

# 65

## Instrumentation for Cell Mechanics

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### 65.1 Background

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Mechanical forces are essential to life at the microscale — from tethering at the junctions between cells that compose a tissue to externally applied loads arising in the cellular environment. Consider the perturbations from acoustic sounds that affect the mechanosensors on auditory hair cells in the inner ear, the contractile forces that a dividing cell imparts on itself in order to split into two daughter cells during cytokinesis, or the bone and muscle loss that occurs from the reduced loads in microgravity [1,2]. Mechanical forces are particularly important in the cardiovascular and musculoskeletal systems [3]. Increased shear stress in the blood flow leads to the dilation and restructuring of blood vessels [4]. The immune response of leukocytes requires that they adhere to and transmigrate through the endothelial barrier of blood vessels [5]. The forces required between leukocytes and endothelium, and between neighboring endothelial cells, in order to execute such complex events have become an important avenue of research [6,7]. Arterial hypertension causes the underlying vessel walls to constrict, preventing local aneurysms and vessel failure [3]. Long-term exposure to such hypertension leads to increased thickening and stiffening of the vessel walls causing vessel stenosis. In the skeletal system, exercise-induced compressive forces increase bone and cartilage mass and strength while subnormal stresses, from bedrest, immobilization, or space travel, results in decreased bone mass [2]. Despite the clear demonstration that mechanical forces are an essential factor in the daily life of many cells and tissues, the underlying question remains to understand how these forces exert their effects.

A key insight to these areas of study has been that nearly all of the adaptive processes are regulated at the cellular level. That is, many of the tissue responses to forces are actually cellular responses. The contraction and hyperproliferation of smooth muscle cells embedded within the arteries in response to hypertension causes the vessel wall constriction and thickening. Changes in bone mass result from

both changes in the production of new bone cells and the metabolic capacity of the existing bone cells. For example, mesenchymal stem cells have been found to differentiate into bone-producing osteoblasts if they are allowed to experience mechanical stresses generated between the individual cells and their local surroundings, but become fat-storing adipocytes when such stresses are eliminated [8]. Thus, an understanding of the importance of forces to medicine and biology must first derive from a better characterization of the forces acting at the single cell level. To begin to explore and characterize the forces experienced and generated by cells (cellular forces), engineers are taking a two-pronged approach. First, they are developing a better understanding of the cell as a mechanical object; and second, they are employing new tools for analyzing cellular forces in the micro and nanoscale.

The measurement of cellular forces is a difficult task because cells are active. That is, they continually change and adapt their mechanical structure in response to their surroundings. The primary mechanical elements in cells are polymers of proteins — in particular, actin, tubulin, and intermediate filament proteins — that are collectively called the cytoskeleton. These cytoskeletal scaffolding structures are continually disassembling and reassembling, realigning, renetworking, contracting, and lengthening. Perhaps one of the most fascinating and simultaneously challenging aspects of characterizing these mechanical rearrangements is that they often occur in direct response to mechanical perturbations. If one pulls on a corner of a cell to measure its material response, it will adjust to the perturbation with reinforcement at the point of applied force. If one places a cell on a soft substrate, it will adjust its shape to achieve a balance between its contractile forces generated by its cytoskeleton and the adhesion forces at its extracellular foundation. The dynamic response of cells to such forces makes the characterization of the natural state of cells difficult. Nonetheless, one of the major goals of mechanobiology is not only to characterize cellular mechanics, but also to identify the mechanism by which cells sense, transduce, and respond to mechanical forces. Whether the mechanosensor itself is an individual protein, a network of structures, or some novel control process remains to be determined. Due to the intimate interaction between the properties of the cell and the techniques used to measure them, we will first provide a brief introduction to the mechanics of the cells themselves, followed by the techniques used to measure the forces that they generate. In addition, since cells are quite small, we will provide a brief discussion of scaling laws and emphasize the technical challenges associated with measuring forces at this length scale.

## 65.2 Cellular Mechanics

The behavior and function of a cell is dependent to a large degree on the cytoskeleton which consists of three polymer filament systems — microfilaments, intermediate filaments, and microtubules. Acting together as a system, these cytoskeleton filament proteins serve as the scaffolding in the cytoplasm of the cell that (1) supports the delicate cell membrane, (2) tethers and secures organelles in position or guide their transport through the cytoplasm, and (3) in conjunction with various motor proteins, the machinery that provides force necessary for locomotion or protrusion formation [1]. Microfilaments are helical polymers formed from actin that organize into parallel bundles for filopodia extensions and contractile stress fibers or into extensively cross-linked networks at the leading edge of a migrating cell and throughout the cell cortex that supports the cell membrane (Figure 65.1b). Microtubules have tubulin subunits and form long, hollow cylinders that emanate from a single centrosome, which is located near the nucleus (Figure 65.1c). Of the three types of cytoskeletal filaments, microtubules have the higher bending resistance and act to resist compression [9]. Intermediate filaments form extensive networks within the cytoplasm that extend circumferentially from the meshwork structure that surrounds the nucleus (Figure 65.1d). These rope-like filaments are easy to bend but difficult to break. They are particularly predominant in the cytoplasm of cells that are subject to mechanical stress, which highlights their role in tissue-strengthening [10]. Since these three subcellular structures have distinct mechanical properties and varying concentrations between cells, the measured cellular forces and mechanical properties of a particular cell may not be the same as the next cell.

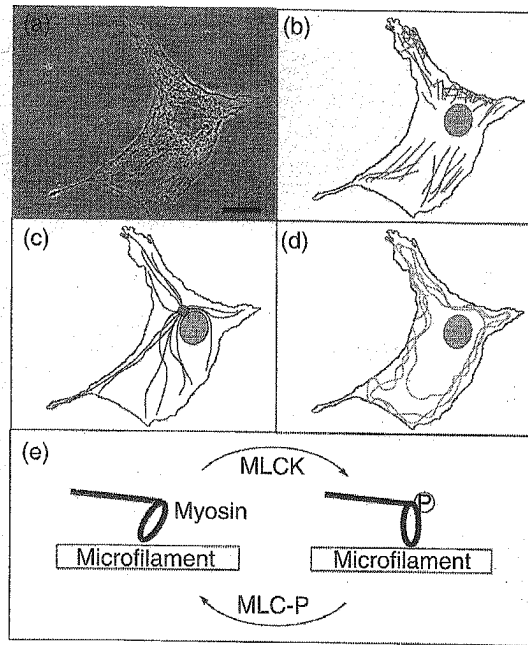
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**FIGURE 65.1** Cells have cytoskeletal structures that determine their force generation machinery. (a) Phase contrast image of 3T3 fibroblast (bar: 10  $\mu\text{m}$ ). The diagram shows the general shape and location of (b) microfilaments, (c) microtubules, and (d) intermediate filaments. (e) Schematic of conventional actomyosin motor for cellular forces.

Microfilaments, when coupled with the motor protein myosin, generate the cellular forces that influence the function of the cell and surrounding tissue. Often, skeletal muscle cells come to mind when one thinks of cellular force generation. Cardiac muscle, striated muscle, smooth muscle, and myoepithelial cells, all of which originate from a common precursor myoblast cell, employ a contractile system involving actin as the filament and myosin as the motor protein. Myosin binds to the microfilaments and moves along it in a step-wise linear ratchet mechanism as a means to generate contractile force. Although well studied in muscle, the same actomyosin contractile apparatus found in skeletal muscle cells is present in nearly all cell types. Myosin changes its structure during cycles of phosphorylation, regulated by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLC-P), to create power strokes that advance the head of the protein along the filament (Figure 65.1e). Each cycle advances the myosin head about 5 nm along the actin filament and produces an average force of 3 to 4 pN [11]. The interaction of myosin and actin is not a constant machine but instead is cycled as dictated by the upstream signaling pathways that regulate MLCK and MLC-P.

A highly researched aspect of cellular forces that highlight their dynamic nature is cell locomotion. These forces, aptly named traction forces, are medically relevant for the metastasis of cancer and occurs when a sessile cell develops the ability to migrate from the tumor and into the bloodstream. Additionally, embryonic development, tissue formation, and wound healing exemplify the physiological relevance of cell migration. The mechanism that generates cell locomotion depends on the coordination of changes in cell shape, via restructuring of the cytoskeletal filaments, and shifting of adhesion sites to the extracellular matrix. When a cell moves forward, it extends a protrusion at the leading edge via microtubule formation and actin polymerization to create new adhesion sites, which are called focal adhesions or focal contacts (Figure 65.2). These nonuniformly distributed, punctate structures link the cytoskeletal filaments and the motor proteins to the substrate and are present at both ends of a migrating cell. After forming new attachments, the cell contracts to move the cell body forward by disassembling contacts at the back end. The locomotion is continued in a treadmill fashion of front protrusion and rear contraction. On account of its dynamic response, a cell does not have uniform mechanical properties and certainly cannot be regarded as a Hookean material with time-independent and linear material properties. As a result, a classical continuum model for the cell has not provided significant insight into the basis for the mechanical properties of a cell.

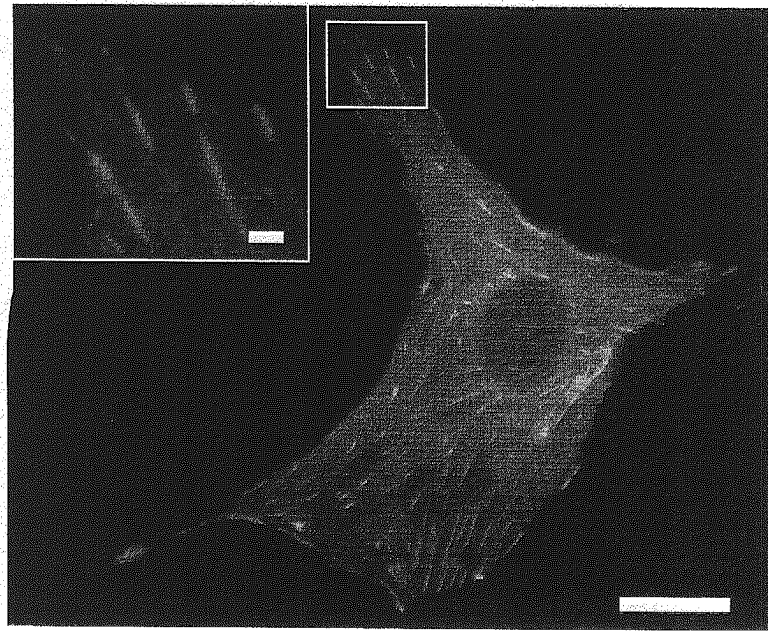


FIGURE 65.2 Immunofluorescence staining of focal adhesions (bar: 10  $\mu\text{m}$ , inset bar: 2  $\mu\text{m}$ ).

To understand both the signaling pathways that control these cytoskeletal processes and the resultant mechanics of cell force-generation, researchers have begun to develop methods to measure these forces. To coordinate a global response with other cells of the tissue to a particular stimulus, there exists a complex mechanism of communication between the cells within a tissue. Often cellular signaling, or communication, is mediated by the release of soluble chemicals or growth factors. When these chemicals diffuse across populations of cells, each cell detects the signal via specific receptors and responds accordingly. One of the most challenging aspects of studying cellular mechanics, however, is that cells are also able to detect physical changes even under a constant chemical environment, and respond with changes in cellular function or mechanical state. The sensing and translation of mechanical signals into a functional biochemical signal is known as mechanotransduction. One of the sites in which mechanotransduction occurs is at focal adhesions. A critical engineering challenge in understanding how cells sense and control cellular forces is to characterize the nanonewton forces at these adhesion sites, and correlate these forces with the intercellular signaling response of mechanotransduction.

Based on these insights about the cells and their intracellular structure, it is clear that how one chooses to measure cellular forces can affect the measurements themselves. Since the mechanical properties of a cell are non-Hookean, one can consider the cell to have a history or a memory. Similar to hysteresis of materials, the loadings of forces on a cell have a time-dependent response and so a cell is often modeled as a viscoelastic material — a soft glassy material. As a result, the procedural conditions of a particular experiment must be examined for a time-dependent response. Thus, the observer needs to consider whether to measure the range of forces exerted by one cell to obtain the direct time-response but with a limited data population, or to interrogate a large number of cells with many cellular forces and only report the average value, thereby disregarding the effect that transitions between loading conditions have on a cell. These considerations should act as a simple warning that one must treat each new system, device, and resulting data on cellular mechanics with a healthy degree of guarded skepticism. Despite such concerns, investigators have begun to develop numerous tools that ultimately will address these issues.

### 65.3 Scaling Laws

In addition to the difficulty in measuring the nanonewton-scale forces that cells exert on the extracellular matrix or at cellular interconnects, the spots where these forces are applied are subcellular (micrometer

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and nanometer-scale) in dimension. Microscale detection is required in order to measure the different traction forces that are applied at the front vs. the back of a migrating cells. Moreover, since mechanical stresses appear to be sensed and applied at individual focal adhesions (ranging in size from 0.1 to  $2 \mu\text{m}^2$ ), it is pertinent to have spatial resolution in the submicron range (Figure 65.2). To obtain the microscale measurement power for studying cellular mechanics, researchers have turned to new techniques and tools. However, in their development, one must consider the impact of scaling laws on the design of the tool. At the micrometer or nanometer scale, the ratio of surface area to volume dramatically differs from the length scale to which we are accustomed. Thus, surface forces dominate over body forces since the former scales with the inverse of the square of the length ( $L^{-2}$ ) while the later scales with the inverse of the length to the third power ( $L^{-3}$ ). For example, adhesion forces and fluid shear forces are often more critical to the function of a cell than those of gravity [2,4]. The microscale forces that compose the environment of a cell are difficult to measure with the types of tools that are typically used at the macroscale.

Not only must the designer of these new tools consider the types of forces they want to measure, but also how they will transfer the data to some readable form. Using a microscope to read the measurements from the tool is a noninvasive technique that does not require direct connections to the substrate. However, the technique does require that the substrate be optically transparent, which limits materials available for fabrication, and has optical limitations in resolving below hundreds of nanometers. Despite the limitations, coupling microscopy with a force sensor does provide improved measurement read-out capabilities over other techniques such as electronics, which have sensitivity limitations due to the low signal to noise ratio from thermal and charge fluctuations in the aqueous environment and integration complexity in constructing the electrical components on the same substrate as the force sensors.

In scaling down to the cellular level, the development of the measuring instruments becomes dependent on experimental materials and microfabrication. Typical hard materials used in strain gauges and springs do not bend under nanonewton loadings with the same displacement that is required for measurement sensitivity. On account of this, soft materials are employed in the construction of the microsensors, even though these thin-film materials are not as fully characterized as their bulk material counterparts. Additionally, as the surface to volume ratio increases at the microscale, the effect of the different chemical composition at the surface of the material, such as the native oxide layers of iron, copper, or silicon, may have more dramatic effects on the overall material properties. In building devices with these materials, the microfabrication techniques used must have good reliability for repeatable and uniform measurements on the device. Even though the equipment used in microfabrication is engineered to deposit material with uniform properties and thickness across the device, tolerance issues are still pertinent because of the topological effect that a micrometer defect can have on the environment that the cell senses. Most of the microsensors are made one at a time or in limited batches and consistency in the fabrication methods is critical for repeatable measurements. In conclusion, the devices detailed in the following sections are powerful measurement tools for detecting the nanoscale cellular forces but are still prototypes, in which consistency in their construction is important to corroborating the scientific discoveries that they provide.

## 65.4 Measurement of Cellular Force

Studying the forces that cells exert on their microenvironment and their corresponding biological mechanisms generally involve culturing cells on flexible substrates that the cells physically deform when applying their contraction or traction forces. When the stiffness of the substrate has been characterized, then optical measurement of the substrate distortion reports the cellular force. A relationship between force and displacement holds whether the substrate is a film of polyacrylamide gel that distorts under the force of a contracting cell adhered to it or silicone microcantilevers that are deflected under the forces of a migrating cell. In the early 1980s, Albert Harris first pioneered the method of measuring cellular forces on thin films of silicone that wrinkled upon the force of the adherent cells and has since evolved into devices that use microfabrication techniques to obtain improved precision of their force sensors.

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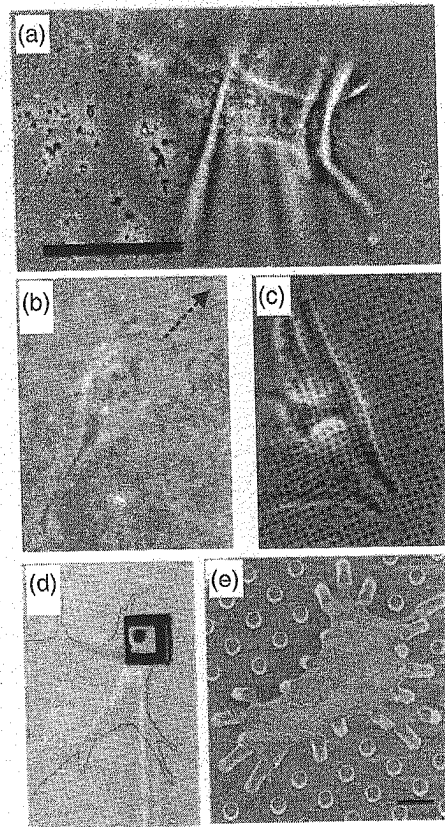
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### 65.4.1 Membrane Wrinkling

The thin membranes of liquid silicon rubber were cross-linked when exposed briefly to flame so that a thin skin of rubber was cured to  $\sim 1 \mu\text{m}$  thickness on top of the remaining liquid rubber that served as the lubricant layer between the glass coverslip [12,13]. Cells could be cultured on the silicone rubber, which is optically transparent and nontoxic, and as they spread on the skin surface, the adhesion forces they applied to the skin were strong enough to produce wrinkles and fold in the skin (Figure 65.3a). Directly underneath the cell, the wrinkles were circumferential with the cell boundary indicating that compressive forces created the folds in the membrane. At areas surrounding the adherent cell, the wrinkles projected out along radial lines from the cell boundary along the axes of tension forces. No observation of the cell pushing against the silicone membrane has been observed. This technique was a breakthrough in that cellular forces had not been experimentally observed and that qualitative measurement of the different regions of compression and tension could be observed simultaneously.

The membrane wrinkling technique has recently been improved upon with an additional fabrication step to reduce the stiffness of the substrate for increased wrinkles and folds and semi-quantitative



**FIGURE 65.3** Techniques for the measurement of cellular forces. (a) Adherent fibroblast cell exerts forces strong enough to wrinkle the underlying silicone rubber membrane (black bar:  $50 \mu\text{m}$ ). (Reproduced from Harris, A.K., P. Wild, and D. Stopak, *Science*, 1980, **208**: 177–179.) (b) Traction forces from migrating fibroblast (arrow indicates direction) are measured from the displacement of fluorescent microparticles embedded in a polyacrylamide substrate. (Reproduced from Munevar, S., Y.-L. Wang, and M. Dembo, *Biophys. J.*, 2001, **80**: 1744–1757.) (c) Contracting fibroblast distorts the regular array of micropatterned fluorescent dots. (Reproduced from Balaban, N.Q. et al., *Nat. Cell Biol.*, 2001, **3**: 466–472.) (d) Bending of horizontal microcantilever locally reports the traction force of a subcellular region during fibroblast migration. (Reproduced from Galbraith, C.G. and M.P. Sheetz, *Proc. Natl Acad. Sci., USA*, 1997, **94**: 9114–9118.) (e) Local contraction forces of smooth muscle cell are measured with an array of vertical elastomeric microcantilevers that deflect under cellular forces (black bar:  $10 \mu\text{m}$ ). (From Tan, J.L. et al., *Proc. Natl Acad. Sci., USA*, 2003, **100**: 1484–1489. With permission.)

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measurement of cellular forces in the hundreds of nanonewtons range. After the flame curing, the membranes were exposed to UV irradiation to weaken the cross-linking of the silicon sheets [14,15]. Applying a known tip-force from a glass pipette on the surface of the membrane and measuring the resultant wrinkles correlated the distortion and force relationship. Specifically, the researchers determined that the length of the wrinkles formed from the pipette was linear with the applied force and called it the “wrinkle stiffness.” Using this new technique, they observed that cytokinesis occurs through increased contractility at the equator of the cell, near the cleavage furrow. The traction force drops as the two daughter cells pinch apart. The newly formed cells migrate away from each other resulting in an increase in traction wrinkles until a strong enough force is generated to rupture the intercellular junction. The observed elastic recoil when the daughter cells break their junction causes them to rapidly separate and there is a relaxation in the surrounding wrinkles. Furthermore, the increased number of wrinkles in this technique allows for the measure of the different subcellular forces that occur during live cell migration. At the lamellipodium of a migrating cell, the wrinkles were radial and remain anchored to spots, possibly focal adhesion, as the cell advanced forward. Once these spots were located at the boundary between the lamellipodium and cell body, the wrinkles transitioned to compressive wrinkles. At the rear of the cell, the wrinkle forces diminished slower than the decreasing cell contact area so that the shear stress of the cell increased to pull it forward. The forces that occur at different regions of a cells attachment to the substrate reveal the coordination between pulling forces at the front of the cell and detachment forces that act against the migrating cell.

The membrane wrinkling technique is sensitive to cellular forces and can monitor the force changes at regions of interest within the adhesion area of a cell over time, but it is only a qualitative technique. It does not have adequate subcellular force resolution to measure the applied forces at the focal adhesions. Quantification of the force by means of the “wrinkle stiffness” is not an accurate measurement of forces due to the nonlinear lengthening of the wrinkles when forces are applied at multiple locations on the membrane. Moreover, the chaotic buckling of the membrane has a low repeatability, which makes matching the wrinkle patterns or lengths between experiments inaccurate.

#### 65.4.2 Traction Force Microscopy

To address these issues, traction force microscopy, a technique employing a nonwrinkling elastic substrate, was developed for cell mechanics [16]. The device layout is similar to Harris et al. in that a thin, highly compliant polymer membrane is cured on a glass coverslip on which cells are cultured, except that the membrane is not allowed to wrinkle. In addition to silicone rubber, polyacrylamide membranes have been used as the flexible membrane for cell attachment [17,18]. Instead of wrinkles, fluorescent beads with nanometer diameter were embedded into the material during the fabrication to act as displacement markers (Figure 65.3b). Fixing the sides of the membrane to the edges of the coverslip enables a prestress to be added to the membrane, which suppresses the wrinkling but enables adequate flexibility to allow in-plane traction forces to create visible displacements of the beads. Subtracting the position of the beads under the forces that the cell exerts and the position once the cell was removed from the surface determined the small movements of the beads between the two states, that is, relative displacement field. The corresponding force mapping of the cell is translated from the displacement field, which involves complex mathematical methods requiring the use of a supercomputer. The results provide spatial resolution of  $\sim 5 \mu\text{m}$  to measure the forces at smaller areas underneath the cell.

In obtaining the corresponding force map, the beads do not move as an ideal spring, in which the displacement is directly proportional to the applied force. Instead, many beads move in response to a single traction force because of the continuous membrane and their movement diminishes as a function of distance from the point of traction. As a result, many force mappings may be possible solutions for the measured displacement field. Appropriate constraints must be applied to the calculation for the solution to converge to a proper solution. Additionally, the displacement beads are discrete markers that are randomly seeded with a nonuniform density, resulting in the lack of displacement information in regions of the cell. To postulate on the magnitude and direction of the forces in the area between beads, a grid

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meshing approximation is superimposed on the cell area during the force calculation in order to solve for the force and displacement relationship at all regions. In fact, the placement of mesh nodes in these sparse areas leads to an ill-posed problem in solving for the force map because often more force points are introduced than there are displacement data due to the random seeding of beads underneath the cell area. Despite these limitations, the solution for the membrane displacement is well addressed in linear elastic theory [19]. The membrane can be regarded as a semi-infinite space of an incompressible, elastic material with tangential forces applied only at the boundary plane. Under these assumptions, the displacement field,  $d(\mathbf{m})$ , and the stress field,  $T(r)$  are related by an integral relation:

$$d_i(\mathbf{m}) = \iint G_{ij}(\mathbf{m} - \mathbf{r}) T_j(\mathbf{r}) d\mathbf{r} \quad (65.1)$$

where  $i, j \leq 2$  for the two-dimensional half-space and  $G_{ij}(\mathbf{m} - \mathbf{r})$  is Green's function that relates the displacement at position  $\mathbf{m}$  resulting from the point force at position  $\mathbf{r}$ . Obtaining the stress field requires inverting Equation 65.1, which is not always a unique solution because often there are not enough beads to determine all the force points. To address this problem, regularization schemes are used to apply additional criteria in selecting the solution of the inversion operation. These criteria include incorporating the constraint that the sum of all of the traction forces must balance, that the forces are only applied at the limited points of focal adhesions, and that the least complex solution be used.

In contrast to the random seeding of beads, microfabricated regular arrays of fluorescent beads have been imprinted onto the elastomeric substrate for improved force tracking [23]. The deformation of the marker pattern on the substrate is readily observed under the microscopy during the recording of a cell's forces (Figure 65.3c). The patterns are formed with Si and GaAs molds to create sub-micron spot diameters with 2 to 30  $\mu\text{m}$  spacing. The calculation for the force mapping is similar to the random seeding but with significant reduction in the number of possible solutions due to the uniform density of displacement markers throughout the cell area. The simplification of the problem makes the calculation readily attainable on a standard PC. Moreover, the regular pattern improved measurement power of the technique to a force resolution of 2 nN.

### 65.4.3 Micro-Cantilever Force Sensors

In the previous methods, the use of a continuous membrane for measuring cell forces has the inherent disadvantage that the discrete forces applied at the focal adhesions are convoluted with distribution of displacements. Since the force calculation is not direct, constraints and selection criteria are required in order to solve for the appropriate force mapping. The lack of a direct, linear technique to transduce the physical substrate deformation into unique traction force readings has necessitated the use of microfabricated devices to measure cellular forces. An innovative approach is the use of microcantilevers that act as force transducers. The first demonstration of these sensors is a horizontal cantilever fabricated on a silicon wafer where the cell bends the cantilever in the plane of the traction force as it migrates across it (Figure 65.3d) [20,21]. Since the sensor is mechanically decoupled from the substrate, the deflection of the cantilever directly reports only the local force. The simple spring equation relates the visually measured deflection of the cantilever beam,  $\delta$  to the cellular traction force:

$$F = K\delta \quad (65.2)$$

where  $K$  is the measured spring constant for the cantilever. The devices are constructed out of a polysilicon thin-film that is deposited on top of a phosphosilicate glass sacrificial layer. Once the sacrificial layer is etched away, the beam is freestanding and fully deflected under the force of a cell. These fabrication steps are labor-intensive and expensive, hence these devices are often reused between experiments. Even though this technique has quick force calculation, the horizontal design of the cantilever restricts the measurements to forces along one axis and only a single location on the cell.

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Modifying the design to a high-density array of vertical cantilevers improved both the spatial resolution of the force sensor and the scope of possible experiments [22]. With each cantilever placed perpendicular to the plane of traction forces, the spacing between each sensor is significantly reduced (Figure 65.3e). These devices are made from silicone rubber that has cylindrical cantilevers formed from a microfabricated mold. The cost per device is inexpensive once the reusable mold has been built and so the devices are disposable. The tips of the cantilevers are coated with extracellular matrix proteins so that cells attach and spread across several cantilevers. The bending of the posts is easily measured under a microscope as the cells probe the tips and apply traction forces. As with the horizontal design, the deflection of the posts is related by a simple relationship between force and displacement:

(65.1)

$$F = \left( \frac{3EI}{L^3} \right) \delta \quad (65.3)$$

where  $E$  is the modulus of elasticity of the silicone rubber,  $I$  is the moment of inertia, and  $L$  is the length of the cantilevers. However, the deflection of the post is not limited to one axis, the force reported is a true vector quantity in which force mappings are possible with an equivalent resolution to those from traction force microscopy. With the close proximity between sensors and measuring independence between them, the array of vertical cantilevers can examine cells at a higher population density than previous methods. Moreover, the technique allows for more relevant studies than previously possible because the forces of large monolayers of cells can be measured. This technique does expose the cell to a topology that is not akin to *in vitro* conditions, which may have an affect on its biological response.

## 65.5 Conclusion

The mechanical force that cells experience in the environment directly regulate their function in healthy tissue. Through the sensing of these forces, cells interact with these mechanical signals through biological responses and mechanical force generation of their cytoskeletal structures and motor proteins. The engineering of deformable substrates to measure the cellular forces has provided powerful insight into the protein interactions associated with mechanotransduction. However, despite these advances, these devices have several issues that need to be addressed in order to overcome their limitations. First, one needs to consider how the cell reacts to the new environment that the tooling presents it. The nonplanar topology and high compliance of the vertical microcantilever substrate may cause the cell to react to an environment that is physiologically irrelevant. Additionally, chemical composition of substrate or deposited extracellular matrix may have a direct effect on what signaling pathways are activated during its mechanical response. Second, since the devices used in cellular mechanics studies are prototypes, they may be lacking in adequate calibration between samples or quality control in device fabrication. These variations are significant if the cell population studied is not sufficiently large, as in the case of expensive or labor-intensive techniques. Lastly, the construction of these devices needs to be simple enough so that widespread use is possible. A large collective effort can be used to screen the numerous protein interactions that occur during the mechanotransduction signaling pathways. In this manner, the understanding of the interaction between mechanical forces and biological response can provide valuable insight into the treatment of diseased states of tissue or cancer.

(65.2)

To achieve this goal, there are many future directions that the techniques described can be advanced to. Foremost is the integration of cellular mechanics instrumentation with other fluorescent microscopy techniques, such as fluorescent recovery after photobleaching (FRAP), GFP protein-labeling, and fluorescent resonant emission transfer (FRET). These optical techniques allow one to detect proteins at the single molecular level, and in combination with force mapping, provide a correlation between molecular activity and observable mechanics. The incorporation of nanotechnology materials or devices may provide powerful new sensors that improve both spatial resolution and force measurement. Since the size of a focal adhesion is ten to hundreds of nanometers and the force of a single actomyosin motor is

few piconewtons, the ability to resolve these structures would provide greater insight into the mechanical behavior of cells. Additionally, the constructions of three-dimensional measurement techniques, be it in gels or more complex sensing devices, would extend the current two-dimensional understanding of force mechanics into an environment more pertinent to cellular interactions in living tissue. One early attempt has been made where two traction force substrates have been used to sandwich a cell while providing some understanding of three-dimensional forces, the substrates are still planar and constrain how the cell organizes its cytoskeleton and adhesions along those planes. Lastly, strong exploration into the development of devices or techniques that are usable for *in vivo* studies of mechanotransduction would open new areas of treatment for diseases in the cardiovascular and skeletal systems.

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