35. Cellular Nanomechanics

Numerous applications of nanotechnology have been developed to probe the unique mechanical properties of cells. In addition, since biological materials exhibit such a wide spectrum of properties, they offer new concepts for nonbiological biomimetic applications. In this chapter, the viscoelastic properties of a cell and its subcellular compartments are described. First, a qualitative picture is presented of the relevant building blocks: the cytoskeleton, cell membrane, nucleus, adhesive complexes, and motor proteins. Next, the various methods used to probe cellular and subcellular mechanics are described, and some of the quantitative results presented. These measurements are then discussed in the context of several theories and computational methods that have been proposed to help interpret the measurements and provide nanomechanical insight into their origin. Finally, current understanding is summarized in the context of directions for future research.

35.1 Overview

35.1.1 The Importance of Cell Mechanics in Biology and Medicine

All living things, despite their profound diversity, share a common architectural building block: the cell. Cells are the basic functional units of life, yet they are themselves comprised of numerous components with distinct mechanical characteristics. To perform their various functions, cells undergo or control a large range of intra- and extracellular events, many of which involve mechanical phenomena or may be guided by the forces experienced by the cell. These micro- and nanomechanical phenomena range from macroscopic events such as the maintenance of cell shape, motility, adhesion, and deformation, to microscopic events such as how cells sense mechanical signals and transduce them into a cascade of biochemical signals, ultimately leading to a host of biological responses.
Cell mechanics plays a major role in biology and physiology. The ability of a cell to perform its function often depends on its shape, and shape is maintained through structural stiffness. In the blood circulation system, red blood cells, or erythrocytes, exist in the form of biconcave disks that are easily deformed to help facilitate their flow through the microcirculation and have a relatively large surface-to-area ratio to enhance gas exchange. White cells, or leucocytes, are spherical, enabling them to roll along the vascular endothelium before adhering and migrating into the tissue. Because their diameter is larger than some of the capillaries they pass through, leucocytes maintain excess membrane in the form of microvilli so that they can elongate at constant volume and not obstruct the microcirculation. Airway epithelial cells are covered with a bed of cilia, finger-like cell extensions that propel mucus along the airways of the lung. Lastly, the cytoskeletal structure in muscle cells is specifically organized to actively generate forces and to sustain large strains. In each of these examples, the internal structure of the cell along with the cell membrane provide the structural integrity to maintain the particular shape needed by the cell to accomplish its function, although the specific components of the structure are highly variable and diverse.

Cell mechanics also plays an important role in cell migration. Migration is critical during early development, but also in fully differentiated organisms. For example, in wound repair when cells from the surrounding, undamaged tissue migrate into the wound, in angiogenesis (i.e., the generation of new blood vessels), or in combating infection when cells of the immune system transmigrate from the vascular system across the vessel wall and into the infected tissues.

Cell migration processes occurs in several stages that include:

1. **Protrusion**, the extension of the cell at the leading edge in the direction of movement
2. **Adhesion** of the protrusion to the surrounding substrate or matrix
3. **Contraction** of the cell that transmits a force from these protrusions at the leading edge to the cell body, pulling it forward
4. **Release** of the attachments at the rear, allowing net forward movement of the cell to occur [35.1–4]

Cellular mechanics and dynamics are critical modulators at each of these steps.

The importance of cell mechanics in biology is most apparent in mechanotransduction, i.e., the ability of the cell to sense and respond to externally applied forces. Most cells are able to sense when a physical force is applied to the cell, and respond through a variety of biological pathways leading to such diverse effects as changes in membrane channel activity, up- or downregulation of gene expression, alterations in protein synthesis, and altered cell morphology. The signaling cascades that become activated as a consequence of mechanical stress have generally been well characterized. However, the initiating process by which cells convert the applied force into a biochemical signal is much more poorly understood, and only recently have researchers begun to unravel some of these fundamental mechanisms. Some studies have suggested that a change in membrane fluidity acts to increase receptor mobility, leading to enhanced receptor clustering and signal initiation. Stretch-activated ion channels or strain-induced activation of G proteins represent other means of mechanotransduction. Similarly, disruption of microtubules or conformational changes of cytoskeletal proteins that alter their binding affinities have been proposed as cellular mechanosensors. Yet others have focused on the role of the glycocalyx, a layer of carbohydrate-rich proteins on the cell surface, in the response of endothelial cells to fluid shear stress. Another potential mechanism is that forced deformations within the nucleus could directly alter transcription or transcription factor accessibility. Constrained autocrine signaling is yet another mechanism whereby the strength of autocrine signaling is regulated by changes in the volume of extracellular compartments into which the receptor ligands are shed. Changing this volume by mechanical deformation of the tissues can increase the level of autocrine signaling [35.5]. Finally, others have proposed conformational changes in intracellular proteins in the force transmission pathway connecting the extracellular matrix with the cytoskeleton through focal adhesions (FAs) as the main mechanotransduction mechanism. While all or a subset of these theories may contribute to mechanotransduction, little direct evidence has been presented in their support. For reviews of this topic, see [35.6–12].

### 35.1.2 Examples Drawn from Biology and Pathophysiology

Many studies during the past two decades have shed light on a wide range of cellular responses to mechanical stimulation. It is now widely accepted that stresses experienced in vivo are instrumental in a wide spectrum of pathologies. One of the first diseases found to be linked to cellular stress was atherosclerosis, where
it was demonstrated that hemodynamic shear influences endothelial function, and that conditions of low or oscillatory shear stress are conducive to the formation and growth of atherosclerotic lesions. Even before then, the role of mechanical stress on bone growth and healing was widely recognized, and since then, many other stress-influenced cell functions have been identified. Mechanotransduction of muscle cells is pivotal to exercise-induced muscle growth (hypertrophy), and defects — caused for example by mutations in mechanosensitive proteins — can result in muscular dystrophies. Asthma is another particularly salient example where the epithelial cells lining the airways are subjected to stresses as the pulmonary airways constrict during breathing, and airway wall remodeling. Further knowledge of the mechanisms by which cells respond to such forces could enhance our understanding of these diseases.

35.2 Structural Components of a Cell

Aside from providing the outer boundary of a cell and nucleus, membranes enclose numerous intracellular structures as well, and the discussion in this section pertains to all of these.

35.2.1 Membranes

The term membrane generally refers to the phospholipid bilayer and the proteins associated with it. The phospholipids contained in the membrane are arranged in two layers, or leaflets, with their hydrophobic tails pointing inward and their hydrophilic heads outward. Together, they constitute a bilayer $\approx 6$ nm in thickness (Fig. 35.1).

![Fig. 35.1 A model of the lipid bilayer, showing the hydrophilic heads (polar head groups) on the exterior surfaces and the hydrophobic tails (fatty acid tails) pointing in. Also shown are examples of transmembrane or integral membrane proteins. The total thickness of the bilayer is $\approx 6$ nm (after [35.13])](image)

In addition to the phospholipids, the membrane contains glycolipids and cholesterol. While the amount of glycolipids is small, constituting only $\approx 2\%$ of the total lipid content, cholesterol is a major membrane constituent, $\approx 20\%$ by weight, a value that remains quite constant among different cell types. The specific membrane composition is critical for determining membrane structural integrity; for example, both the bending stiffness and the viscosity of the lipid bilayer are strongly dependent on the cholesterol content. Cellular membranes also contain many membrane-associated proteins, which account for $\approx 50\%$ of the membrane by weight but, because of their relatively large molecular weight, only $\approx 1–2\%$ of the number of membrane molecules. Membrane proteins serve a variety of functions, ranging from signaling to transport of ions and other molecules across the membrane to cell–cell and cell–matrix adhesion.

The plasma membrane has associated macromolecular structures on both intra- and extracellular sides, giving rise to a three-layer composite construction. On the intracellular side, the membrane is physically attached to a cortex or the cytoskeleton. The cortex is a dense, filamentous structure that lends stiffness to the membrane, and can also interact with various transmembrane proteins, often impeding their free diffusion either by steric interactions or direct chemical bonding. In some cells, the cortex is simply a region of dense cytoskeletal matrix in the vicinity of the bilayer. In others, it exhibits a distinctly different structure or composition; for example, erythrocytes possess a cortex comprised of a network of spectrin tetramers linked by actin filaments. This network is attached to the membrane by ankyrin and the integral membrane protein band 3. This spectrin network accounts for much of the bending stiffness exhibited by the red cell membrane.
Most cells are coated by a glycocalyx, which has been shown to extend as far as 0.5 μm from the surface of endothelial cells, where it forms a compressible barrier separating circulating erythrocytes and leucocytes from the endothelial membrane. The glycocalyx is comprised of short oligosaccharide chains, glycoproteins, glycolipids, and high-molecular-weight proteoglycans, all organized into an interconnected network with an overall negative charge. Although its function is not completely understood, it apparently plays a role in macromolecular transport across the endothelium and is an important factor in the interaction between bloodborne cells and the endothelium. Studies have demonstrated that the glycocalyx in a capillary is readily compressed by a passing leucocyte, yet is sufficiently rigid to prevent flowing erythrocytes from approaching the endothelial surface.

35.2.2 Cytoskeleton

The cytoskeleton is the network of biopolymers that permeates the cell and largely account for its structural integrity. At high magnification, this network appears to be comprised of several distinct types of intertwined filaments with a variety of interconnections, as can be seen in the micrograph in Fig. 35.2. The apparent stiffness of the network, as that of other fibrous materials, depends fundamentally upon the elastic properties of the constituent fibers.

The cytoskeletal matrix is primarily comprised of three constituents, actin microfilaments (∼7–9 nm in diameter), microtubules (24 nm), and intermediate filaments (∼10 nm) (Table 35.1). These form a complex interconnected network that exists in a state of constant flux, especially when the cell is dividing, migrating or undergoing other dynamic processes. All are polymers built from protein subunits, held together by noncovalent bonds. They also share the common feature that they cross-link, often with the aid of other proteins, to form bundles and lattice networks. The higher-order structures formed by these polymers, as we will see, are critical determinants of cytoskeletal elasticity and can vary substantially between different cells.

Actin Microfilaments

Actin filaments play an essential role in virtually all types of motility. Actin–myosin interactions are of obvious importance in muscle, but are also instrumental in the migration and movement of nonmuscle cells. Actin polymerization is thought to be one of the factors initiating cell migration through the formation of filopodia or lamellipodia [35.15].

Actin is one of the most prevalent proteins found in the cell, ranging in concentration from 1–10% by weight of total cell protein in nonmuscle cells, to 10–20% in muscle. Molecular actin is comprised of 375 amino acids (molecular weight 43 kDa) and is found in at least six forms that differ from each other only slightly. Of these, four are found in muscle, the other two in nonmuscle cells. Actin exists either in globular form (G-actin monomers) or filamentous form to form bundles and lattice networks. The higher-order structures formed by these polymers, as we will see, are critical determinants of cytoskeletal elasticity and can vary substantially between different cells.

### Table 35.1

<table>
<thead>
<tr>
<th></th>
<th>Diameter (2a) (nm)</th>
<th>Persistence length ( l_p ) (μm)</th>
<th>Bending stiffness ( K_b ) (N m^2)</th>
<th>Young’s modulus ( E ) (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin filaments</td>
<td>9–10</td>
<td>15</td>
<td>( 7 \times 10^{-26} )</td>
<td>1.3–2.5 \times 10^9</td>
</tr>
<tr>
<td>Microtubules</td>
<td>25</td>
<td>6000</td>
<td>( 2.6 \times 10^{-23} )</td>
<td>1.9 \times 10^9</td>
</tr>
<tr>
<td>Intermediate filaments</td>
<td>10</td>
<td>1–3</td>
<td>( 4–12 \times 10^{-27} )</td>
<td>1.3 \times 10^9</td>
</tr>
</tbody>
</table>
(F-actin polymer), with the balance between the two being a highly dynamic process that is finely regulated by a variety of different factors. F-actin is a long, flexible filament, \( \approx 9–10 \) nm in diameter. Subunits (monomers) are organized into a double-stranded helix having structural and functional polarity (pointed or negative, and barbed or positive ends) and a half-pitch of \( \approx 37 \) nm (Fig. 35.3a). At the barbed end, an adenosine triphosphate (ATP) binding cleft is exposed, allowing for binding of monomer and linear growth of the filament. ATP binding and hydrolysis play a critical role in regulating actin dynamics and controlling the length of the actin filament.

The F-actin filaments can further organize into quaternary structures such as bundles or a lattice network with the aid of actin binding proteins (ABP). The bundles, also referred to as stress fibers, are closely packed parallel arrays of filaments, connected to each other by several members of the ABP family (e.g., \( \alpha \)-actinin, fascin, and scruin). Stress fibers can vary in size, but are typically several hundred nanometers in diameter. Actin stress fibers tend to form when the cell requires additional strength, such as in endothelial cells, in response to an elevated shear stress, or in migrating fibroblasts. These fibers often concentrate around and attach to focal adhesion sites, and are therefore critical to cell adhesion. Actin networks consist of an interconnected matrix of F-actin filaments, the junctions of which are often seen to be nearly orthogonal. At least two distinct types of network are observed – cortical (membrane-associated and more planar in nature) and non-membrane-associated, which possess a more isotropic three-dimensional structure. The formation of bundles and networks is facilitated by a variety of cross-linking proteins such as filamin, which forms a V-shaped polymer that connects two actin filaments nearly at right angles.

The elastic properties of actin filaments have been measured in a variety of ways: by axial stretch [35.16], twisting [35.17, 18], and bending [35.19]. By all methods, single actin filaments were found to exhibit a Young’s modulus in the range of \( (1.3–2.5) \times 10^9 \) N/m\(^2\). This range compares favorably with that measured for silk and collagen [35.20] and is also roughly consistent with predictions based on van der Waals bonding between surfaces [35.14].

**Intermediate Filaments**

Intermediate filaments, which form \( \approx 10 \) nm-diameter fibers, are much less studied than actin and not as well characterized. Perhaps this is due to the fact that there is no single molecular constituent that comprises the intermediate filaments (IFs); instead, there are more than 50 different IF genes that have been identified.

Intermediate filaments constitute \( \approx 1\% \) of total protein in most cells, but can account for up to 85% in cells such as epidermal keratinocytes and neurons [35.21]. While they come in many varieties, they share a common structural organization. All have a large central \( \alpha \)-helical rod domain flanked by amino- and carboxy-terminal domains. Assembly occurs by the formation of dimers into a coiled coil structure. Then the dimers assemble in a staggered antiparallel array to form tetramers that connect end-to-end to form apolar protofilaments. These protofilaments assemble into a rope-like structure containing \( \approx 8 \) subunits each (Fig. 35.3c) [35.21]. Although intermediate filaments are more stable than microfilaments, they can be modified by phosphorylation. Intermediate filaments exhibit a lower bending stiffness than either microfilaments or microtubules, as evidenced by their persistence length of only 1–3 \( \mu \)m. Linker proteins such as bullous pemphigoid antigen 1 (BPAG1) and plectin contain both actin and IF binding domains, providing a means by which these networks can be linked. Evidence also exists for plectin binding to microtubules.

Intermediate filaments are often found surrounding the nucleus and extending outward to the plasma membrane. In epithelial cells, keratin filaments connect to the plasma membrane at desmosomes and hemidesmosomes and help them to withstand mechanical stress.

**Microtubules**

Microtubules are important in determining cell shape, and they play a critical role in separating chromosomes during mitosis. Microtubules are central to the motion of cilia and flagella. Compared with either microfilaments or intermediate filaments, microtubules are rigid structures, but exist in a dynamic equilibrium, much as do microfilaments. Microtubules take the form of hollow cylinders with \( \approx 25 \) nm outer diameter and 14 nm inner diameter. The tubular structures are comprised of tubulin, a globular dimer consisting of two 55 kDa polypeptides, \( \alpha \)- and \( \beta \)-tubulin. The dimers polymerize to form microtubules that consist of 13 linear protofilaments forming a hollow-cored cylinder (Fig. 35.3b). The filaments are polar, having a rapidly growing end and a slowly growing end, mediated by hydrolysis of guanosine triphosphate (GTP) after polymerization. If hydrolysis occurs too quickly, before new GTP-bound tubulin can bind to the end, the microtubule might disassemble. Depending on the rate of hydrolysis and rate
Fig. 35.3 (a) Schematic showing the polymerization of G-actin monomers to form F-actin. (b) α- and β-tubulin organized into microtubules. (c) Organizational structure of an intermediate filament of GDP-bound tubulin addition to the end, the microtubule can either grow or shrink [35.22]. In fact, free ends tend to alternate between periods of steady growth and disassembly in a stochastic manner.
During interphase, microtubules tend to be anchored at their negative ends to the centrosome, located near the nucleus. From there they extend to all parts of the cell, suggesting a strong role in maintaining the structural integrity of the cell and providing a cellular highway system for cargo transported by motor proteins such as the kinesins and dyneins. In many of these situations, the microtubule is thought to play a structural role that relies on it having a high bending stiffness, which was found to be on the order of $2.6 \times 10^{-23} \text{ N m}^2$ [35.20].

### 35.2.3 Nucleus

The nucleus is the distinguishing feature of eukaryotic cells and directs and controls deoxyribonucleic acid (DNA) replication, ribonucleic acid (RNA) transcription and processing, and ribosome assembly [35.23]. Most eukaryotic cells contain a single nucleus. However, some specialized cells (e.g., red blood cells) become anucleate during their maturation, while other cells such as skeletal and cardiac muscle cells can become multinucleated due to cell fusion. With a diameter in the range $\approx 5–20 \mu \text{m}$, the nucleus is the largest cellular organelle. The nucleus is separated from the cytoplasm by the nuclear envelope, which consists of two lipid bilayers, the inner and outer nuclear membrane, and the underlying nuclear lamina, a dense protein network consisting mostly of lamin proteins that control the nuclear shape, size, and stability (Fig. 35.4). The outer nuclear membrane is continuous with the endoplasmic reticulum and connects to the inner nuclear membrane at the nuclear pores, thus enclosing the perinuclear space. Nuclei typically contain a few thousand nuclear pores, comprised of hundreds of proteins that form the nuclear pore complex that controls transport between the nucleus and the cytoplasm [35.24].

The nuclear interior contains the packaged DNA in the form of chromatin as well as diverse intranuclear compartments referred to as subnuclear bodies that include nucleoli, Cajal bodies, and promyelocytic leukemia bodies (PML) bodies. These subnuclear bodies are not surrounded by membranes but self-organize through processes only incompletely understood. Chromatin can be organized into two distinct forms, the dense and transcriptionally silent heterochromatin often located at the nuclear periphery, and the gene-rich and transcriptionally active euchromatin. Chromatin is comprised of 30 nm fibers that arise from regular wrapping of DNA around histone octamers to form nucleosomes resembling beads on a string and subsequent further compaction facilitated through the linker histone H1. This process allows the $\approx 2 \text{ m}$ of human DNA to be packaged into a nucleus a few micrometers in diameter. The chromatin fibers in turn form higher-order structures such as euchromatin and heterochromatin, which can be dynamically regulated by biochemical modification (e.g., by methylation, acetylation or phosphorylation) of histone proteins and DNA. Furthermore, chromatin fibers from single chromosomes often form distinct and nonoverlapping chromosome territories. In addition to chromatin and nuclear bodies, the nuclear interior also contains several structural proteins, including nuclear actins, myosin, spectrin, and nucleoplasmic lamins A and C [35.25–29]. However, it remains unclear what intranuclear structures (often referred to as nuclear matrix) these proteins form within the nuclear interior and how such structures could contribute to nuclear processes such as transcription.

Importantly, the nucleus cannot be viewed in isolation from the surrounding cytoskeleton. The molecular mechanism by which the nucleus is connect to the cytoskeleton has puzzled researchers for many years, as it was unclear how forces required for nuclear positioning and anchoring could be transmitted across the 50 nm-wide perinuclear space. Recent work in *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammalian cells led to the discovery of two new fami-

![Fig. 35.4 Schematic drawing of a mammalian cell nucleus. The nucleus is surrounded by the inner and outer nuclear membranes and the underlying nuclear lamina. Nuclear pores allow transport between the nuclear interior and the cytoplasm. The nuclear interior consists of densely packed heterochromatin, mostly located at the nuclear periphery, the transcriptionally active euchromatin, and several intranuclear structures such as Cajal and PML bodies and the nucleoli. Not shown are intranuclear structures formed by lamins and other proteins that constitute the nuclear matrix.](image-url)
lies of nuclear envelope proteins that are ideally suited
to transmit forces from the cytoskeleton across the nu-
clear envelope to the nuclear interior [35.30–39]. These
findings have led to the current model of nuclear–cytoskeletal coupling (Fig. 35.5), in which large nesprin isoforms located on the outer nuclear membrane can

![Diagram of nuclear cytoskeletal coupling](image)

**Fig. 35.5** Nuclear cytoskeletal coupling. Schematic drawing of the current model of nuclear cytoskeletal coupling in mammalian cells. The nesprin 1 and nesprin 2 giant isoforms contain an N-terminal actin-binding domain that can interact with cytoskeletal actin filaments. Shorter isoforms of nesprin 1 and 2 do not contain an actin-binding domain, but it is thought that the large spectrin repeat regions (brown and grey spheres) can interact with other cytoskeletal elements. Nesprin 3 can directly bind to plectin, which can associate with intermediate filaments. Nesprins can interact with the inner nuclear membrane proteins SUN1 and SUN2 across the perinuclear space through their C-terminal KASH (Klarsicht, ANC-1, Syn homology)-domain. SUN proteins can in turn bind to the nuclear lamina, nuclear pore complexes, and possibly other, yet to be identified, proteins at the inner nuclear membrane. These proteins interact with chromatin and intranuclear proteins, thus completing the physical link between the cytoskeleton and the nuclear interior.

bind to cytoskeletal F-actin and intermediate filaments. At the same time, nesprins physically interact across the perinuclear space with Sad1p/UNC-84 (SUN) proteins, which are located at the inner nuclear membrane. There, SUN proteins can bind to lamins, chromatin, and other as-yet unknown nuclear envelope proteins, thus creating a physical link between the cytoskeleton and the nucleus [35.32]. Due to this intricate coupling between the nucleus and the cytoskeleton, defects in nuclear envelope proteins can have direct effects on cytoskeletal structure and mechanics. For example, fibroblasts lacking the nuclear envelope proteins lamin A and C have reduced cytoskeletal stiffness and disturbed actin, vimentin, and microtubule organization [35.40–42], and mutations in nesprins, similar to lamins, can result in muscular dystrophies [35.43].

**35.2.4 Cell Contractility and Motor Proteins**

All muscle cells use the molecular motor comprised of actin and myosin to produce active contraction. These are arranged in a well-defined structure, the sarcomere, and the regularity of the sarcomeres gives rise to the characteristic striated pattern seen in skeletal muscle cells and cardiac myocytes. Importantly, even nonmuscle cells contain contractile machinery, which they use for a variety of functions such as maintaining cell tension, changing cell shape, and cell migration. Prominent bundles of actin filaments, called stress fibers, are contractile and house myosin filaments. The macromolecular organization of stress fibers bears similarities to sarcomeres – actin filaments are in parallel arrangement with the filament polarity alternating alongside; laterally a space of $\approx 10 \text{ nm}$ is maintained that situates parallel bipolar myosin II filaments in between. The myosin head moves toward the positive pole of the actin filaments during a power stroke, producing a net contractility for an alternating-polarity arrangement. Stress fibers (like sarcomeres) have a troupe of proteins, e.g., $\alpha$-actinin, filamin, troponin, caldesmon, and tropomyosin, which control the arrangement of actin and myosin, and regulate their interaction. The formation and strengthening of stress fibers is intricately linked to the mechanism by which cells respond to their force environs. First, stress fibers are known to form between points in the cell where actin myosin contractility is resisted. Typically these are hot-spots of protein activity known as focal adhesions, where the actin cytoskeleton gets anchored to transmembrane integrins, which are anchored to the matrix proteins. The focal adhesion also includes a cascade of proteins that relay...
biochemical signals, regulate its strengthening, and organize the cytoskeleton for the growth of stress fibers (like cross-linking actin and recruiting myosin).

35.2.5 Adhesion Complexes

Adhesion complexes are collections of proteins forming a physical linkage between cytoskeleton and extracellular matrix (ECM) and between cells. A cell can use focal adhesions to gain traction on the ECM during the process of spreading and migration. The assembly of focal adhesions is a dynamical process that is closely regulated by the mechanical and chemical cues that the cell experiences. Both intracellular and extracellular mechanical stresses transmitted through focal adhesions are important in the formation of a focal adhesion complex, whereas the release of stress results in the turnover of a focal adhesion. The myosin-mediated contractile force transmitted to the ECM and the tension applied to the adhesion complex is necessary for promoting focal adhesion development. In contrast, disruption of myosin activity effectively inhibits the formation of focal adhesions. In the absence of myosin contractile force, externally applied mechanical forces can also promote the formation of focal adhesions. This force-regulated focal adhesion assembly allows a cell to probe the mechanical stiffness of its surroundings and respond accordingly, for example, by migrating in the direction of increasing substrate stiffness. Adhesion complexes also play an important role in helping tissues form by holding the cells together. The cells of most tissues are bound directly via cell–cell junctions. Cell–cell adhesion complexes are found in many different types, depending on tissue and cell type, and serve in both mechanical coupling of cells as well as intercellular transport.

35.3 Experimental Methods

35.3.1 Methods of Force Application

Measuring cellular or subcellular biomechanics often requires the application of precisely controlled forces to single or multiple cells and quantification of the induced deformation, although some techniques rely on detecting forces generated by the cells or on observing particles within the cytoplasm subjected to thermal motion. Consequently, experimental methods can be divided into active (Table 35.2, Fig. 35.6) and passive techniques. In the active techniques, applied forces are generally in the same range as physiological forces acting at the cellular and molecular level (Table 35.3), and induced displacements are on the nanometer or micrometer scale. For most methods – whether they are active or passive – the cellular deformations are detected based on computer-based image analysis of bright-field or fluorescence microscopy images, but some techniques also apply quadrant photodiode detectors for faster and higher-resolution tracking of microspheres embedded in the cytoplasm.

Active Measurements

Atomic Force Microscopy. Atomic force microscopy (AFM) – only developed in 1986 – is now routinely used in cell biology to image cells, measure cytoskeletal stiffness, and quantify single-molecule interactions. In an atomic force microscope, a small tip attached to

<table>
<thead>
<tr>
<th>Method</th>
<th>Typical force range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic force microscopy</td>
<td>10 pN–100 nN</td>
</tr>
<tr>
<td>Microindenter</td>
<td>1–100 nN</td>
</tr>
<tr>
<td>Microplate stretcher</td>
<td>1–100 nN</td>
</tr>
<tr>
<td>Magnetic bead microrheology (twisting)</td>
<td>10 pN–1 nN</td>
</tr>
<tr>
<td>Magnetic bead microrheology (pulling)</td>
<td>100 pN–10 nN</td>
</tr>
<tr>
<td>Optical traps</td>
<td>1–500 pN</td>
</tr>
<tr>
<td>Micropipette aspiration</td>
<td>1–100 nN</td>
</tr>
<tr>
<td>Substrate strain</td>
<td>1–30% strain</td>
</tr>
<tr>
<td>Shear flow</td>
<td>1–100 Pa</td>
</tr>
<tr>
<td>MEMS devices</td>
<td>0.5–1500 nN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological force</th>
<th>Force range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force generated by motor proteins (e.g., kinesin, myosin)</td>
<td>≈ 1–10 pN</td>
</tr>
<tr>
<td>Force transmitted by protein–protein interactions</td>
<td>≈ 1–200 pN  (rate dependent)</td>
</tr>
<tr>
<td>Force required for (partial) protein unfolding</td>
<td>≈ 100 pN</td>
</tr>
<tr>
<td>Force generated by migrating or contracting cells</td>
<td>≈ 1 nN–10 μN</td>
</tr>
</tbody>
</table>
Part D

Bio-/Nanotribology and Bio-/Nanomechanics

Experimental methods for cellular force application. Overview of techniques to apply precisely controlled forces or deformations to cells for active cellular biomechanics measurements: (a) atomic force microscopy, (b) microindentation or cell poking, (c) parallel microplate stretcher, (d) pulling magnetic bead micro rheology (single-pole magnetic trap), (e) optical trap, (f) micropipette aspiration of adherent cell, (g) substrate strain experiments, (h) flow chamber for fluid shear-stress application, (i) micromachined device, in which the cell is plated onto microscopic platforms that are then moved apart, (j) cell plated on micropillars that deform (i.e., deflect) under the cellular traction force. This passive technique can be modified by embedding small magnetic particles in some of the pillars, which can then be actively manipulated to apply a highly localized force onto the basal cell surface.

A flexible cantilever is controlled with (sub-)nanometer precision to carefully probe (i.e., indent) the cell surface. Deflection of the cantilever can then be used to infer the indentation depth and calculate the applied force. The tip of the cantilever typically has the shape of a pyramid (with a tip radius of \( \approx 20 \) nm), but some applications use small polystyrene beads (0.1–2 \( \mu \)m diameter) attached to the cantilever or tipless probes to provide a larger contact area with the cell. The mechanical properties of the probed cell (e.g., Young’s modulus or shear modulus) are inferred from the force–indentation curves, often assuming a Hertz model for linear elastic, isotropic material [35.44], although several modifications have been proposed to account for example for the finite thickness of thin cell extensions such as lamellipodia [35.45]. These theoretical models often provide a surprisingly good fit to the experimental data, despite the fact that cells are nonisotropic, nonhomogeneous, and nonlinear elastic materials. One benefit of the atomic force microscope is that the usable force range spans several orders of magnitude (Table 35.2), depending on the spring constant of the selected cantilever. Therefore, AFM can also be used for single-molecule measurements, for example, to measure bond strength. In this case, the AFM probe is functionalized with low concentrations of the protein of interest and then brought into contact with the appropriate binding partner immobilized on a rigid surface. After binding occurs, the AFM tip is then carefully retracted until the molecules dissociate, which results in a sudden drop of force. Rupture forces at different velocities can be directly inferred from AFM data.

**Microindenter and Microplate Stretcher.** These custom-made devices are closely related to the AFM principle. In the case of the microindenter, a small (\( \approx 10–100 \) \( \mu \)m-diameter) probe is used to poke single cells while measuring induced cytoskeletal and nuclear deformations under a fluorescence microscope. The applied force is measured with a sensitive force transducer attached to the indenter. For the microplate stretcher, cells are placed between a rigid, piezo-controlled plate and a thin, flexible plate and allowed to adhere to both plates. The slides are then slowly moved apart, thus stretching the cell between the plates while imaging the experiment under a microscope. Here, the induced deflection of the thin plate is used to infer the applied force. The forces that can be achieved with these tools are higher than that of a typical atomic force microscope and are sufficient to induce significant deformations of an entire cell.

**Magnetic Twisting Cytometry.** In this technique, mechanical measurements are based on the displacements of small (\( \approx 0.2–5 \) \( \mu \)m diameter), ferromagnetic or paramagnetic beads attached to the cell surface and subjected to a magnetic force. Early versions of this technique [35.46, 47] used two orthogonal magnetic fields, one to magnetize the ferromagnetic particles with a brief, intense pulse, and the second one to induce...
a twisting, magnetic torque to the beads. The induced bead rotation is then measured by a change in the orientation of the induced magnetic field or through microscopic observations. The latter approach offers the advantage that it can detect rotation and displacement of individual beads, so that loosely attached beads that might rotate freely can be excluded from the overall measurements. In a related variation of this technique, often referred to as magnetic trap or tweezers, paramagnetic beads on the cell surface or inside the cytoplasm are manipulated by a single- or multipole electromagnet controlled through a computer. The force acting on a paramagnetic bead inside a magnetic field is given by the equation \( F = \mu_0 \chi V \nabla (H \cdot H) \), where \( F \) is the magnetic force, \( \mu_0 \) is the permeability constant, \( \chi \) is the volume susceptibility, \( V \) is the bead volume, and \( H \) is the external magnetic field strength. Thus, the applied force increases with increasing field strength and increasing field gradient. For a single-pole magnetic trap, the magnetic field decays rapidly with increasing distance from the tip of the pole. This results in a steep gradient and large forces near the tip that exponentially decay away from the tip, requiring careful calibration of the magnetic trap. One of the limitations of single-pole magnetic traps is the unidirectional force direction, i.e., forces can only be exerted in the direction towards the magnetic trap (pulling), but this can be overcome by using multipole magnetic traps. Another limitation is that the bead localization on the cell is random, so care must be taken to only compare results from cells with similar bead positions (e.g., on the nucleus, the nuclear periphery or the lamellipodia). Lastly, the induced bead rotation and displacement are strongly dependent on the bead attachment angle, i.e., how deep the magnetic bead is embedded in the cell surface, requiring careful controls or confirmation by confocal three-dimensional (3-D) reconstruction.

**Optical Traps/Tweezers.** This technique is similar to the magnetic trap experiments, as the induced displacement of microscopic beads (50–1000 nm) attached to the cell surface or inside the cytoplasm subjected to a precisely controlled force is used to determine the mechanical properties of the cytoskeleton. The major difference is that, in an optical trap, a focused laser beam is used to position and displace a bead with high refractive index on the cell, allowing precise bead manipulation in all directions. The optical trap acts as an elastic spring with a tunable spring constant, so the force applied to the bead can also be controlled with high precision (Chap. 32). Another advantage of the optical trap system is that multiple beads can be independently controlled by splitting the laser beam, so that cells can, for example, be stretched between two beads that are slowly moved apart. The biggest limitation of using optical traps in cell mechanics experiments is that the maximal force level is limited to \(< 1 \text{nN}\), as larger forces would require higher laser power that could excessively heat the cell. While the small forces generated by an optical trap are ideally suited for single-molecule studies and are sufficient for experiments that measure, for example, membrane tethers, they are often too small to induce large-scale cellular deformations, especially when probing stiffer cells such as myocytes.

**Micropipette Aspiration.** In these experiments, single adherent or suspended cells are partially aspirated into a micropipette with a \( \approx 2–10 \mu\text{m} \)-diameter opening by applying precisely controlled suction pressure (typically 100–10 000 Pa). The aspirated cell is imaged on a microscope and cellular deformations such as the aspirated tongue lengths are computed from the cell geometry. In a technique called fluorescent/confocal-imaged microdeformation, fluorescent labeling of specific intracellular components such as the nucleus, the nuclear lamina or nucleoli is used to provide additional information on the subcellular deformations during micropipette aspiration [35.48–51]. One potential limitation of the micropipette aspiration technique is that the interpretation of the experiments is not always straightforward. Analytical or computational models are often necessary to derive material properties from the geometric measurements of the aspirated cells and the applied pressure, and the underlying assumptions may at times be difficult to validate. However, this technique has been proven very useful when studying cells with relatively homogeneous structural organization such as red blood cells or neutrophils [35.49, 52–54].

**Substrate Strain.** Unlike the other techniques, these experiments do not apply controlled forces to single cells, but instead use carefully controlled strain application to induce deformation in cells plated on a flexible substrate. Generally, cells are plated on transparent, elastic silicone membranes coated with extracellular matrix proteins and subjected to uniaxial or biaxial strain. Depending on the particular experiment, the strain can be held constant or varied over time (e.g., cyclic strain application). Strain levels normally do not exceed 30% in order to avoid damaging the cells, but the exact levels are cell-type dependent. Cells are imaged under a microscope before, during, and after strain application,
and small markers or fluorescently labeled components of the cells are used to calculate intracellular deformations and applied membrane strain. Since the cell is firmly attached to the extracellular matrix on the silicone membrane through cell surface receptors which are connected to the cytoskeleton, the cytoskeleton will experience strain levels comparable to the applied substrate strain, whereas the stiffer nucleus typically deforms significantly less [35,41,55,56]. The advantages of this technique are that force can be applied to several cells at once and that the strain application closely resembles physiological mechanical stress, e.g., in muscle cells or endothelial cells subjected to blood vessel expansion. The major limitations are that this technique is only suitable for adherent cells and that the applied forces cannot be determined directly.

**Shear Flow.** The most commonly used devices for shear stress application are the cone and plate rheometer and flow (or perfusion) chambers. Cone and plate rheometers allow precise control over the applied shear stress, but are generally not equipped to image cells during shear stress application, making it difficult to visualize induced cellular deformations. On the other hand, flow chambers are routinely used to study cellular deformations under shear stress [35,57] or to investigate cellular responses to shear stimulation such as calcium influx or cytoskeletal remodeling. Parallel-plate flow chambers are made of transparent glass slides separated by a thin spacer/gasket (the channel height is often \( \approx 100 \mu m \)) and can thus apply precisely controlled fluid shear stress to a cell monolayer plated on the bottom slide while simultaneously imaging the cells on a microscope. The shear stress at the cell surface can be calculated for a Newtonian fluid in the parallel plate geometry as \( \tau = 6Q\mu/wh^2 \), where \( \tau \) is the fluid shear stress at the wall (and cell surface), \( Q \) is the flow rate, \( \mu \) is the viscosity, \( w \) is the channel width, and \( h \) is the channel height. The shear stress can thus be adjusted to physiological shear stress levels (\( \approx 0.1-10 \) Pa) by altering the height of the flow chamber or modulating the flow rate, and experiments can be carried out with either constant or pulsatile flow. However, the actual shear stress at the cell surface might deviate from the predicted wall shear stress, as small variations in the cell height and topology can cause local variations, which might in fact contribute to the alignment of endothelial cells to the flow direction.

**Micromachined Devices.** Within the last decade, microfabrication has enabled the design of numerous custom-designed devices to measure cellular mechanics, including microelectromechanical systems (MEMS). Typically, these devices contain an actuator to apply precisely controlled forces or deformations to single cells and a force sensor, often comprised of an element with known spring constant that deforms under the applied load. In some cases, these two components can be combined into a single element. One advantage of micromachined devices is the ability to directly control force application and sense the applied forces with high precision without requiring elaborate assumptions of cellular structure and mechanical properties. Also, by appropriately tuning the geometry of the force sensor, relatively high forces (up to 1500 nN) can be measured, allowing measurements of forces required to detach adherent cells from the substrate. The major disadvantages are the still relatively high costs and the need for specialized equipment in the fabrication process.

**Passive Measurements**

In contrast to the active measurements, these experiments measure forces generated by the cells themselves or quantify the random motion of particles embedded in the cytoplasm subjected to thermal fluctuations. Typical examples of passive measurement techniques include particle tracking of beads in the cytoplasm and traction force microscopy. In passive bead microrheology, small (0.1–2 \( \mu m \)) beads are injected into the cytoplasm or taken up by endocytosis and are then tracked with high spatial and temporal resolution with a laser beam and quadrant photodetector. The complex cytoplasmic shear modulus \( G(s) \) can then be computed from the unilateral Laplace transform of the measured mean-squared displacement \( \langle \Delta r^2(s) \rangle \) using a generalized Stokes–Einstein equation as \( G(s) = k_B T/(\pi as \langle \Delta r^2(s) \rangle) \), where \( s \) is the Laplace frequency, \( k_B \) is the Boltzmann constant, \( T \) is the temperature, and \( a \) is the bead radius [35,58]. Traction force microscopy was originally based on wrinkles generated in thin silicone sheets by the contraction of cells plated on top of these sheets, but has subsequently been refined for more quantitative force determination by plating cells on polyacrylamide gels with fluorescent beads embedded in the gel. The mechanical stiffness of the polyacrylamide gel can be tuned based on the cross-linker concentration, and microscopic measurements of the induced displacement of beads near the surface can be converted into cellular forces exerted on the gel by elastic theory [35,59]. Most recently, a new generation of traction force microscopy has emerged in which cells are coated on a fine grid of micro-
fabricated micropillars made of polydimethylsiloxane (PDMS) [35.60]. In this case, the stiffness of micropillars can be controlled by adjusting the cross-sectional area or length of the micropillars, and the cellular force exerted on each micropillar can be measured based on the deflection of the pillar using beam bending theory. This technique offers the advantage that force application is localized to individual micropillars, and that the geometry and stiffness can be independently adjusted.

### 35.3.2 Rheological Properties

The rheological properties of the cell, such as its viscoelastic properties and its diffusion parameters, are key to the cell’s ability to accomplish its diverse functions in health and disease. A wide range of computational and phenomenological models as well as experimental techniques have been proposed over the past two decades to describe the cell, giving rise to several, often contradictory, theories for describing the rheology of the cytoskeleton. The highly heterogeneous structure of the cytoskeleton, coupled with a small linear response regime [35.62] and active dynamics and continuously remodeling, present a major challenge for quantitative measurements and descriptions of its rheology [35.63].

Nonetheless, a wide range of computational models exist for cytoskeletal rheology and mechanics, ranging from continuum to discrete descriptions of the cytoskeleton (see reviews in [35.64]). A major challenge in cytoskeletal rheology and mechanics is how to relate experimental observations to theoretical and phenomenological models. Much effort has recently focused on the interesting rheological behavior of cells, measured by one of the methods described above. Most often, cellular viscoelastic properties are expressed in terms of the complex shear modulus (see Viscoelastic Solid/Poroelastic Solid), the real part $G'$ indicating the elastic component, and the imaginary part $G''$ the viscous component. While each measurement method has its drawbacks, perhaps the most comprehensive data have been obtained through magnetic twisting cytometry (Fig. 35.6). These measurements have shown that a cell exhibits a relatively simple power-law behavior over much of the frequency domain, with [35.61]

$$G' + iG'' = G_0 \left( \frac{\omega}{\omega_0} \right)^{x-1} \frac{1 + i\eta}{\Gamma(2-x)} \times \cos \left[ \frac{\pi}{2} (x-1) \right] + i\omega \mu , \quad (35.1)$$

where $\Gamma$ is the Gamma function, $\mu$ is the viscosity, and $\eta = \tan((x-1)\pi/2)G_0$, $\omega_0$, and $x$ are parameters of the model. All but $x$, however, have been found to be nearly constant in all experiments. This turns out to be similar to the behavior of soft glassy materials, although the fundamental basis for this behavior remains a topic of some debate.

The data in Fig. 35.7 [35.61] apply to small deformations in the linear regime. However, linearity persists up to surprisingly large deformations. Much of what we know about nonlinear effects has been obtained from experiments on reconstituted gels, typically of actin with one or more actin cross-linking proteins. From these experiments, the following observations have been made:

1. Linearity persists up to $\approx 30\%$ strain.
2. Values of $G'$ and $G''$ in this linear regime are often orders of magnitude lower than are measured in cells.
3. Strain stiffening is observed at strains $> 30\%$, followed by a precipitous drop in $G'$ at strains of $\approx 70\%$, presumably indicating rupture or unfolding of the cross-linkers.

All of these issues continue to be actively studied in the hope of generating a comprehensive understanding of cytoskeletal rheology.
35.3.3 Active Force Generation

The cytoskeleton is an active structure that maintains cell shape and facilitates its motion. The cytoskeletal network is in a nonequilibrium state that drives motor proteins as force-generating features in cells. The cytoskeleton is activated by these molecular motors, which are nanometer-sized force-generating proteins, e.g., myosin. To better understand the nature of such active force-generation systems, researchers have developed simplified models of the active cytoskeleton by mixing actin with actin binding proteins (e.g., filamin and α-actinin) and molecular motor proteins myosins [35.65]. Such a reconstituted synthetic cytoskeleton exhibits local contractions reminiscent of living cells. Tension generated by contraction can lead to drastic increase of the cytoskeletal stiffness. Such models demonstrated that a remarkably simple system, with just three components (myosin, actin, and ATP), can reproduce key phenomena also observed in far more complex living cells [35.66].

35.3.4 Biological Responses

The cellular responses to mechanical stimuli take place over several time scales and range from intracellular signaling to changes in cellular morphology and function. The fastest responses occur within seconds to minutes of stimulation and include changes in intracellular ion concentrations (especially Ca^{2+}) through opening of mechanosensitive ion channels and activation of cellular signaling pathways such as nuclear factor-κB (NF-κB), phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt), protein kinase C (PKC), Rho family GTPases, and mitogen-activated protein kinases (MAPKs) [35.67]. These first steps do not require synthesis of new proteins, but involve modification (e.g., phosphorylation) and intracellular translocation of existing proteins. These initial events often trigger activation of mechanosensitive immediate early genes such as egr-1, c-fos, c-jun or c-myc encoding transcription factors that turn on additional downstream genes. These later response genes often include proteins involved in cellular structure (e.g., actin, myosin) or extracellular matrix remodeling. However, cytoskeletal remodeling in response to mechanical stress or strain can start even before synthesis of new proteins as early signaling pathways can also mediate polymerization and depolymerization of cytoskeletal structures. For example, endothelial cells exposed to fluid shear stress begin to align with the flow direction within 20 min, and the process is completed within 24 h. The multifaceted response to mechanical stimulation can effectively mediate several cellular functions, including DNA and RNA synthesis, hypertrophy (increase in cell size), proliferation, apoptosis, migration, and extracellular matrix remodeling.

35.3.5 Nonlinear Effects

As described earlier, cells are composed of intricate networks of filamentous structures, called the cytoskeleton. These networks exhibit unique properties including relatively large shear moduli, strong signatures of nonlinear response in which, for example, the shear modulus can increase drastically under modest strains [35.68, 69]. Models of semiflexible polymer networks have emerged to describe these unique properties and the dynamics of the cytoskeletal networks. These models involve a semiflexible description of the constituent actin filaments [35.70]. Quantitative models of the nonlinear behavior of the cytoskeletal network are essential for understanding the complex, dynamic, and nonlinear behavior of cells, and ultimately the tissues and organs.

35.3.6 Homogeneity and Anisotropy

Cells are inhomogeneous structures composed of various intracellular components, including the lipid bilayer membrane that encases the cell, the actin cortex, which is a dense actin network providing stability for the cell membrane, cytoskeletal networks, and the nucleus, which itself comprises various important substructures. The cytoskeleton is primarily responsible for the structural integrity and stiffness exhibited by a cell. It is composed of a system of highly entangled protein filaments that permeate the microfluidic space of the cytosol. The major components of the cytoskeletal network, i.e., the actin filaments, intermediate filaments, microtubules, and their cross-linking proteins offer an exquisite microenvironment which is highly inhomogeneous and anisotropic in its structure and geometry.
35.4 Theoretical and Computational Descriptions

As is evident from the discussion above, the cytoskeleton is a complex biopolymer network with varying degrees of connectivity, existing in a state of dynamic equilibrium. The dynamic state arises from the ongoing polymerization and depolymerization of the constituent filaments and the changing density of cross-links between filaments of the same or different family. This picture is complicated further by the milieu of other intracellular constituents that may or may not affect structural properties exhibited by the cell.

Our objective in this section is to come to a better appreciation of how the elastic properties and geometric arrangement of the constituent filaments give rise to the material properties observed by the various experimental methods just described. These experiments have shown that the elastic modulus of cells can vary considerably from one cell type to another. Cells of the epidermis, for example, require greater structural integrity than the red blood cells subjected to the relatively low shear stresses in the blood. Even within a given cell type, the elastic properties can change. Skeletal muscle, for example, changes its modulus by over an order of magnitude within a small fraction of a second. Other cells change too, but more often over a longer time period, in response, for example, to changes in their mechanical environment.

According to the various measurements that have been made, cells seem to range in shear modulus in the range $10 \text{--} 10000 \text{ Pa}$, and therefore exhibit a stiffness somewhat lower than collagen gels (or common gelatins) at low concentration or relaxed skeletal muscle. This wide range of moduli probably says more about differences in the models used as a basis to infer the shear modulus from the data than it does about real cell-to-cell variations. At best, these numbers inferred from experiment should be viewed as measures of an effective stiffness, and comparisons between different measurement methods and interpretation should be made with caution. Other biological materials (e.g., bone, wood) exhibit a much higher modulus, but not because of their cellular content. Rather, their high stiffness is due to the calcification in bone and the collagen matrix found in wood and most plants. In tissues, too, the stiffness we measure is more often associated with the extracellular matrix with its elastin and collagen, than the resident cells. Even within the cytoskeleton, individual filaments (i.e., F-actin, intermediate filaments, and microtubules) exhibit moduli much greater than the measured bulk modulus of the cytoskeleton. In the next section we explore different approaches that have been developed to relate the properties of the individual fibers to those of the assembled network.

35.4.1 Continuum Models

Elastic Solid

In several of the experiments described earlier, the assumption of a homogenous and isotropic elastic solid is used to infer a value for an effective Young’s modulus for the cell. It is not our intention here to provide a comprehensive description of elasticity theory, but rather to outline some of the basics. For a full derivation of the governing equations, the reader is referred to any of a number of excellent textbooks [35.71]. Assuming equilibrium conditions (i.e., all forces balance each other) and a material that can be modeled as a Hookean (i.e., linear) elastic solid, the constitutive equations relating the mechanical stress and strain can be written in tensor notation as $\tau_{ij} = C_{ijkl} \varepsilon_{kl}$, where the summation convention is used. Here $\tau_{ij}$ are the elements of the stress tensor, $\varepsilon_{kl}$ are the elements of the strain tensor, and $C_{ijkl}$ is the 81-element coefficient matrix. In the case of an isotropic material, the coefficient matrix reduces to just two independent elastic constants, with the constitutive equation now reduced to the following simplified form:

$$\tau_{ij} = 2Ge_{ij} + \lambda \varepsilon_{kk} \delta_{ij}, \quad (35.2)$$

where $\lambda$ and $G$ are the Lamé constants with $G$ being termed the shear modulus. Recall that this expression can also be written in the inverted form

$$\varepsilon_{ij} = \left( \frac{1 + \nu}{E} \right) \frac{\tau_{ij}}{E} - \left( \frac{\nu}{E} \right) \tau_{kk} \delta_{ij}. \quad (35.3)$$

Here the two material constants are now given as $\nu$, the Poisson’s ratio, and $E$, the Young’s modulus. When using these to solve a particular problem, a set of appropriate boundary conditions also needs to be posed. For example, in the case of measurements by an indentation probe, the displacement of the surface in contact with the probe might be given. In addition, it would be necessary to specify that the opposite side of the cell is held fixed, and that the unsupported sides and the region on the upper surface not in contact with the probe have zero applied stress. Given these, the equations above provide a complete solution.

The use of the above equations presumes that the response of the cytoskeleton can be represented by an
elastic continuum lacking any discernable microstructure. The validity of this clearly depends upon the length scale of interest in the problem, and is a serious concern in the context of cytoskeletal mechanics where the typical length scale (spacing distance between filaments) may be on the order of 10–100 nm. This becomes comparable to the linear dimension of the region of interest in some of the experimental procedures (e.g., cell poking, AFM measurements) and needs to be kept in mind. Furthermore, while this is a convenient approach for analyzing how cells deform under loading, it provides no insight into the relationship between the macroscopic properties that are measured and the elastic properties of the constituent matrix elements.

**Viscoelastic Solid/Poroelastic Solid**

A viscoelastic material is to an elastic material as a spring and dashpot network is to a system containing only springs. In both the discrete and continuous systems, the instantaneous displacement or strain is a function of the stress history. Similarly, the instantaneous stress depends on the strain history. This simple realization, in combination with the assumption that all functions describing the material properties are continuous, leads to a set of generalized constitutive equations for a viscoelastic solid.

Many of the experiments performed on cells have been interpreted in the context of either an elastic or viscoelastic model. It might be argued, however, that like bone, cartilage, and many other biological tissues, it may be more appropriate to view the cytoskeleton as a poroelastic material. While attempts to apply a poroelastic model to studies of cell mechanics are just beginning, it remains to be seen whether a viscoelastic or poroelastic description is more appropriate. For a description of poroelastic theory, the reader is referred to [35.74].

**Oscillatory Simple Shear.** A useful example of viscoelastic behavior derives from the case in which the upper surface of a sample is being oscillated sinusoidally in the plane of the surface so that the shear strain satisfies \( \varepsilon_{21} = \varepsilon_{21}^* \sin \omega t \) and the material experiences an oscillatory shear stress. In this case, the stress in the viscoelastic material will also vary sinusoidally, but out of phase with the strain. The relationship between stress and strain is often described by introducing the complex shear modulus (as introduced above), \( G^* = G' + iG'' \), satisfying the following expressions

\[
\tau_{21}(t) = \varepsilon_{21}^* (G' \sin \omega t + G'' \cos \omega t), \quad (35.4)
\]

\[
\tau_{21}(t) = \tau_{21}^* \sin(\omega t + \phi)
= \tau_{21}^* \cos \phi \sin \omega t + \tau_{21}^* \sin \phi \cos \omega t, \quad (35.5)
\]

so that \( G', G'' \), and \( \phi \), the phase lag, are related to the amplitudes of strain and stress through the expressions

\[
G' = \omega \int_0^\infty G(t') \sin \omega t' \, dt' = \left( \frac{\tau_{21}^*}{\varepsilon_{21}^*} \right) \cos \phi, \quad (35.6)
\]

\[
G'' = \omega \int_0^\infty G(t') \cos \omega t' \, dt' = \left( \frac{\tau_{21}^*}{\varepsilon_{21}^*} \right) \sin \phi. \quad (35.7)
\]
Note that $G'$ represents the component of stress in phase with the imposed strain and $G''$ the out-of-phase component. For a purely elastic material (in which strain and stress are in phase), $G'$ is simply the shear modulus $G$ and $G''$ is zero. On the other hand, it can be shown that for a viscous Newtonian fluid, $G'$ is zero, and $G'' = \mu \omega$, where $\mu$ is the Newtonian viscosity.

It should be noted that these results could also have been obtained by expressing the time-varying strain as a complex quantity. In so doing, the shear modulus is complex, and $G'$ and $G''$ are, respectively, the real and imaginary parts of the stress–strain ratio.

### 35.4.2 Biopolymer Models

Cytoskeletal networks can be viewed as a polymer gel in which the matrix is considered to consist of relatively straight segments connecting junctions where the filaments are either chemically cross-linked, or effectively so due to entanglements (Fig. 35.8a). Using concepts from polymer physics [35.75] the force required to change the length of one segment of a polymer filament (F-actin, for example) of length $L_e$ by an amount $\delta$ can be expressed as

$$F_T \approx \frac{k_B T L_e^2}{L_c^4} \delta \approx \frac{K_p^2}{k_B T L_e^2} \delta,$$

(35.8)

where $k_B$ is Boltzmann’s constant, $T$ is temperature, $K_p$ is the persistence length, and $L_e$ is the distance between the points where the tension is applied (or, the distance between points of entanglement or cross-linking between network filaments). This expression arises from a consideration of the curvature of the polymer resulting from Brownian or thermal fluctuations, and is based on the assumption that thermal energy is equally partitioned among the different modes of oscillation. The polymer filaments are therefore assumed to be bent prior to the application of stress. Externally imposed forces either increase or decrease the end-to-end length of these filaments, and the deformation produced depends both on the intrinsic bending stiffness of the filaments and on their initial degree of curvature due to thermal fluctuations.

For a network comprised of such filaments in which the distance separating points at which physical bonds exist between the filaments or regions of entanglement is $L_c$, the change in filament length between bonds due to a shear strain $\theta$ is $\delta \propto \theta L_c$. Making use of the fact that the number of filaments per unit area parallel to the surface on which the stress is applied scales inversely with the square of the characteristic mesh spacing $\xi$, the shear stress required to produce a strain $\theta$ is given by

$$\tau \propto F_T \frac{\text{(number of filaments)}}{\text{(unit area)}} \propto \frac{k_B T L_e^2 \rho}{(L_c^3 \xi^2)}$$

(35.9)

from which a shear modulus of the (elastic) network $G_n$ can be determined as the ratio of stress to strain

$$G_n \propto \frac{\tau}{\theta} \propto \frac{k_B T L_e^2}{(L_c^3 \xi^2)}.$$

(35.10)

The scaling of $G_n$ clearly depends on both the distance between cross-links or entanglements and the mesh size. As the concentration of polymer increases, $\xi$ decreases and so likely does $L_c$, at least in terms of the degree of entanglement. As the concentration of actin binding protein (ABP) increases, $L_e$ will decrease. The mesh spacing $\xi$ also depends on the monomer concentration or solid fraction $\Phi$ (monomer volume/total volume). Assuming that the filaments are stiff and homogeneously dispersed through the medium, this relationship can be expressed as $\xi \propto a/\Phi^{1/2}$, where $a$ is the monomer or filaments radius. Substituting yields

$$G_n \propto \frac{k_B T l_p^2 \Phi}{(L_c^3 a^2)}.$$

(35.11)

In the limit of a highly cross-linked network, in which case $L_c \approx \xi$, this leads to

$$G_n \propto k_B T l_p^2 \left(\frac{\Phi}{a^2}\right)^{5/2}.$$

(35.12)

Alternatively, we can express this in terms of the density of cross-links $\rho_c$. Note that, if $\rho_c$ is the number of cross-links per unit volume, it should vary inversely with the volume associated with each bond or entanglement, i.e., as $L_c^{-3}$. Combining this with the expressions (35.10–35.12) gives

$$G_n \propto k_B T l_p^2 \frac{\Phi \rho_c}{a^2}.$$

(35.13)

The Young’s modulus of the network $E_n$ can be obtained by a similar procedure, and can be shown to scale in the same manner as $G_n$.

### 35.4.3 Cellular Solids

The theory of cellular solids was developed for the purpose of relating the macromechanical properties of low-density cellular materials to their microstructural characteristics. The approach used is based on...
the concept that the material can be modeled as being comprised of many unit cells, one representation of which is shown in Fig. 35.8. When a cellular solid is stressed under tension or compression, the fibers act like struts and beams that deform under stress. The unit cell model in Fig. 35.8 has struts or fiber elements of length $L$ and cross-section of radius $a$.

The relative density of the material is defined by the volume fraction of solid material $\Phi$. This is calculated as the solid volume contained within a unit cell ($\propto a^2 L$) divided by the total unit cell volume ($\propto L^3$) or $\Phi \propto (a/L)^2$. Beam theory gives the deflection $\delta$ of a beam of length $L$ subject to a force $F$ acting at its midpoint as

$$\delta \propto \frac{FL^3}{E_f I} ,$$

(35.14)

where $E_f$ is the stiffness of the beam constitutive material and $I$ is the beam’s second moment of area. The moment of area for a beam of thickness $a$ is given by $I \propto a^4$. The stress $\tau$ is the force per unit area, or $\tau \propto F/L^2$. The strain is related to beam deflection $\delta$ by $\varepsilon \propto \delta/L$. Using these, combined with the expressions above, the network Young’s modulus or elastic modulus can be expressed as

$$E_n = \tau / \varepsilon = c_1 E_f I L^4 ,$$

(35.15)

where $c_1$ is a constant of proportionality, or

$$\frac{E_n}{E_f} = c_1 \Phi^2 .$$

(35.16)

Data from a wide range of materials and cell geometries give a value for $c_1$ of $\approx 1$ [35.72]. A similar analysis for cellular materials subjected to shear stresses results in an expression for the network shear modulus $G_n$ [35.72]:

$$\frac{G_n}{E_f} \propto c_2 \Phi^2 ,$$

(35.17)

where $c_2 \approx 3/8$. If the material is linearly elastic and isotropic, these values lead to a value of $1/3$ for the Poisson ratio $\nu$.

### 35.4.4 Tensegrity

Networks can also derive their structural integrity from an interaction between members that are in compression and members in tension. Some familiar large-scale examples include the circus tents and the geodesic dome. In the case of the circus tent, the rigidity of the structure is due to the balance between the tent poles in compression and the ropes anchored to the ground in tension. The rigidity of the structure, in this case, is related to the elastic characteristics of the tension elements.

Ingber [35.76] first proposed that the cytoskeleton behaves like a tensegrity structure with the microtubules acting in compression and the F-actin microfilaments acting in tension. In support of this concept, microtubules have been shown to be capable of supporting compressive loads and the F-actin network exhibits behavior at junctions consistent with their being in tension. Intermediate filaments may also be involved, although their contributions at this stage are unclear.

Analysis of a fully three-dimensional tensegrity network begins, as in the case of the cellular solids model, with a unit cell consisting of an interconnected system of elements in tension in balance with other elements in compression. When the three-dimensional tensegrity network of Fig. 35.8c is used, analysis shows that prestress plays an important role. Intuitively, it is not surprising that networks with greater pretension in the elastic members should exhibit greater resistance to deformation. The effect of prestress increases the network Young’s modulus for small strains, and only in the limit of infinite prestress does $E_n$ become independent of pre-stress. Wang and co-workers [35.77] have shown that the cytoskeleton exhibits this same tendency, becoming increasingly stiff when, for example, the cell passes from a spherical (low prestress) to flattened (high pre-stress) state.

### 35.5 Mechanics of Subcellular Structures

#### 35.5.1 Cell–Cell and Cell–Matrix Adhesions

Cells can adhere to their surroundings by either non-specific or receptor–ligand (specific) bonding. Though both mechanisms are likely active in most situations, receptor–ligand bonding is the stronger of the two, by a considerable margin, and is therefore the most relevant mechanically. In situations for which either the receptors or their ligands are not present, however, such as might be the case in certain in vitro experiments,
nonspecific binding can be important as well, and can produce a net attractive force per unit area of $\approx 100$ Pa at a separation distance of $\approx 25$ nm.

Models for Receptor–Mediated Adhesion

Cell–cell adhesion has obvious similarities to adhesion of cells to substrates, the surrounding extracellular matrix, and other cells, so a similar approach can be used. This requires, in addition, consideration of such factors as the distribution, type, and density of receptor–ligand bonds or potential bonds, and the elastic properties of the structures to which they are anchored. On the intracellular side, this involves the series of couplings that link the receptor to the cell. In the simplest case, this might simply be a link to the lipid bilayer if the receptor has no intracellular connections. More typically, especially for couplings with a structural role, it involves a series of proteins ultimately linking the receptor to the cytoskeleton.

The typical setting in vivo is one in which cells adhere to other cells or to the extracellular matrix. Adhesions are more easily probed, however, through in vitro experiments, where cell adhesion more often occurs to an artificial substrate mediated through one of several extracellular proteins that are used to coat the surface. Generally, either collagen or fibronectin is used. Cells are often adhered to these substrates, but to produce a more controlled environment, rigid beads are sometimes coated with the appropriate receptors so that one specific receptor–ligand interaction can be probed. While these systems are useful as models of certain adhesion phenomena, it is important to recognize in the interpretation of these experiments that, when bound to a rigid substrate or bead, the binding proteins cannot freely diffuse, as they would in a more natural environment. In particular, the formation of focal adhesions would not occur in bead–substrate experiments because the receptors would be constrained from aggregating.

It is useful to consider a single adhesion bond, for example, one linking the actin matrix of the cytoskeleton to a $\beta_1$ integrin, and the integrin receptor binding to the extracellular matrix beyond the cell membrane. If the bond is stressed, as for example if the cell experiences a force relative to the ECM, it will at first stretch an amount dictated by the level of force in the bond and the stiffness of the complex. Each bond, as well as each protein in the bond complex, can be thought of as having a certain stiffness, giving rise to a picture in which several springs are considered connected in series. Forces acting on the adhesion complex are transmitted via this series of bonded proteins between the cytoskeleton (CSK) and the ECM, producing local deformations and stresses in the corresponding matrices that decay with distance from the adhesion site. On the intracellular side, these forces are transmitted via a complex involving vinculin, $\alpha$-actinin, paxillin, and talin. Attachment to the extracellular matrix is mediated by an arginine–glycine–aspartic acid (RGD) sequence (in fibronectin for example), which in turn, has binding sites for collagen and fibrin. If the force is sufficiently strong, above a certain threshold value $F_0$ say, and applied for a sufficiently long time, the bond might be severed (decouple). Typical values of this threshold force lie in the range of $10–100$ pN [35.78], but depend, as well, on the rate at which the force is increased. Under more rapid force application, the threshold is high compared with when the force is increased slowly. This simply reflects the fact that detachment is a stochastic process and that, at any given level of force, there exists a finite probability of detachment, and this probability increases with time.

We need also to consider that, in equilibrium, a fraction $\Phi = K(1 + K)^{-1}$ (where $K$ is the equilibrium constant) of the receptors will be bound at any given time, and that the receptor–ligand complexes are continually cycling between the bound and unbound states, characterized by their respective rate constants. On a larger scale, numerous adhesion sites typically act in parallel, each contributing some amount to the total adhesion force. This gives rise to an average local stress $\tau$ that can be thought of as the product of the average force per bond times the bond density. This stress is typically nonuniform. In the adhesion of a cell to a flat substrate, the bonds near the periphery of the contact zone determine the strength of the cell against detachment, and when the cell is subjected to a detaching force, the stresses are concentrated in this peripheral region. In the vicinity of the edge of adhesion, several factors will influence the stress distribution, including the mechanical properties of the membrane (in particular the bending stiffness $K_B$), the receptor density, the spring constants of the bond, and the bond strength.

The interplay between receptor–ligand bonding and membrane stiffness also determines whether a cell will spread over a surface or peel away from it under a given tension. Membrane bending stiffness acts to maintain the membrane and substrate within close proximity near the edge of contact. Spreading can occur only if their separation distance is within the extended length of the receptor ligand bond over a distance comparable to the linear spacing between neighboring bonds. Under high tension, the surfaces rapidly diverge and spreading is
Transient Cell Adhesion

Concepts of transient adhesion and release are of particular importance in the case of leukocyte rolling, adhesion, and transmigration across the endothelial layer of a blood vessel. In addition, cell migration through extracellular matrix or along a substrate requires the ability of the cell to both form and release adhesions. One problem that has received considerable attention is the interaction between an adherent or rolling leukocyte and the adhesion receptors on the endothelium. These studies are complicated by the three-dimensional nature of the flow, the compliance of the interacting surfaces, and receptor–ligand dynamics. In a series of recent studies, Hammer and coworkers [35.79] have addressed several of these issues, computing the viscous force and torque acting upon a sphere near to a planar wall from the mobility matrix [35.80] and using the Bell model of receptor binding as described above [35.81], but neglecting the effects of cell deformation.

A Monte Carlo method is employed at each time step in the calculation to determine bond formation or breakage. In these studies, the nature of the leukocyte–endothelial interaction is characterized as firm adhesion, rolling adhesion, bimodal adhesion or no adhesion, and mapped as a function of the two parameters of the Bell model. Most literature values for the Bell model parameters for a variety of selectin receptors (known to be instrumental in leukocyte rolling) correspond to the rolling adhesion regime for typical shear rate of 100 s⁻¹. An interesting, but counterintuitive, result from this study was the observation that, as the bond stiffness was increased from the value used in most calculations (100 dyn/cm), adhesiveness decreased, which the authors attribute to reduced deflection for a given level of force, leading subsequently to more rapid dissociation.

35.5.2 Cell Membranes

For the purpose of analysis, we treat the cell membrane as a homogeneous two-dimensional (2-D) plate or sheet completely enclosing the cytoplasm. The membrane referred to here can be thought of either as the lipid bilayer by itself, or more typically, as the bilayer plus the associated cortex of cytoskeletal filaments and the glyocalyx on the extracellular surface. In addition, though not explicitly recognized in the analysis, transmembrane proteins and their attachments to the intracellular and extracellular milieu are included in terms of their influence on the continuum properties of the model. Were it not for these, the membrane would exhibit little resistance to shear deformation.

In qualitative terms, the lipid bilayer can be thought of as a two-dimensional fluid, within which the individual lipid molecules, or other molecules embedded in the membrane, are relatively free to move about by diffusion or directed motion. Phospholipid molecules in either of the two layers resist being pulled apart, however, so each layer is highly inextensible. This also contributes to the bending stiffness, which is low in absolute terms but high for such a thin layer, since bending requires one layer to expand while the other is compressed. By contrast, the two layers readily slide relative to each other. These qualitative notions are put in more quantitative terms in the next section.

Types of Deformation

Any deformation of the membrane can be thought of, in general terms, as a superposition of several simpler deformations. For small strains in which linearization is appropriate, the principle of superposition is rigorously valid. For larger strains, however, linear theory breaks down and superposition can only be used as a rough, qualitative guide in visualizing combined influences. Here we present the three primary types of deformation: pure extension, pure bending, and pure shear.

Pure Extension. In discussing the extensional stiffness of the membrane, we need to distinguish the behavior at low tension from that at high tension. As an extensional stress is first applied at the edges of a lipid bilayer, the projected or apparent membrane area first increases while the actual or true membrane area remains constant. This results from the suppression of out-of-plane undulations. Forces acting to resist membrane flattening originate from entropic effects analogous to those seen in the case of a flexible polymer as its end-to-end distance is increased – many more membrane configurations exist with undulations compared with the single, perfectly flat state. Only when these undulations have been eliminated does the true membrane area, proportional to the surface area per molecule group, begin to increase, and this is associated with a relatively abrupt increase in extensional stiffness. For now, we consider only the stiffness of a flat membrane and leave the discussion of undulations of entropic origin to a later point in the chapter. Hence we initially neglect all entropic effects, or equivalently, consider the membrane to be at
zero temperature. Based in this assumption, consider an infinitesimal plate initially of area \( A_0 = L_0^2 \) that is deformed by a uniform normal stress \( \tau_{11} = \sigma_1 = \tau_{22} = \sigma_2 \) applied to its edges (Fig. 35.9a) to a new area \( A \). The expressions relating stress and strain in two dimensions, and in the absence of stresses normal to the \( x_1-x_2 \) plane, can be written as

\[
\sigma_\alpha = \frac{E}{1-\nu^2}(\epsilon_\alpha + \nu\epsilon_\beta),
\]

(35.18)

where the length of one edge is \( L_\alpha = L_0(1+\epsilon_\alpha) \); note that the subscripts \( \alpha, \beta \) are used here rather than \( i, j \) to distinguish stresses and strains in two dimensions from those, more generally, in three. Thus, whereas \( i \) and \( j \) can be either 1, 2 or 3, \( \alpha \) and \( \beta \) are restricted to being either 1 or 2. When this stress is uniform in the plane of the membrane (the \( x_1-x_2 \) plane) it can be replaced, without loss of generality, by a surface tension \( N_\alpha \) (force per unit length) defined as \( \sigma_\alpha h \), where \( h \) is the thickness of the membrane. These can be combined in the case when \( N_1 = N_2 = \) constant and, consequently, \( \epsilon_1 = \epsilon_2 = \epsilon \) to give

\[
N = \frac{Eh}{1-\nu^2} \epsilon.
\]

(35.19)

In terms of a plate stretched uniformly in both directions, we can define the areal strain as

\[
\frac{\Delta A}{A_0} = \frac{A - A_0}{A_0} = \frac{L_0^2(1+\epsilon)^2 - L_0^2}{L_0^2} \approx 2\epsilon,
\]

(35.20)

where the last approximation is appropriate for small strains. By combining this result with (35.19), we can define the area expansion modulus \( K_e \) (units of N/m) using

\[
N = \frac{Eh}{2(1-\nu)} \frac{\Delta A}{A_0} = K_e \frac{\Delta A}{A_0}.
\]

(35.21)

Note that, although we used the continuum structural equations in our analysis, the final result can also be viewed as simply the definition of the area expansion modulus and applies regardless of whether or not the membrane can be modeled as a continuum.

Experimental measurements of \( K_e \) lie in the range of 0.1–1 N/m for various types of lipid bilayers and \( \approx 0.45 \) N/m (450 dyn/cm) for red blood cell membranes [35.82]. These numbers suggest that cell membranes are quite resistant to extension and, for that reason, are often treated as inextensible. This discussion neglects the effects of thermal fluctuations in the membrane that give rise to a much more compliant behavior at the smallest areal strains. When surface stress is sufficient to smooth out most thermal fluctuations, the cell or vesicle will exhibit the large moduli given here.

This high resistance to area change is in large part due to the energy penalty associated with exposing the hydrophobic core of the membrane to water that occurs as the spacing between individual amphiphilic molecules is increased (for a detailed description of bilayer structure and thermodynamics, see [35.83]). Continuing to increase extensional stress, the lipid bilayer eventually ruptures, but at very small extensional strains, in the vicinity of 2–3% [35.84]. Note that a bilayer in a lipid vesicle, for example, stretches primarily by increasing the area per molecule since recruitment of additional material to the membrane occurs very slowly.

Using these expressions, the surface tension at which the membrane would rupture can be computed to be \( \approx 0.06 \) N/m if we use a value near the higher end of the observed range \( K_e = 1 \) N/m. Cells also often exhibit an intrinsic surface tension. Reported values are small, however, lying in the range of \( \approx 10^{-5} – 10^{-4} \) N/m [35.85].

---

**Fig. 35.9a–c** Membrane mechanics. (a) A membrane, initially of area \( A_0 \), subjected to a uniform extensional stress along its edges (\( \sigma_1 = \sigma_2 \)), causing an increase in area to \( A \). (b) Application of equal moments \( M_1 \) at the two edges of a membrane sheet. (c) Shear deformations produced by the application of nonequal surface tensions on the boundaries of a membrane sheet. The inner square experiences shear strains as shown by the arrows.
**Pure Bending.** By contrast, lipid bilayers exhibit very low bending stiffness, so low that it is often neglected in models of membrane mechanics. It can be important in certain situations, however, and is essential, for example, in analyzing the thermal fluctuations of vesicles. Bending stiffness arises from the same type of molecular interactions that cause extensional stiffness. When an initially flat bilayer is bent, the hydrophilic head groups on the outside of the bend move further apart while on the inside, intermolecular spacing decreases; both represent departures from the equilibrium, unstressed state and require energy.

Returning to our simple continuum plate model, consider a bending moment applied to the two ends, causing the plate to curve slightly (Fig. 35.9b). If the bending is due to moments applied at the two ends about the $x_2$-axis, then the bending moment per unit length is related to the deflection by

$$M_\alpha = -\frac{E t^3}{12(1-\nu^2)} \left( \frac{\partial^2 u_3}{\partial x_\alpha^2} \right) = -K_b \left( \frac{\partial^2 u_3}{\partial x_\alpha^2} \right),$$

(35.22)

where $K_b$ is termed the **bending stiffness** having units of Nm. Implicit in this expression are the assumptions that there exists a mid-plane (the **neutral plane**) on which the in-plane stress and strain are both zero, and that straight lines perpendicular to this mid-plane remain straight and normal to this surface after deformation.

Typical values for the bending stiffness $K_b$ lie in the range of $10^{-19}$ N m ($10^{-12}$ dyn cm) for a red blood cell or lipid bilayers [35.84]. This value is larger, on the order of $(1–2) \times 10^{-18}$ N m [35.86], for other cell types (e.g., neutrophils, endothelial cells) that possess a more extensive cortex.

**Pure Shear.** Shear deformations arise when a membrane is stretched in one direction by a surface tension $N_1$ (units of force/length) while the lateral surface contracts under a lesser tension $N_2$, at constant surface area and in the absence of bending (Fig. 35.9c). Surfaces oriented at 45° to the boundaries experience pure shear stresses of magnitude $(N_1 - N_2)/2$. When subjected to shear stresses in the plane of the membrane, a pure lipid bilayer behaves essentially as a liquid. It exhibits a membrane viscosity in that it poses a resisting force proportional to the **rate of shear deformation**, but only a small shear modulus to **static** shear deformations. It is not clear, in fact, whether or not pure lipid bilayers exhibit a nonzero shear modulus. Typical cell membranes do exhibit a shear modulus, however, largely due to the cortex of cytoskeletal filaments that lie on the intracellular side of the membrane. In a red blood cell this matrix, as discussed above, consists of interconnected filamentous spectrin and actin with attachments to the membrane via ankyrin. The equations relating shear force per unit length of membrane to shear deformation (Hooke’s law) can be expressed as $N_{12} = \tau_{12}h = 2G\varepsilon_{12} = K_s\varepsilon_{12}$, where we define the membrane shear modulus $K_s$ in units of N/m. Typical values lie in the range $(6–9) \times 10^{-6}$ N/m for a red blood cell membrane. A pure lipid bilayer exhibits a **viscous** resistance to shear deformations characterized by a shear viscosity of $\approx 10^{-6}$ N s/m [35.53].

### 35.5.3 Cell Nuclei

Measurements based on micropipette aspiration, atomic force microscopy, and substrate strain experiments suggest that the nucleus is $\approx 2–10$ times stiffer than the surrounding cytoskeleton [35.55, 87–89]. What makes the nucleus so hard to deform? This question may in part depend on the particular mechanical load application. In cellular compression experiments, the highly condensed chromatin provides a large resistance to nuclear compression. In contrast, the nuclear lamina can act as an elastic molecular shock absorber [35.90] that seems ideally suited to sustain large strain when the cell or nucleus is stretched. This idea is supported by micropipette aspiration experiments on isolated nuclei that reveal that, in condensed nuclei, chromatin is the major contributor to nuclear stiffness, whereas the nuclear lamina carries much of the applied mechanical load in swollen nuclei that closely resemble nuclei in intact cells [35.48]. Similarly, condensed nuclei were found to be much stiffer than swollen nuclei [35.48]. Based on the nuclear structure, the mechanical contributions of the nucleus can be separated into three interconnected components: the nuclear membranes, the nuclear lamina, and the nuclear interior, which is mostly comprised of chromatin but could also contain a nuclear matrix of lamins, actins, and other proteins. Each of these structural components has its own physical properties. The specific contributions have been addressed in experiments that induced osmotic swelling of isolated nuclei by varying the buffer salt concentration to separate the nuclear envelope from the underlying chromatin [35.48, 90]. Additional insights have come from micropipette aspiration experiments, in which specific components of the nucleus (e.g., the lamina, the nuclear membrane or the chromatin) are fluorescently labeled and monitored during aspiration. These experi-
ments have revealed that the nuclear interior behaves as a compressible, viscoelastic gel and can be compacted by more than 60% during micropipette aspiration before becoming resistant to further compression [35,51]. The reduction in nuclear volume during micropipette aspiration results in buckling of the nuclear envelope outside the pipette [35.48, 51], further demonstrating the tight physical attachment between the nuclear envelope and the chromatin interior. Nucleoli and other intranuclear structures deform along with the nuclear interior in these experiments [35.50, 51], but might have slightly different mechanical stiffness. The viscoelastic nature of the chromatin also appears responsible for the persistent plastic deformations of nuclei seen after micropipette aspiration of isolated nuclei [35.50, 91]. In contrast to the gel-like nuclear interior, the nuclear lamina itself behaves as a two-dimensional elastic shell. This can be illustrated when monitoring the fluorescence intensity of green fluorescent protein (GFP)–lamin A incorporated into the nuclear lamina during micropipette aspiration experiments. While the fluorescence intensity is very uniform in the nonaspi-rated section of the nucleus, the fluorescence intensity decays exponentially inside the aspirated segment, as the nuclear lamina is stretched under the aspiration pressure. Such a fluorescence intensity profile can be well fitted by the predicted strain profile of a solid elastic shell [35.51, 92]. Unlike the nuclear lamina, the nuclear membranes have fluid-like characteristics, i.e., they do not show any induced strain during micropipette aspiration as they can flow in response to pressure application [35.51]. Further illustrating the fluid-like nature is the fact that small membrane vesicles continue to form blebs in the aspirated section of the nucleus as long as the suction pressure persists [35.51]. Consequently, the nuclear membrane cannot carry much of the mechanical load, leaving the nuclear interior and the nuclear lamina as the major contributors to nuclear stiffness.

In addition to the various structural components, the differentiation state of a cell can also modulate the mechanical properties of its nucleus. A recent study that probed nuclei from human embryonic stem cells and differentiated cells found that nuclei from embryonic stem cells were significantly more deformable than nuclei from differentiated cells and became up to sixfold stiffer relative to the cytoplasm during differentiation [35.50]. While some of this increase in nuclear stiffness could be caused by changes in chromatin structure and organization, much of it can be attributed to a stiffer lamina through increased expression of lamins A and C, as independent experiments have shown that lamins A and C are the main contributors to nuclear stiffness [35.95].

Overall, micropipette aspiration has been the most widely used technique to probe nuclear mechanics, although cellular strain experiments, AFM, microbead rheology, and parallel-plate compression experiments have also been successfully applied to measure nuclear stiffness (Table 35.4). While some groups have described continuous creep of aspirated nuclei into the micropipette [35.48, 50, 90, 96], others [35.51, 88, 92] found that the nucleus stabilized after an equilibration period of \(\approx 10\) s. These differences may be due to variations in the experimental setup or the particular cell type studied. Depending on the experimental technique used, various mathematical models have been applied to describe the induced nuclear deformations. For micropipette aspiration experiments, the creep compliance is often inferred from the following equation:

\[
J(t, \Delta P) = \Phi \frac{4\pi}{3} \frac{L}{D} \frac{1}{\Delta P},
\]

where \(\Delta P\) is the applied pressure difference, \(L\) is the aspirated tongue length, and \(D\) is the micropipette diameter, and the dimensionless parameter \(\Phi\) (value = 2.1) accounts for the effects of the micropipette geometry [35.97]. The challenge in finding analytical expression for the mechanical behavior of the nucleus is that the nucleus is comprised of various, structurally very different domains, as discussed before. Finite-element modeling can address some of these complex interac-

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Experimental method</th>
<th>Nuclear stiffness</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondrocytes</td>
<td>Micropipette aspiration</td>
<td>1–2 kPa</td>
<td>[35.88]</td>
</tr>
<tr>
<td>Bovine endothelial cells</td>
<td>Parallel-plate compression</td>
<td>5–8 kPa</td>
<td>[35.87]</td>
</tr>
<tr>
<td>Monkey kidney epithelial cells</td>
<td>AFM and micropipette aspiration</td>
<td>(\approx 1–10) kPa*</td>
<td>[35.48]</td>
</tr>
<tr>
<td>Mouse fibroblasts</td>
<td>Intranuclear particle tracking</td>
<td>18 Pa</td>
<td>[35.93]</td>
</tr>
<tr>
<td>Human cervical cancer cells (HeLa)</td>
<td>Magnetic tweezers</td>
<td>250 Pa</td>
<td>[35.94]</td>
</tr>
</tbody>
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Table 35.4 Reported values for nuclear stiffness. (\(^*\) Power-law rheology, not single spring constant; the nuclear envelope expansion modulus was measured to be \(\approx 325\) mN/m for these cells, after [35.48])
tions [35.98], but often has to make assumptions on the specific material parameters that are difficult to verify. An alternative approach is to directly compare nuclear deformations under identical experimental conditions, e.g., the aspirated lengths of the nucleus during a constant applied pressure gradient in micropipette aspiration experiments or the induced nuclear strain under a given applied substrate strain in cell strain experiments [35.99].

### 35.5.4 Mechanosensing Proteins

While the complex biochemical pathways have been explored in considerable detail by biologists during the past several decades, work has only recently begun to understand the mechanical intracellular pathways for the propagation of force through the cell. For example, Geiger and Bershadsky [35.100] have mapped the complex and interconnected pathways that can be traced from the extracellular domain to the cytoskeletal matrix within the confines of a focal adhesion, a small portion of which is depicted in Fig. 35.10. Forces transmitted to the cell, either by tethering to the extracellular matrix or via ligand-coated beads in various in vitro experiments, are transmitted via the integrins (or other transmembrane receptors) through a collection of membrane-associated proteins, ultimately linking up with the cytoskeleton and propagated throughout the cell. Many of the proteins that form this pathway are known signaling molecules and several have been implicated in mechanotransduction. The process by which changes in protein conformation give rise to protein clustering at a focal adhesion or initiate intracellular signaling, however, remains largely unknown. This has called for close examination of the individual proteins or protein complexes that transmit the forces throughout the cell and their interconnections. Consider one example pathway from Fig. 35.11. It can be seen that integrins bind to talin, which in turn connects with F-actin either directly or via vinculin. Talin and integrin also bind to focal adhesion kinase (FAK). This force pathway alone gives rise to several possibilities for mechanotransduction.

Even in the absence of biochemical signals, mechanical stimulation can still elicit the formation of viable focal adhesions [35.101]. These focal adhesions are produced as a result of direct molecular conformational changes in focal adhesion-forming molecules. Force-induced conformational changes in talin, vinculin, and focal adhesion kinase (FAK) initiate focal adhesion formation by anchoring integrin molecules to actin filaments and recruiting other focal adhesion-forming molecules to the site of interaction. Other mechanosensing molecules such as filamin and α-actinin stabilize actin filaments through mechanobracing and making scaffolds. The conformational changes in the focal adhesion-initiating molecules and the actin filament scaffold bracing molecules demonstrate the mechanisms of molecular mechanotransduction. In the following, we will briefly review some recent work in molecular dynamics simulation of some mechanosensing proteins, namely talin interaction with vinculin [35.102, 103], focal adhesion kinase interaction with paxillin [35.104], and molecular mechanics of actin binding proteins α-actinin [35.105] and filamin [35.106].

**Talin**

Talin interacts with the membrane-bound integrin molecule at its head domain, and with vinculin in its tail.
domain. It has been implicated as a site of molecular activation by external force. The forces acting on the cells from the ECM are transmitted from the integrin molecules to the talin head and then to all regions of the talin tail (Fig. 35.11). The talin tail has 11 vinculin binding sites (VBS), some of which are on amphipathic alpha helixes and are buried in the hydrophobic core of the talin tail. VBS1 is one of the cryptic binding sites buried in the hydrophobic core of the TAL5 region of talin’s tail. TAL5 has several surface polar residues that are sites of hydrogen bonding with other secondary structures in the rod domain. Transduced external forces from the ECM are applied to the TAL5 domain via these residues [35.107]. In order to simulate the force transduction through the cryptic VBS1 of TAL5, Lee et al. [35.103] applied forces averaging 21.2 pN at the residues near the N-terminal near the talin head while constraining fixed the polar residues at the C-terminal, which interact with the adjacent secondary structure domain. The external locally applied force caused TAL5 to elongate, H1 to move in the direction of the pulling force, H1 to apply torque to H4 via its hydrogen bonds, and H4 to rotate, exposing the VBS1 (Fig. 35.12). Once exposed to solvent the VBS1 is activated and can interact with vinculin. The force-induced activation of VBS sites in the talin tail together with force-induced activation of vinculin can anchor the stimulated integrin molecules to the actin cytoskeleton and thereby initiate focal adhesions.

An alternative mechanism was recently proposed by Hytönen and Vogel [35.108] which involves the unfolding of the rod domain in order to expose the vinculin binding site VBS1. Using steered molecular dynamics, Hytönen and Vogel demonstrated that the talin rod can be fragmented into three helix subbundles, which is followed by the sequential exposure of vinculin-binding helixes to water. The unfolding of a vinculin-binding helix into a completely stretched polypeptide might then inhibit further binding of vinculin.

**α-Actinin**

One major actin filament cross-linker is α-actinin. α-Actinin is a cross-linking molecule that produces...
a scaffold for parallel actin filaments, such as those in stress fibers or in muscle z-disk formations. \(\alpha\)-Actinin is an antiparallel homodimer with three major domains, an actin binding domain, a calmodulin homology domain, and a central rod domain. In order to act as a molecular scaffold, cross-linking actin filaments in a stressed cytoskeletal network \(\alpha\)-actinin must have a semiflexible structure. By exploring the natural frequencies of \(\alpha\)-actinin and its response to external forces, Golji et al. [35.105] characterized the flexible and rigid regions of \(\alpha\)-actinin and have demonstrated the molecular conformational changes that underlie the semiflexible cross-linking and mechanical bracing of \(\alpha\)-actinin.

**Microtubules**  
As yet another mechanosensing protein system one can name the microtubule filaments [35.109]. The microtubule cytoskeleton, which is comprised of individual microtubules typically nucleated from a microtubule organizing center near the nucleus, usually forms a radial network that radiates outward to the cell periphery. These microtubules are inherently polarized due to their head-to-tail assembly, and this allows for processive movement of cargo via motor proteins that move specifically towards either the plus or the minus end. Microtubular filaments resist mechanical deformation and facilitate transport of intracellular cargo via coupling to molecular motors. These filaments are typically in compression, effectively balancing the tensile forces exhibited by the actin–myosin system [35.76].

**Stretch-Sensitive Ion Channels**  
Another class of mechanosensing proteins are the mechanosensitive ion channels, e.g., the mechanosensitive channel of large conductance (MscL), which has been studied extensively [35.110, 111]; molecular dynamic simulation has been used to show how stresses in the cell membrane act directly on the channel and cause it to change its conductance [35.112]. These channels have emerged as another potential gateways for the cell to sense and respond to environmental stimuli.

### 35.6 Current Understanding and Future Needs

Progress is rapidly being made on both the experimental and computational fronts to further understand the nanomechanics of the cell. Using either AFM or optical tweezers, controlled force applications in the pN range and displacement measures in the nm range are already within current capabilities. Optical traps were recently used in a clever experimental assay system to measure the rupture force of a complex formed by an actin-binding protein (namely, filamin or \(\alpha\)-actinin) linking two actin filaments [35.113]. At the same time, single-molecule fluorescence measurements provide the opportunity to monitor single binding events. On the computational side, technical and technological barriers are being slowly surmounted. Molecular dynamics can be applied to proteins to predict their conformational change under force, and docking simulations provide a means for determining binding affinities in different conformational states. The barriers to progress lie primarily in our lack of atomistic models with adequate resolution for those proteins of greatest interest, located in the force transmission pathway. These tend to be difficult to crystallize, so few structures are available. Moreover, due to their size, simulations are computationally intensive, especially if the presence of water molecules is included explicitly. Despite these constraints, some progress can be made by using subdomains of the proteins of interest, provided that their functionality can be demonstrated.

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