of substrate rigidity on cell behavior [34]. Typically, substrate rigidity is altered either by changing the gel density, in the case of hydrogels derived from natural ECM proteins, or by changing the cross-linker concentration, in the case of ECM analogs such as PA gels. However, altering the density of hydrogels also changes the amount of ligand while cross-linker concentration also

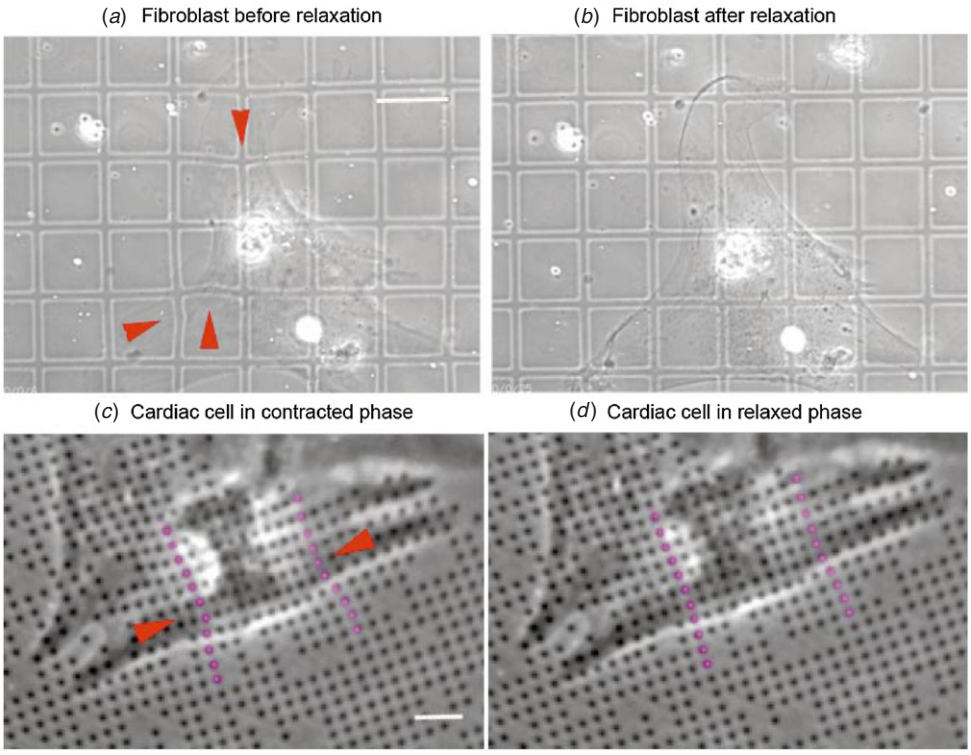


Figure 2. Cells plated on patterned elastomers. (*a*) Phase-contrast image of a rat cardiac fibroblast creating distortions (arrowheads) by applying force to the elastomer. (*b*) The same cell as in (*a*) 10 min after relaxation. The regular grid pattern is regained after relaxation. (*c*) Micrograph of a contracting cardiac myocyte plated on the elastomer with embedded photoresist pattern of dots. The arrowheads and the magenta dots underline the pinching action of the contraction on the elastomer. Images reprinted by permission from Macmillan Publishers Ltd [28]. Copyright (2001).

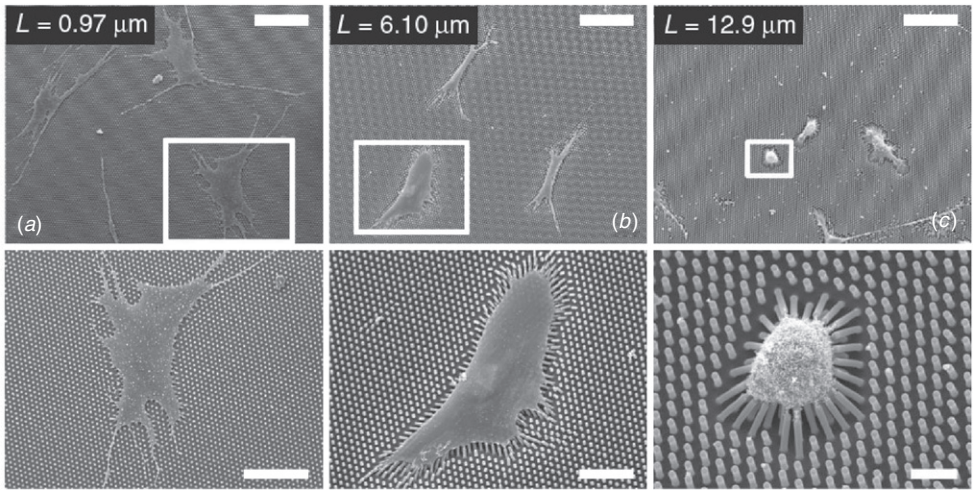


Figure 3. Scanning electron micrographs of human mesenchymal stem cells plated on PDMS micropost arrays. The diameters of the posts were the same (1.83 μm) but the lengths (L) were different, as indicated in the figure. Note that the deflection is substantially larger for the 12.9 μm micron length posts (*c*), which were almost 1000 times softer than the 0.97 μm posts (*a*). Images at the bottom are magnifications of the boxed regions in the top images. Images reprinted by permission from Macmillan Publishers Ltd [34]. Copyright (2010).

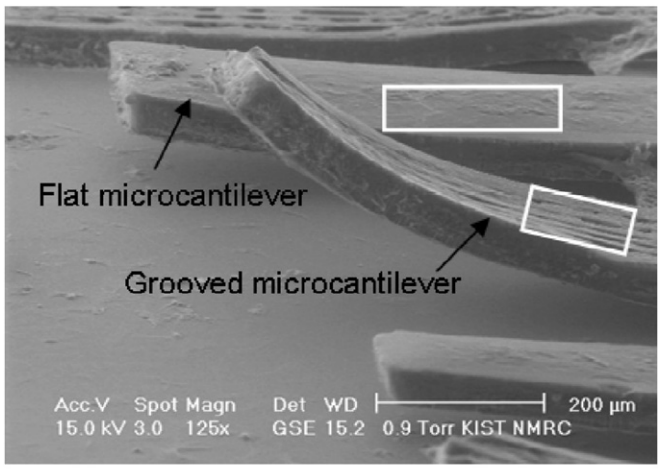
affects porosity, surface chemistry and binding properties of immobilized adhesive ligands. Therefore, it is not possible to isolate the effect of substrate rigidity on cell behavior. In the case of mPADs, on the other hand, the rigidity of the posts can be altered simply by changing their geometry while keeping all other factors constant. Using this approach (figure 3), Fu *et al* [34] showed that micro post rigidity affects cell morphology, focal adhesions, cytoskeletal contractility and stem cell differentiation. Furthermore, the study indicated that early changes in cytoskeletal contractility could predict later stem cell fates in single cells.

4. Measurement of cell contractile forces

MEMS sensors have also been used to measure the forces exerted by single cardiac myocytes and how their contractility

depends on sarcormeric density, structure and organization. Till the advent of MEMS sensors, force measurements of cardiac muscle have been typically restricted to the tissue level due to scaling problems associated with interfacing single cardiac cells with standard force transducers. To measure the contractile forces exerted by single cardiac cells, Lin *et al* [35] developed a 3D polysilicon force sensor. The cardiac cells were held at the two ends by polysilicon clamps that were suspended by a pair of microbeams. Contractile forces were calculated by measuring the deflection of the microbeams optically. It was found that the maximum force generated by the cardiac cell was about 12.5 μN, which was correlated with optically imaged striation pattern periodicity. Furthermore, the variation in cell contractility with calcium ion concentration was measured by immersing the sensor into microfluidic chambers.

Optically transparent polymer-based devices have also been used to study cardiac cells. For example, Park *et al* [36] used PDMS cantilevers to measure contractile forces exerted by multiple self-organized cardiac myocytes. In this set up, cardiomyocytes, attached to the surface of the PDMS cantilever, produced bending when they contracted. The in-plane and out-of-plane motions of the cantilevers were measured optically and the system was modeled as a sheet of cardiomyocytes attached to thin cantilever beams. The contractile forces exerted by the myocytes were calculated by analyzing this hybrid biopolymer system using Stoney's equation and finite element analysis. Based on the analysis, the stress exerted by the cardiomyocyte sheets on flat cantilevers was found to be 2–5 nN μm^{-2}, confirming previous studies. Later, an additional aspect to this technique was introduced by engraving grooves along the long axis of the cantilevers (figure 4). It was shown that myocytes grown on grooved cantilevers had more organized actin filaments and elongated nuclei compared to myocytes grown on flat cantilevers [37]. Of interest, the stress exerted by myocytes on the grooved cantilevers was higher (4–7 nN μm^{-2}), clearly showing a link between cytoskeletal organization and force production. Apart from cantilever-based systems, mPADs have also been used to investigate the contractility of cardiomyocytes. These studies have revealed the dependence of contractility on cardiac cell morphology and sarcomeric structure, as well as the effect of pharmacological interventions [31, 38].



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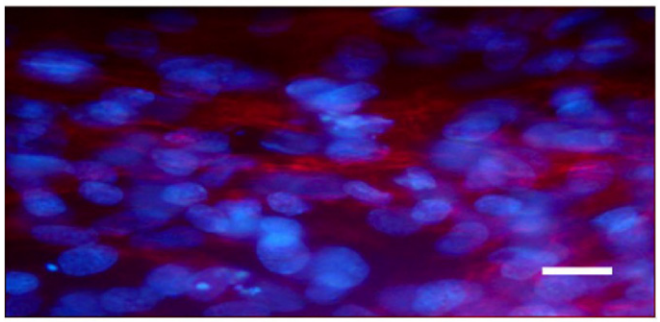

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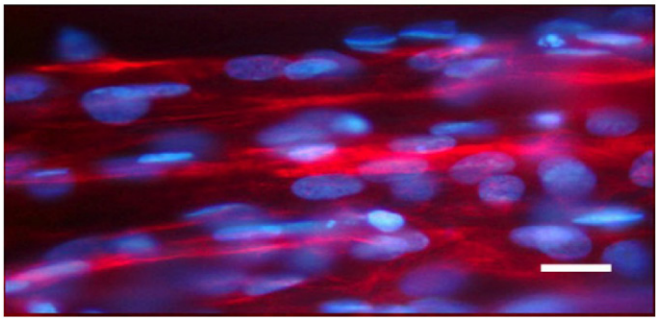

(*c*)

Figure 4. Cardiomyocytes cultured on PDMS cantilevers. (*a*) SEM image of a flat and a grooved cantilever deformed by cardiomyocytes. Stained images of cardiomyocytes grown (*b*) on a flat microcantilever and (*c*) on a grooved microcantilever. Cells were stained with TRITC (tetramethyl rhodamine iso-thiocyanate) phalloidin to show actin filaments (red) and nuclei are stained with DAPI (4,6-diamidino-2-phenylindole) (blue). Note the elongated nuclei and highly organized actin filaments on the grooved cantilever. Images reprinted from [37]. Copyright © 2006 IOP Publishing Ltd, with permission.

5. Measurement of cell and cytoskeletal stiffness

Several studies have shown that elastic and viscoelastic properties of diseased cells can differ substantially from normal cells [39–41]. The shear modulus of red blood cells infected with malaria parasite *Plasmodium falciparum* is ten times larger than normal red blood cells. Similarly, energy dissipation increases whereas elastic modulus decreases in pancreatic cancer cells treated with sphingosylphosphorylcholine, a bioactive lipid that influences cancer metastasis [42]. Changes in cell stiffness can affect shape and mobility, which in turn can influence disease state and severity *in vivo*. Understanding the link between cell mechanical properties and biological function can provide critical insights into disease progression and potentially offer new diagnostic tools [43].

MEMS force sensors offer significant advantages in the measurement of cell mechanical properties because of their small size and high force resolution. A dual-axis feedback controlled electrostatic actuator was developed by Sun *et al* [44] for mechanical characterization of single cells. The system featured a silicon probe driven by comb-drive actuators, which remained in air, and capacitive sensors for displacement

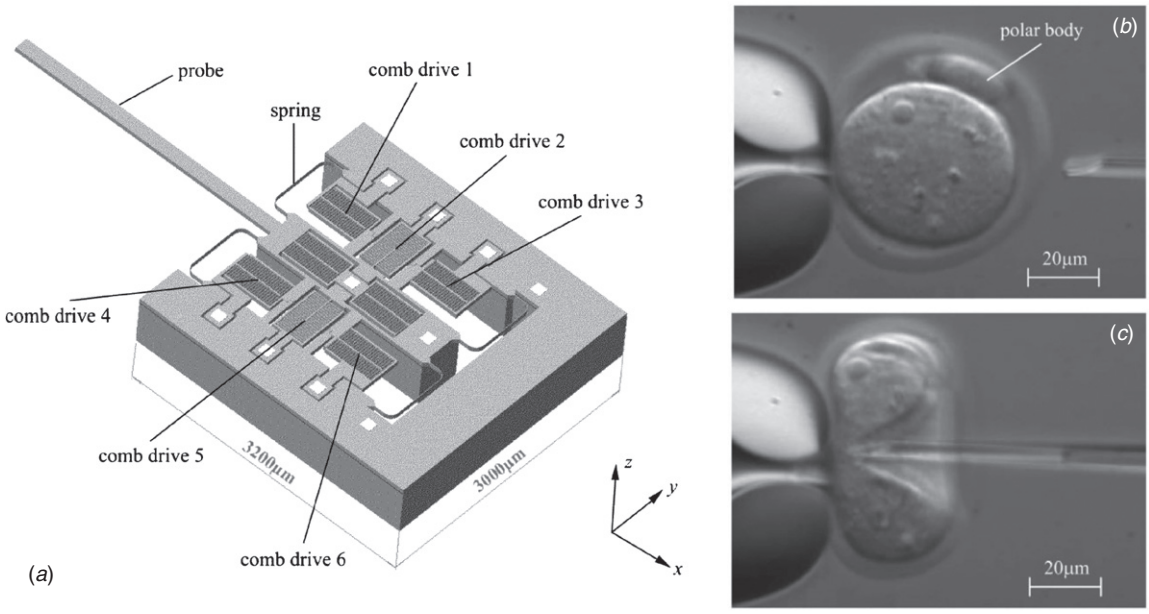

Figure 5. (*a*) Schematic of a dual-axis feedback controlled electrostatic actuator. The comb-drive actuators, which remain in air, are used for actuation and capacitive sensors are used for force measurement. (*b*) Image of an undeformed mouse embryo zone pellucida. (*c*) A deformed mouse embryo zone pellucida. The applied force is 12.7 μN, and the indenter displacement is 52.3 μm. Images reprinted from [44]. Copyright © 2003 IEEE, with permission.

measurement. Using this system (figure 5), it was found that the zona pellucida of mouse oocyte became 2.3 times harder after fertilization, presumably to prevent subsequent sperm from penetrating. Yang and Saif [45] developed a silicon-based force sensor to study the mechanics of single cells. The sensor was specifically designed to apply and measure large deformations and forces, a feature not present in other systems, to mimic cell response to injuries and large strains. The sensor comprised a probe attached to a pair of flexible beams, and was mounted on an external actuator to apply deformation on the cells through the probe. The force response of the cell was calculated by optically measuring the beam deflection. The force–deformation response of fibroblasts was found to be linear, reversible and repeatable even for large deformation [46]. However, treatment with cytochalasin D, an actin depolymerizing agent, reduced the cell stiffness to almost zero.

More recently, Serrell *et al* [47] fabricated a bioMEMS device similar to a displacement controlled uniaxial tensile testing machine to measure the properties of single cells. They found that the force response of a single fibroblast was linear until de-adhesion occurred at a force of 1.5 μN. Mukundan *et al* [48] developed an electrostatic comb-drive actuator capable of operating in highly conductive liquid media. This on-chip actuation system was designed to be operated in microfluidic chambers so that the behavior of adherent cells could be measured under combined mechanical and biochemical stimuli. This system was integrated with a planar force sensing system to measure the response of Madine–Darby canine kidney cells. The average stiffness of the cells was found to be about 85 nN μm^{-1}, in agreement with previous studies.

6. MEMS for *in vivo* cell mechanics studies

While most of the MEMS sensors and platforms have been developed for *in vitro* studies, there has been increasing interest in measuring cell mechanical behavior *in vivo*. Rajagopalan *et al* [49] developed a class of ultra soft silicon-based force sensors for cell mechanics studies (figures 6(*a*) and (*b*)). These sensors comprised a series of flexible beams attached to a probe to deform and measure cell forces. The forces were obtained by optically measuring the deflection of the beams with respect to a reference. Because the beams were connected in series, the sensors had very low stiffness (0.1–1 nN μm^{-1}) and yet were capable of measuring forces up to hundreds of nanonewton. The sensors further had the advantage of a linear force–displacement relationship over the entire measurement range.

An essential requirement for using MEMS sensors in biological studies is their ability to operate in an aqueous environment. This is a major challenge for soft force sensors because they have to withstand extremely large capillary forces during their immersion and removal from aqueous solutions. To circumvent this problem, Rajagopalan *et al* [49] came up with a novel approach to insulate the force sensors from capillary forces. The key idea was to keep the sensors immersed in liquid at all times so that they avoid the air–liquid interface where capillary forces act. During the fabrication process, the flexible beams on the force sensor were connected together by a thin aluminum film. After the force sensor was detached from the wafer, the bottom side of the sensor was attached to a thin glass slide. The glass slide with the sensor was then immersed into a base developer (AZ-327 MIF). During immersion, the aluminum film protected the beams against damage from capillary forces. After immersion,

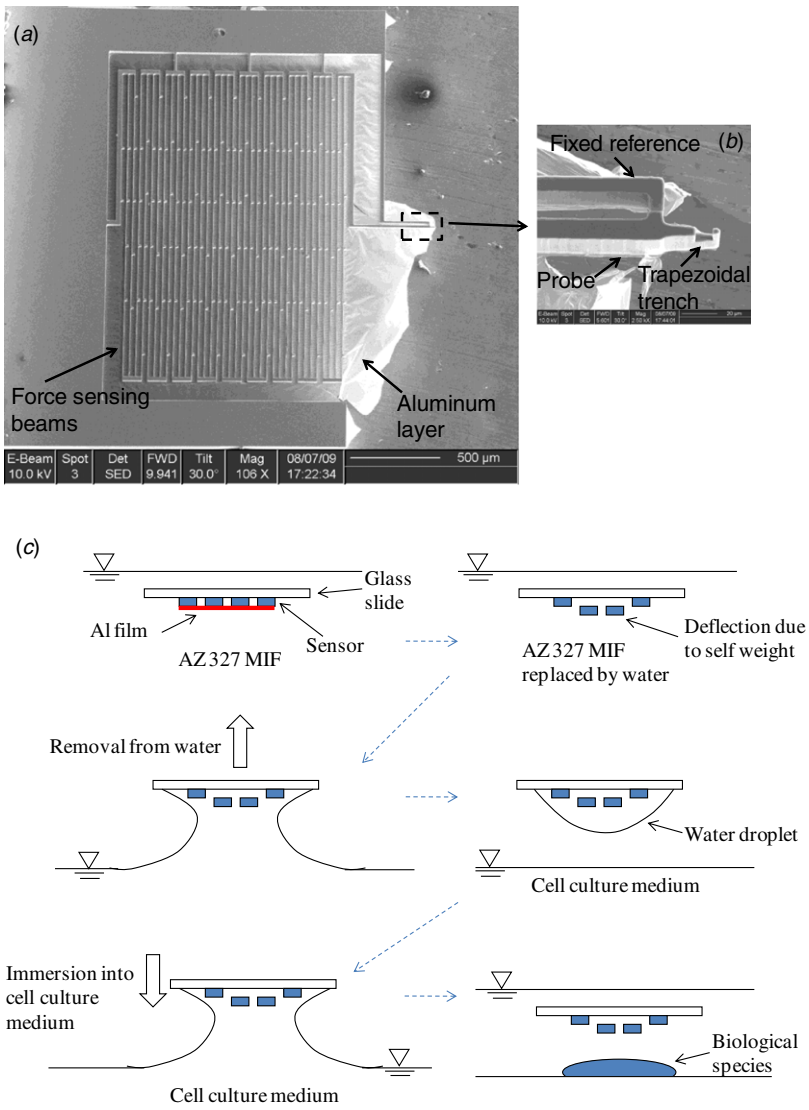

Figure 6. Scanning electron micrograph of a cantilever-based silicon force sensor. Because the beams are connected in series, the sensors have very low stiffness (0.1–1 nN μm^{-1}) but can measure forces up to hundreds of nanonewton [18]. (*b*) Magnified view of the probe and the reference beam. A trapezoidal trench was cut into the probe using focused ion beam milling to enable easier gripping of the axons. (*c*) Schematic of the process by which the force sensor is used for biological studies. Because the glass slide retains a droplet of water, the sensor never experiences any capillary forces. Images reprinted from [49]. Copyright © 2010 IEEE, with permission.

the developer etched the aluminum film and released the flexible beams and simultaneously exposed the hydrophilic native silicon dioxide layer. Then the developer was replaced with water by repeated dilution. When the glass slide was removed from water it retained a droplet of water, thereby keeping the sensor inundated. Similarly, when the sensor was immersed into the cell medium for biological experiments, the cell medium first contacted the water droplet and enveloped the sensor. Therefore, the sensor did not experience any damage either during immersion or removal from liquid (figure 6(*c*)), since it never encountered the liquid/air interface or the liquid meniscus.

A recent study [8] revealed that vesicle clustering in the presynaptic terminal of the neuromuscular junction in *Drosophila* embryos is dependent on mechanical tension in the axons. Vesicle clustering disappeared with loss of mechanical tension and was regained upon restoring tension. In addition, increase in tension appeared to increase the vesicle density at the synapse, suggesting that mechanical tension could be a signal to modulate synaptic plasticity *in vivo*. To verify if neurons modulated axonal tension *in vivo*, Rajagopalan *et al* [18] used these soft MEMS sensors to study the mechanical behavior of axons in live *Drosophila* embryos. Their experiments showed that *Drosophila* neurons maintain an axonal rest tension of 1–13 nN. Furthermore, when the tension in the axons was suddenly diminished, the neurons actively generated force to restore tension, sometimes to a value close to their rest tension. The neurons also showed passive viscoelastic behavior in response to applied deformation (figure 7). Of interest, these results were almost in exact agreement with the *in vitro* behavior of neurons [50–52], suggesting that mechanical tension may strongly influence neuronal behavior *in vivo*.

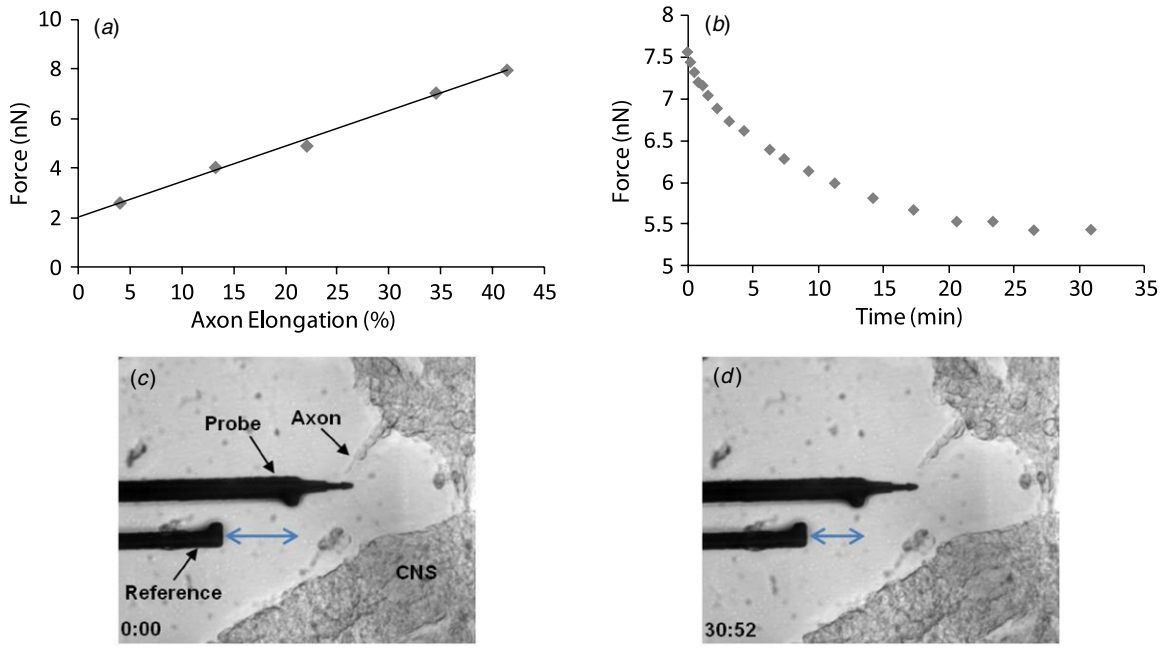

Figure 7. (*a*) Force–deformation response of a *Drosophila* axon during loading. The response is linear even up to 50% axon elongation. Extrapolation of the force–deformation curve to zero deformation results in a positive force value, indicating the presence of a rest tension. (*b*) Force relaxation in the axon shown in (*a*) which is characteristic of a viscoelastic solid. Images reprinted from [18]. Copyright © 2010 The Biophysical Society, with permission. (*c*) and (*d*) Optical images showing the relaxation of axonal force over time, as indicated by the reduction in probe displacement (double arrows) with respect to the reference. Images reprinted from [49]. Copyright © 2010 IEEE, with permission.

7. Microsystems for studies of cellular organisms

A majority of mechanobiology studies using micro systems have primarily focused on the measurement of cell forces and displacements under different microenvironments. However, several studies have shown that the application of external forces lead to changes in internal structures and activities of cells. For example, when dictyostelium cells are aspirated by micropipette it leads to a redistribution of myosin II, which likely plays a mechanosensory role during cytokinesis [53]. Similarly, endothelial cells subjected to stretching show an increase in voltage-gated K^+ current [54], and laterally indented fibroblasts exhibit actin agglomeration [55].

Motivated by these results, there has been increased interest in studying the mechanical properties and the effect of external forces on the development of cellular organisms. Kim *et al* [56] developed a micromechanical force sensing system to measure the change in the mechanical properties of the chorion membrane in zebrafish embryos during early development. They found that the chorion's elastic modulus at the pre-hatching stage was lower than at the blastula stage, indicating a mechanical softening during development. Their study further suggested that the chorion softening was the effect of proteolytic enzymes released during the pre-hatching stage. By integrating a PDMS cell holding device with a microrobotic cell manipulation system, Liu *et al* measured the indentation force–deformation curves for zebrafish [57] and mouse embryos [58] as well as the penetration force for cell injection. A significant difference was found in the force–deformation slope of healthy and fragmented mouse embryos, suggesting that mechanical property measurements can be used to identify embryonic defects during cell injection.

In a recent work, Nam *et al* [59] developed an explicit force-feedback control system to exert indentation force on cellular organisms while simultaneously measuring their impedance. Using zebrafish embryos as a test model they showed that the application of controlled external forces leads to a significant change in the impedance of the embryos. Based on their results they suggested that the impedance change was due to changes in the activity of pore canals in the chorion. These engineered systems represent a new class of mechano-control approaches that aim to manipulate the physiological properties of cellular organisms by direct control of external forces exerted on them.

In addition to manipulating cellular organisms, MEMS sensors have also found use in understanding locomotion of small animals and how their body mechanics is coupled to the mechanosensory system. Park *et al* [60] developed MEMS piezoresistive displacement clamp and studied the body mechanics of *C. elegans*. Piezoresistive cantilevers were used as force–displacement sensors that were coupled to a feedback system to apply defined load profiles. This system was capable of delivering forces between 0.01 and 1000 μN over a large bandwidth (0.1 Hz to 100 kHz), which traditional tools such as optical tweezers and AFM are incapable of providing. Their observations showed that the force–displacement response of *C. elegans* nematodes was linear and suggested that the contribution of the shell to the nematode stiffness dominates over that of the internal hydrostatic pressure. Doll *et al* [61] developed a two-axis micro strain gauge force sensor to measure the tactile

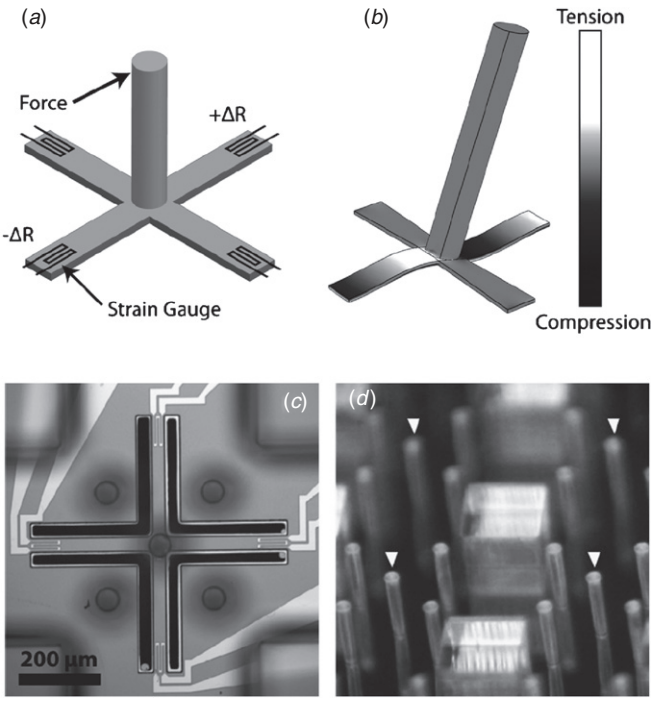

Figure 8. A SU-8 force sensing pillar array for biological measurements. (*a*) A lateral force applied at the tip of the pillar bends the four cantilever beams on which the pillar is suspended. The bending strain is transduced at the base of the cantilever using metal strain gauges. (*b*) Finite element analysis showing that bending induces alternating regions of compressive and tensile stress in the cantilever beams. (*c*) A single device viewed from the top. (*d*) An array of finished devices. The force sensing pillars, indicated by the arrows, are surrounded by passive spacer pillars and posts. Images reprinted from [61] reproduced by permission of The Royal Society of Chemistry.

sensitivity and interaction forces during locomotion by small organisms such as nematodes (figure 8). The device, made with SU-8 photoresist, was transparent and compatible with light microscopes, allowing behavioral experiments to be combined with quantitative force measurements. Using this device, they characterized the interaction forces generated in wild-type *C. elegans* during locomotion.

8. Summary

MEMS-based sensors and microsystems offer significant potential for studies of cell mechanobiology. The microsystems discussed in this paper, which is by no means comprehensive, provide examples of how the geometry, mechanical properties and force and displacement capabilities can be tuned to investigate specific biological systems. However, a large majority of these systems are two dimensional and cannot mimic the three-dimensional micro environment cells experience *in vivo*. Studies have shown that the behavior of cells on three-dimensional gels is substantially different from two-dimensional substrates. Creating more realistic three-dimensional environments for cells and measuring cell generated forces in such an environment is an essential step toward understanding how mechanical forces affect cell function *in vivo*. Insights from such studies will also be invaluable for creating appropriate conditions for *in vitro* tissue engineering.

Another area that presents significant challenges is the measurement of cell and tissue mechanical properties *in vivo*. As discussed earlier, there are very few examples [8, 18] of MEMS-based sensors being used for *in vivo* studies. For MEMS sensors to be successfully adapted to *in vivo* studies, creating biocompatible platforms is essential. Already, mechanically flexible silicon electronics have been developed for multiplexed measurement of electrical signals on three-dimensional surfaces of soft tissues in the human body [62]. Similarly, bioresorbable substrates have been used in conjunction with ultra thin electronics for *in vivo* neural mapping studies on feline animal models [63]. By leveraging such technology, it may be possible to construct microsystems capable of real time measurement of cell mechanical properties *in vivo*.

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References

[1] Engler A J, Sen S, Sweeney H L and Discher D E 2006 Matrix elasticity directs stem cell lineage specification *Cell* **126** 677–89
[2] Pelham R J Jr and Wang Y 1997 Cell locomotion and focal adhesions are regulated by substrate flexibility *Proc. Natl Acad. Sci. USA* **94** 13661–5
[3] Yeung T, Georges P C, Flanagan L A, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V and Janmey P A 2005 Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion *Cell Motil. Cytoskeleton* **60** 24–34
[4] Zheng J, Lamoureux P, Santiago V, Dennerll T, Buxbaum R E and Heidemann S R 1991 Tensile regulation of axonal elongation and initiation *J. Neurosci.* **11** 1117–25
[5] Puig-De Morales M, Grabulosa M, Alcaraz J, Mullol J, Maksym G N, Fredberg J J and Navajas D 2001 Measurement of cell microrheology by magnetic twisting cytometry with frequency domain demodulation *J. Appl. Physiol.* **91** 1152–9
[6] Franze K *et al* 2009 Neurite branch retraction is caused by a threshold-dependent mechanical impact *Biophys. J.* **97** 1883–90
[7] Desmoulière A, Chaponnier C and Gabbiani G 2005 Tissue repair, contraction, and the myofibroblast *Wound Repair Regen.* **13** 7–12
[8] Siechen S, Yang S, Chiba A and Saif T 2009 Mechanical tension contributes to clustering of neurotransmitter vesicles at presynaptic terminals *Proc. Natl Acad. Sci. USA* **106** 12611–6
[9] Weiss L and Schmid-Schonbein G W 1989 Biomechanical interactions of cancer cells with the microvasculature during metastasis *Cell Biochem. Biophys.* **14** 187–215
[10] Bao G and Suresh S 2003 Cell and molecular mechanics of biological materials *Nat. Mater.* **2** 715–25
[11] Suresh S, Spatz J, Mills J P, Micoulet A, Dao M, Lim C T, Beil M and Seufferlein T 2005 Connections between single-cell biomechanics and human disease states: gastrointestinal cancer and malaria *Acta Biomater.* **1** 15–30
[12] Van Vliet K J, Bao G and Suresh S 2003 The biomechanics toolbox: experimental approaches for living cells and biomolecules *Acta Mater.* **51** 5881–905

[13] Li N, Tourovskaia A and Folch A 2003 Biology on a chip: microfabrication for studying the behavior of cultured cells *Crit. Rev. Biomed. Eng.* **31** 423–88
[14] Bashir R 2004 Biomems: state-of-the-art in detection, opportunities and prospects *Adv. Drug Deliv. Rev.* **56** 1565–86
[15] Khademhosseini A, Langer R, Borenstein J and Vacanti J P 2006 Microscale technologies for tissue engineering and biology *Proc. Natl Acad. Sci. USA* **103** 2480–7
[16] Kim D, Pak K W, Park J, Levchenko A and Sun Y 2009 Microengineered platforms for cell mechanobiology *Ann. Rev. Biomed. Eng.* **11** 203–33
[17] Yang S and Saif M T A 2007 Mems based force sensors for the study of indentation response of single living cells *Sensors Actuators* A **135** 16–22
[18] Rajagopalan J, Tofangchi A and Saif M T A 2010 *Drosophila* neurons actively regulate axonal tension *in vivo Biophys. J.* **99** 3208–15
[19] Whitesides G M, Ostuni E, Takayama S, Jiang X and Ingber D E 2001 Soft lithography in biology and biochemistry *Annu. Rev. Biomed. Eng.* **3** 335–73
[20] Beningo K A and Wang Y-L 2002 Flexible substrata for the detection of cellular traction forces *Trends Cell Biol.* **12** 79–84
[21] Sniadecki N J, Anguelouch A, Yang M T, Lamb C M, Liu Z, Kirschner S B, Liu Y, Reich D H and Chen C S 2007 Magnetic microposts as an approach to apply forces to living cells *Proc. Natl Acad. Sci. USA* **104** 14553–8
[22] Weibel D B, DiLuzio W R and Whitesides G M 2007 Microfabrication meets microbiology *Nat. Rev. Microbiol.* **5** 209–18
[23] Galbraith C G and Sheetz M P 1997 A micromachined device provides a new bend on fibroblast traction forces *Proc. Natl Acad. Sci. USA* **94** 9114–8
[24] Galbraith C G, Yamada K M and Sheetz M P 2002 The relationship between force and focal complex development *J. Cell Biol.* **159** 695–705
[25] Wang Y L and Pelham R J Jr 1998 Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells *Methods Enzymol.* **298** 489–96
[26] Munevar S, Wang Y-L and Dembo M 2001 Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts *Biophys. J.* **80** 1744–57
[27] Wang J H and Lin J 2007 Cell traction force and measurement methods *Biomech. Model. Mechanobiol.* **6** 361–71
[28] Balaban N Q *et al* 2001 Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates *Nat. Cell Biol.* **3** 466–72
[29] Tan J L, Tien J, Pirone D M, Gray D S, Bhadriraju K and Chen C S 2003 Cells lying on a bed of microneedles: an approach to isolate mechanical force *Proc. Natl Acad. Sci. USA* **100** 1484–9
[30] Du Roure O, Saez A, Buguin A, Austin R H, Chavrier P, Siberzan P and Ladoux B 2005 Force mapping in epithelial cell migration *Proc. Natl Acad. Sci. USA* **102** 2390–5
[31] Zhao Y and Zhang X 2006 Cellular mechanics study in cardiac myocytes using PDMS pillars array *Sensors Actuators* A **125** 398–404
[32] Sniadecki N J and Chen C S 2007 Microfabricated silicone elastomeric post arrays for measuring traction forces of adherent cells *Methods Cell Biol.* **83** 313–28
[33] Ganz A, Lambert M, Saez A, Silberzan P, Buguin A, Mège R M and Ladouxt B 2006 Traction forces exerted through N-cadherin contacts *Biol. Cell* **98** 721–30
[34] Fu J, Wang Y, Yang M T, Desai R A, Yu X, Liu Z and Chen C S 2010 Mechanical regulation of cell function with geometrically modulated elastomeric substrates *Nat. Methods* **7** 733–6
[35] Lin G, Pister K S J and Roos K P 2000 Surface micromachined polysilicon heart cell force transducer *J. Microelectromech. Syst.* **9** 9–17
[36] Park J, Ryu J, Choi S K, Seo E, Cha J M, Ryu S, Kim J, Kim B and Lee S H 2005 Real-time measurement of the contractile forces of self-organized cardiomyocytes on hybrid biopolymer microcantilevers *Anal. Chem.* **77** 6571–80
[37] Park J, Kim J, Roh D, Park S, Kim B and Chun K 2006 Fabrication of complex 3d polymer structures for cell–polymer hybrid systems *J. Micromech. Microeng.* **16** 1614–9
[38] Zhao Y, Chee C L, Sawyer D B, Liao R and Zhang X 2007 Simultaneous orientation and cellular force measurements in adult cardiac myocytes using three-dimensional polymeric microstructures *Cell Motil. Cytoskeleton* **64** 718–25
[39] Sleep J, Wilson D, Simmons R and Gratzer W 1999 Elasticity of the red cell membrane and its relation to hemolytic disorders: an optical tweezers study *Biophys. J.* **77** 3085–95
[40] Cooke B M, Mohandas N and Coppel R L 2001 The malaria-infected red blood cell: structural and functional changes *Adv. Parasitol.* **50** 1–86
[41] Dao M, Lim C T and Suresh S 2003 Mechanics of the human red blood cell deformed by optical tweezers *J. Mech. Phys. Solids* **51** 2259–80
[42] Suresh S, Spatz J, Mills J P, Micoulet A, Dao M, Lim C T, Beil M and Seufferlein T 2005 Connections between single-cell biomechanics and human disease states: gastrointestinal cancer and malaria *Acta Biomater.* **1** 15–30
[43] Suresh S 2007 Biomechanics and biophysics of cancer cells *Acta Biomater.* **3** 413–38
[44] Sun Y, Wan K T, Roberts K P, Bischof J C and Nelson B J 2003 Mechanical property characterization of mouse zona pellucida *IEEE Trans. Nanobiosci.* **2** 279–86
[45] Yang S and Saif T 2005 Micromachined force sensors for the study of cell mechanics *Rev. Sci. Instrum.* **76** 1–8
[46] Yang S and Saif T 2005 Reversible and repeatable linear local cell force response under large stretches *Exp. Cell Res.* **305** 42–50
[47] Serrell D B, Law J, Slifka A J, Mahajan R L and Finch D S 2008 A uniaxial biomems device for imaging single cell response during quantitative force–displacement measurements *Biomed. Microdevices* **10** 883–9
[48] Mukundan V and Pruitt B L 2009 Mems electrostatic actuation in conducting biological media *J. Microelectromech. Syst.* **18** 405–13
[49] Rajagopalan J, Tofangchi A and Saif M T A 2010 Linear high-resolution biomems force sensors with large measurement range *J. Microelectromech. Syst.* **19** 1380–9
[50] Joshi H C, Chu D, Buxbaum R E and Heidemann S R 1985 Tension and compression in the cytoskeleton of pc 12 neurites *J. Cell Biol.* **101** 697–705
[51] Dennerll T J, Lamoureux P, Buxbaum R E and Heidemann S R 1989 The cytomechanics of axonal elongation and retraction *J. Cell Biol.* **109** 3073–83
[52] Chada S, Lamoureux P, Buxbaum R E and Heidemann S R 1997 Cytomechanics of neurite outgrowth from chick brain neurons *J. Cell Sci.* **110** 1179–86
[53] Effler J C, Iglesias P A and Robinson D N 2007 A mechanosensory system controls cell shape changes during mitosis *Cell Cycle* **6** 30–5
[54] Fan J and Walsh K B 1999 Mechanical stimulation regulates voltage-gated potassium currents in cardiac microvascular endothelial cells *Circ. Res.* **84** 451–7
[55] Yang S and Saif M T A 2007 Force response and actin remodeling (agglomeration) in fibroblasts due to lateral indentation *Acta Biomater.* **3** 77–87

[56] Kim D, Hwang C N, Sun Y, Lee S H, Kim B and Nelson B J 2006 Mechanical analysis of chorion softening in prehatching stages of zebrafish embryos *IEEE Trans. Nanobiosci.* **5** 89–94

[57] Liu X, Sun Y, Wang W and Lansdorp B M 2007 Vision-based cellular force measurement using an elastic microfabricated device *J. Micromech. Microeng.* **17** 1281–8

[58] Liu X, Kim K, Zhang Y and Sun Y 2009 Nanonewton force sensing and control in microrobotic cell manipulation *Int. J. Robot. Res.* **28** 1065–76

[59] Nam J H, Chen P C Y, Lu Z, Luo H, Ge R and Lin W 2010 Force control for mechanoinduction of impedance variation in cellular organisms *J. Micromech. Microeng.* **20** 025003

[60] Park S, Goodman M B and Pruitt B L 2007 Analysis of nematode mechanics by piezoresistive displacement clamp *Proc. Natl Acad. Sci. USA* **104** 17376–81

[61] Doll J C, Harjee N, Klejwa N, Kwon R, Coulthard S M, Petzold B, Goodman M B and Pruitt B L 2009 SU-8 force sensing pillar arrays for biological measurements *Lab Chip* **9** 1449–54

[62] Viventi J *et al* 2010 A conformal, bio-interfaced class of silicon electronics for mapping cardiac electrophysiology *Sci. Transl. Med.* **2** 24ra22

[63] Kim D H *et al* 2010 Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics *Nat. Mater.* **9** 511–7