Chapter 5 Cytoskeletal Mechanics and Rheology

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Abstract: Cells are basic functional units of life and control a wide range of intra- and extra-cellular activities. They are highly complex structures with unique biomechanical properties to withstand the physiological environment as well as mechanical stimuli. Studies related to the mechanics of single cells are aimed at describing the molecular mechanisms responsible for the physical integrity of the cells as well as their biological functions. These studies have significant implications for biotechnology and human health. Recent advanced and innovative experimental techniques for measuring forces at piconewton resolutions and displacements over nano-meter scales have greatly facilitated this area of research. Moreover, tremendous research efforts have been devoted to the development of multiscale multiphysics computational models for the mechanical properties and functions of cells. This chapter reviews recent numerical and experimental studies in the area of cytoskeletal mechanics and rheology. For this purpose, basic modeling techniques for the mechanics of semiflexible actin filaments as well as various experimental and computational methods for measuring the mechanical behavior of cells are discussed.

Keywords: living cells, cytoskeletal mechanics, semiflexible actin filaments, cell mechanobiology

5.1 Introduction

Cells are the basic functional units of life and control a wide range of intra- and extra-cellular activities. They are dynamic and ever changing systems

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S. Li et al., *Advances in Cell Mechanics*

composed of numerous components with distinct mechanical, chemical and biological properties. Cells alter their mechanical properties in response to external stimuli in order to perform various functions to which mechanics and biology are often intrinsically linked. Many cells migrate both during development as organisms grow and at maturity in health- and disease-related states, e.g. wound repair and atherosclerosis. Moreover, cells sense and respond to externally applied mechanical forces and transduce them into a cascade of biochemical events influencing their behavior. The cellular processes involved in sensing and responding to mechanical stimuli are studied in the fast growing field of mechanotransduction. The ability of a cell to perform the above functions depends on its integrity and its particular shape which, are maintained through the structural stiffness and rheology of the cytoskeleton. A detailed understanding of cytoskeletal mechanics and rheology is required to determine the molecular basis for cellular phenomena such as cell migration and cell adhesion. Cytoskeleton, the primary source of the cell structural integrity and stiffness, comprises of a system of highly entangled protein filaments that permeate the microfluidic space of the cytosol. It behaves elastically in response to quick deformations while behaving similar to a viscous fluid-like material in slow deformations. Actin filaments, intermediate filaments, microtubules, and their cross-linking proteins are the major components of the cytoskeletal network. Microfilaments and microtubules are made of a chain of globular proteins while intermediate filaments are composed of long fibrous subunits (Fig.5.1). These protein filaments are collectively responsible for cell structural properties and motilities.

Microtubules are polymers of tubulin heterodimer, α-tubulin and β-tubulin. The tublin monomers organize themselves into a small hollow cylinder with an outer diameter of approximately 25 nm. Microtubules with half-lives of only a few minutes are highly dynamic and polarized structures undergoing constant polymerization and depolymerization. They radiate from the centrioles, located near nucleus, and interact with motor proteins to move intracellular organelles inside the cell.

There are different intermediate filaments in different cell types; however, they are all formed from the assembly of fibrous proteins such as Keratin. Intermediate filaments consist of a central α-helical domain with over 300 residues. These dimers assemble into a staggered array to form tetramers; the end-to-end connection of tetramers yields protofilaments. An intermediate filament has a ropelike structure and is composed of approximately eight protofilaments. Intermediate filaments are fairly stable and exhibit high resistance to solubility in salts.

The filamentous actin (F-actin) is constructed through polymerization of the globular monomeric actin, G-actin, into two twisted strands which are loosely wrapped around each other. Actin microfilaments are often concentrated beneath the cell membrane and reinforce the cell surface against external forces. F-actin has a diameter of about 8 nm and is polarized with a pointed (minus) and a barbed (plus) end. The polymerization of actin occurs

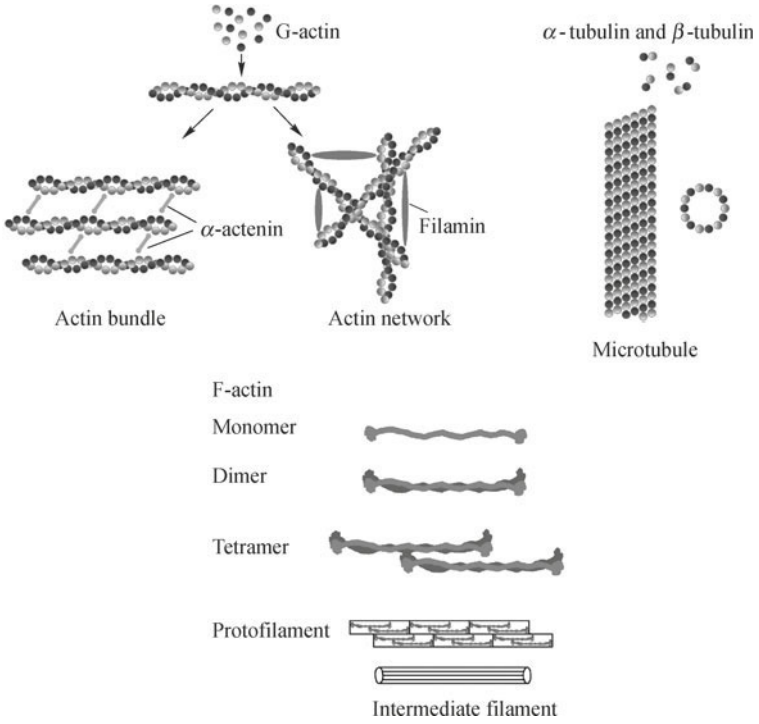

Fig. 5.1 Schematic plot for the structure of an actin filament, a microtubule an intermediate filament (color plot in the book end).

though the hydrolysis of the Adenosine-5′-triphosphate (ATP) of actin subunit to adenosine diphosphate (ADP) plus an inorganic phosphate. Monomer addition and filament growth occur through ATP binding to the positive barbed end whereas depolymerization occurs preferentially at the pointed ends. Filament growth and organization are regulated by many factors, including ionic concentrations, actin binding proteins (ABPs), a variety of capping, binding, branching, and severing proteins. The assembly and disassembly of microfilaments allow a cell to change its shape and to accommodate cellular movement. For example, white blood cells are able to move through capillary walls and enter the damaged tissue through cytoskeletal remodeling.

A plethora of actin monomers along with a large number of actin-monomer-binding proteins are required for the rapid growth of actins in motile cells and also for sudden reorganization of actins in response to intra- and extra-cellular stimuli. These proteins connect actin to microtubules, actin to intermediate filaments or actin to both of these fibers. Actin binding proteins such as α-actinin and filamin organize actin filaments into tertiary structures such as fiber bundles (known as stress fibers) and three-dimensional lattice-like networks. Stress fibers form at focal adhesions, i.e. the points in the cell at which actin myosin contractility is resisted. At the focal adhesion,

the actin cytoskeleton is anchored to transmembrane protein integrins. For the stress fiber growth, the cytoskeletal microstruture is required to be regulated and orginized by recruiting myosin motor molecules and actin cross-linkers. Molecular motor protein myosins belong to actin binding protein category and play an important role in the cytoskeleton dynamic behavior. Myosins are present in all muscle cells forming a well-defined structure, the sarcomere, and produce active contraction. In skeletal muscle cells and cardiac myocytes, the regularly arranged sarcomeres appear in a clearly observable striated pattern. In non-muscle cells these contractile machineries are mainly involved in functions such as maintaining a resting level of cell tension, changing cell shape, and cell migration.

Despite the importance of the cytoskeletal rheology, the relation between the microstructural details and the macroscopic rheological behavior of the cytoskeleton remains elusive. Several fundamentally different models have been proposed for the mechanics and rheology of the cytoskeleton; a thorough review of these models has recently been presented by the authors[97]. Here, we briefly review some of the theoretic and experimental attempts to analyze and understand the mechanics, microrheology, and macrorheology of the cytoskeleton. The structure of this chapter is as follows. First, basic concepts for modeling semiflexible filaments are introduced. The chapter then discusses various experimental techniques used to measure macroscopic behavior of cells under external loads. A brief review of recent computational advancement in cytoskeletal modeling is given in the end.

5.2 Modelling semiflexible filament dynamics

This section discusses a recent methodology to study and characterize the behavior of the semiflexible filaments[8,9]. The cytoskeleton is highly heterogeneous and has an intricate yet diverse structure. Its three broadly important filaments are F-actin, microtubules, and intermediate filaments. These filaments are classified as being semiflexible because of their long persistence length with the respect to their contour length. The persistence of actin, microtubules, and intermediate filaments are about 10 μm, 6 mm, and 1 μm, respectively. The persistence length is the length over which the thermal bending becomes appreciable and the contour angles become correlated[1,2]. For modeling purposes, a semiflexible filament may be represented as a continuously isotropic Euler beam. For any conformation Ω of the semiflexible filament, the total free energy $H(\Omega)$ is given by

$$H(\Omega) = \frac{EI}{2} \int_L \mathrm{d}l \left(\frac{\partial \theta(l)}{\partial l} \right)^2,$$

where L is the contour length, $\theta(l)$ is the filament angle at the distance l along the filament, and E and I denote the filament Young's modulus and cross-

sectional rigidity, respectively. The probability $p(\Omega)$ of finding the filament in a particular configuration Ω is given by Boltzmann distribution,

$$p(\Omega) = \frac{1}{Z}\exp\left(-\frac{H(\Omega)}{K_B T}\right) = \frac{1}{Z}\exp\left[-\frac{EI}{2K_B T}\int_L \mathrm{d}l\left(\frac{\partial\theta(l)}{\partial l}\right)^2\right],$$

where K_B is the Boltzmann constant, T is the absolute temperature, and Z is a normalization constant. From the above equation, the persistence length can be defined as

$$L_p = \frac{EI}{K_B T}.$$

This expression captures the competition between flexural rigidity and thermal energy in determining the conformation or shape of the filament. From equipartition theory, $K_B T/2$ is the mean energy for each degree of freedom (here bending deformation). It can be shown that persistence length is also expressed as

$$< \boldsymbol{t}(l) \cdot \boldsymbol{t}(l+r) >_l = \exp\left(\frac{-r}{2L_p}\right),$$

where $\boldsymbol{t}(s)$ is the tangent vector, r is the distance from initial position l along a filament and $< >_l$ denote averaging over l. In semiflexible polymers, the entropic contribution to the free energy is small and the enthalpic or strain energy contribution plays a more significant role.

Many of artificial polymers can be categorized as being flexible, i.e. the polymer persistence length is much smaller than its contour length and therefore it turns many times in the solution and looks like a relatively compact disordered coil. The theory for the behavior of network formed by these flexible polymers, where the persistence length is small compared with both the length of the polymer and the entanglement distance in the solution, has been well developed[1]. As the persistence length becomes larger and comparable to (or larger than) the contour length, the dynamic of the filaments changes. These filaments are called semiflexible filaments and the properties of the networks formed by these fibers are less developed.

Cytoskeleton is a highly heterogeneous, active and dynamic structure consisting of semiflexible filaments with interconnection lengths of the same order as fiber persistence length. The biophysical properties of the cytoskeleton have been studied and measured in living cells. For example, F-actin networks subjected to oscillatory shear deformation show negative normal stresses as strong as shear stresses[3]. This phenomenon does not appear in the behavior of flexible polymer networks; their response is like most materials where the tendency to expand in the direction normal to the applied shear forces yields positive normal stresses. Another unique property of actin networks is the observation of both stiffening and softening. Unlike simple polymer gels, networks of semiflexible filaments nonlinearly stiffen in order to resist large deformations and maintain the network integrity[4,5] A reversible

stress-softening behavior in compression, essential in preventing catastrophic fracture of actin networks, has also been reported[6]. Flexible and rigid polymers exhibit only monotonic stiffening in compression. The origins of these anomalies and unique macroscopic behavior are under study and there is no comprehensive theory yet. It seems that additional complexities due to nonhomogeneous protein microstructure are required to be considered for the proper description of the cytotskeletal behavior. The underlying filament deformations leading to such a network behavior are still unknown[7].

A new approach has recently been suggested to dynamically simulate semiflexible filaments in a physically consistent but computationally efficient manner[8,9]. The prevalent method for dynamically simulating semiflexible filaments is the string-of-beads idealization. In a weak force field F^P, a particle drifts with a velocity v proportional to the force. The proportionality constant is called the frictional coefficient ξ. Considering the random Brownian forces F^B generated by constant collision with solvent molecules,

$$\xi v(t) = F^P(t) - F^B(t).$$

For fluctuating semiflexible filaments, the conserved force F^P is the internal bending force of the worm model,

$$\xi v(t) = EI\frac{\mathrm{d}^2\theta}{\mathrm{d}t^2} - F^B(t).$$

In order to determine the points on the filament at which the fluctuation, dissipation, and elastic forces are applied and balanced, a filament is often discretized into a string of beads. The viscous and Brownian forces are then applied at each spherical bead center. The discretization of a filament into beads leads to an error in the hydrodynamics. The diffusion coefficient of a rigid representation of a string of beads approaches that of a rigid cylindrical rod only at infinite number of beads[1]. Furthermore, this way of discretization yields errors in determining bending angles and contour length of a continuous filament. In order to tackle these problems and decrease the computational cost, an idealization of a semiflexible filament as a string of continuously flexible rods has been proposed. The Brownian forces due to solvent collision over flexible rods are considered as normal and parallel forces acting on the surface of the rods. Neglecting the hydrodynamic screening and end effects, it is assumed that Brownian force on a segment of a long filament is the same as the force acting on the segment when it is free in the solution. Moreover, the influence of segment bending capacity is neglected in the analysis. Therefore, the Brownian force on a segment that is a part of a flexible filament is obtained by considering a freely diffusing and rigid segment. The resulting force is projected into two perpendicular directions and its variance in each direction is determined by the effective translational and rotational diffusion coefficient of the segment[8]. The Brownian fluctuation dynamics of a semiflexible filament is obtained from balancing simultaneously the Brownian and other forces acting on each segment of the filament. The Brownian

forces are determined from Gaussian distributions with variance determined by the diffusion coefficients. The variance for the net Brownian force F_i in a time interval Δt is given as

$$< F_i F_i >= 4\frac{(K_B T)^2}{D_i}\Delta t,$$

where the diffusion coefficients D_i is calculated from the segment configuration at the beginning of time interval Δt. The direct application of the well established diffusion coefficients of cylindrical rods as well as their bending mechanics to the analysis is the clear advantage of this methodology. The low computational cost of this methodology allows using larger and more realisitic networks to model and investigate the mechanics of cytoskeletal networks with large filament aspect ratios. In the next section, recent experimental techniques and computational methods developed for studying the mechanical properties of the cytoskeleton are briefly reviewed.

5.3 Experimental measurements

Experimentally, the behavior of the cytoskeleton is determined either by monitoring the Browning movements of inherent or introduced particles (passive method) or by direct application of external forces (active measurements)[10,11]. Passive techniques examine the motion of inherent or introduced particles due to thermal fluctuations. If a medium is soft enough, thermal fluctuations of a microscopic probe are measurable and represent the linear response of viscoelastic parameters of the medium surrounding the probe. For example, in passive microrheology, the displacements of micro-sized beads embedded into the cytoskeleton are monitored using either video recordings and particle tracking or laser beam interferometry[12-17]. There are two versions of this technique; one-particle method and two-particle method. In the former, the positions of individual particles are recorded and the complex shear modulus of the environment is calculated from the mean square displacement of the Brownian motion using the Fluctuation Dissipation theory[11]. In this method, active movement of the probe particles may influence the medium viscoelastic properties and cause errors in the measurements. Two-particle microrheology has been developed to avoid this problem by measuring the cross correlation of the displacement fluctuations of two particles located at a given distance from each other.

In active techniques, the mechanics is derived from probing the structure via applying a direct force using optical tweezers, atomic force microscopy, osmotic pressure, and hydrodynamics flow among others[10,11,18,19]. Unlike the passive measurements, the active microrheology methods incorporate applying localized forces at the site of the interrogation. In the next subsections, a summary of these techniques is given.

Nevertheless, before discussing active microrheology methods, a note is in place about frequency-dependent viscoelasticity measurements. Cells show viscoelastic properties at macroscopic scales; experiments based on dynamic mechanical analysis are usually used to study their viscoelasticity. In these tests, a small amplitude oscillatory strain, $\varepsilon_0 \sin(\omega t)$, is applied to a material and the resulting stress, $\sigma_0 \sin(\omega t + \delta)$, is measured where δ is the phase shift between stress and strain, ω is frequency of strain oscillation, and t is time. The in-phase stress response is called the storage elastic modulus, $E'(\omega) = \sigma_0/\varepsilon_0 \cos(\delta(\omega))$, which is a measure of the stored mechanical energy. The out-of-phase response is called the loss elastic modulus, $E''(\omega) = \sigma_0/\varepsilon_0 \sin(\delta(\omega))$, and it measures the energy dissipated as heat in the material. Loss and storage moduli are frequency-dependent, i.e. depending on applied frequencies, a material can have solid-like or liquid-like behavior. Complex variables are used to express modulus as $E^*(\omega) = E'(\omega) + iE''(\omega)$ where i is the imaginary unit. A straightforward and robust approach to characterize the cell microrheology is to determine its complex modulus $E^*(\omega)$ from oscillatory measurements over a wide range of frequencies. The real and imaginary parts of $E^*(\omega)$ represent the elastic energy stored and the frictional energy dissipated within the cell at different oscillatory frequencies, respectively.

5.3.1 Glass microneedles

Glass needles are thin enough to apply small but meaningful forces without damaging the cell. They are first used to apply nanonewton or smaller forces on neurons and to initiate neurite extension[20]. Cells are deformed by poking them with glass needles which are calibrated to determine their bending constant. The calibration process starts with a precalibrated large rod; this rod is used to calibrate a rod slightly smaller than itself. Each rod is then used to calibrate a rod smaller than itself sequentially until the thin microneedle rod is calibrated. In the experiment, two needles are often mounted in a micromanipulator; one needle is calibrated for its bending constant and used as the needle applied to the cell, while the other needle is used as an unloaded reference for bending of the calibrated needle and for possible drift of the micromanipulator system[21,22].

5.3.2 Cell poking

The cell poker is a device to apply forces locally to the surface of live cells using an oscillating glass needle tip[23,24]. The cell is suspended in fluid from a coverslip on top of a vertical glass needle which is attached to a wire needle at its opposite end. The wire needle is coupled to a piezoelectric actuator

which oscillates the glass needle up and down. The glass needle tip indents the cell surface; the applied force is determined by measuring the difference in displacement between the wire needle and the glass needle. The cell poker provides phenomenological information about the viscoelastic properties of cells, about mechanical changes induced by external stimuli, and about the function of the cytoskeleton in the cell mechanical response. Changes in cell shape and cytoskeletal organization associated with physiological processes and due to the physical forces are detectable with the cell poker[25]. Although the cell cytoskeletal network is neither isotropic and homogenous nor much thicker than the amount of indentation, Hertz model may be used to estimate analytically the cell poking results. In this model, the force displacement curves are estimated in terms of Young's modulus and Poisson's ratio of the domain.

5.3.3 Atomic force microscopy

The atomic force microscope (AFM) is essentially a high-resolution and very sensitive cell poker. It was invented about twenty five years ago and is considered as a classical tool for imaging, measuring, and manipulating materials at nanoscale. AFM tip is used to exert controlled forces to the samples and record the corresponding displacements in order to probe the mechanical properties of the material surface. It is operated in both static and dynamic modes such as contact, tapping, and noncontact modes. The cell properties are probed by applying either pushing or pulling forces as long as the tip is strongly bonded to the cell surface. The spatial inhomogeneity of cells is a problem when indentation is done with conventional sharp tips ($\sim$10 nm)[26]. In order to control the inhomogeneities and create a well-defined probe geometry, polystyrene beads of known radius can be attached to the AFM tips[27,28]. The range of measured forces is from 10 pN to 100 nN with estimated Hertz model-based Young's modulus of 0.1–10 kPa. In the taping mode, where cells are probed with a rapidly oscillating AFM tip, it is observed that cells stiffen[29]. AFM has been widely used to probe oscillatory mechanics of cells as well as to estimate their apparent Young's moduli assuming that they are pure elastic materials[30]. At high frequencies, the viscous drag acting on the cantilever should be corrected to obtain reliable measurements of the complex modulus[31]. AFMs have also been used for measuring forces between receptors and ligands; quantification of these forces is essential to develop a better understanding for cell-cell interaction, e.g. rolling of flowing leukocytes on vascular surfaces[32].

5.3.4 Micropipette aspiration

The viscosity and elastic response of cells floating in a solution or attached to a surface can be estimated by pulling on the cell membrane using a small diameter micropipette and measuring the length of aspiration. It is a technique based on the principle of hydrostatic pressure transmission. During the last sixty years, micropipette aspiration has been used to study the mechanical properties of a variety of cells such as monocytes, red blood cells, leukocytes, and erythrocytes[33-40]. Because of the capability of this method to probe cells that are attached to a surface as well as those suspended in solution, it is a powerful method to probe the viscoelasticity properties of non-adherent blood cells; mechanics of suspended leukocytes determines their transportation and retention in the body. Mechanics of cells in suspension is required for better understanding of cancer cell properties; these cells grow in suspension and a canonical feature of them is the loss of anchorage dependence. Micropipette aspiration measures the cortical tension in the cell membrane, the cytoplasmic viscosity and the cell elasticity. Cortical tension is the tangential tension at the cell surface resisting the increase of surface area; it is conceptually similar to surface tension that pulls a water drop into a sphere. Micropipettes can be used to perform creep tests by applying a step pressure and measuring the projection length of the cell into the micropipette as a function of time.

5.3.5 Microplates

The microplate method has been developed to measure viscoelasticity of surface-adherent cells. In this method, the entire cell is probed by applying loads in physiological range. The deformation is accurately measurable and very large deformations can be applied. Basically, the cell is sandwiched between two glass plates; the cell is tightly attached to the bottom plate which is rigid and the top flexible plate is brought in contact with the top cell surface. The rigid plate is then moved to produce compression, extension, or shear which can be measured from the deflection of the flexible microplate[41,42]. This technique is used to measure the creep response (the slow deformation under constant stress) or the stress relaxation (the stress relieve under constant strain)[43]. It is noted that measurements for one single cell at a time can only be performed and the method does not allow regional differences in cell properties to be probed.

5.3.6 Parallel-plate flow chambers

In this method, the fluid flow through a chamber surface coated with a cell monolayer is used to study response of cells to fluid flow; a cellular probe is used to measure this response. Several cell types such as vascular endothelial cells and osteocytes are physiologically exposed to fluid flow and shear stress. Cells sense these external forces and react accordingly; this process is crucial for many regulatory processes. For example, endothelial surface layer has multifaceted physiological functions and behaves as a transport barrier, as a porous hydrodynamic interface in the motion of red and white cells in microvessels, and as a mechanotransducer of fluid shearing stresses to the actin cortical cytoskeleton of the endothelial cell[44]. Endothelial cells adopt an elongated shape in the flow direction if they are subjected to a shear flow. A similar situation exists for osteocytes in bone where mechanosensing controls bone repair and adaptive restructuring processes[45]. It is believed that strain-derived flow of interstitial fluid through lacuno-canalicular porosity mechanically activates the osteocytes. There are three candidates stimulating cells: wall shear stress, streaming potentials, and chemotransport[46,47]. Controlling the wall shear stress and measuring its effect on fluid transport, bone cell nitric oxide, and prostaglandin production can be used to study the nature of the flow-derived cell stimuli[47]. Fluid shear stress rate is also an important parameter for bone cell activation[48].

5.3.7 Optical tweezers

In the optical tweezers technique, controlled manipulative forces are applied to the cells while leaving the cell wall intact[49]. The mechanical properties of living cytoplasm can be studied with minimal damage through application of highly local internal forces. Optical tweezers method is based on trapping a bead with laser beam focalized by a microscope objective. In other words, a laser beam passing through a high-aperture objective lens spatially traps a particle as the scattering force pushing the particle away from the focus point balances the gradient force pulling the particle toward the focus point of the laser. The trapped microbead is partially embedded onto the cell surface and used to apply local forces on the cell. The limited power of the laser bound the applied forces within the range of 1 pN to 100 pN. This method has a wide range of applications ranging from manipulating cells and transporting foreign materials into single cells to delivering cells to specific locations and sorting cells in microfluidic systems. Principal strength of using tweezers for these characterizations includes non-contact force for cell manipulation, high force resolution, wide range of frequencies, and amiability to liquid medium environments. In this method, the microbead usually causes local unwanted remodeling events which alter the structure under study. For further discus-

sion, the reader is referred to a recent review on recent development of optical tweezers[50].

5.3.8 Magnetic traps

In this technique, the ferromagnetic microbeads coated with a protein which binds to the intracellular proteins are used to apply large forces both on the surface of cells and in their intracellular environment. Several cell types such as white blood cells and fibroblasts exhibit elastic moduli of ∼1–10 kPa, therefore, large forces on order of nanonewton are needed to study the deformation of these cells[51]. A high magnetic field is first used to magnetize the beads and create parallel magnetic moments in order to apply a torque. Ferromagnetic particles are used to apply torques and paramagnetic particles to apply force only[52-56]. Forces up to 10 nN may be generated using paramagnetic beads and forces of several piconewtons are generated using ferromagnetic beads[57-59].

5.4 Computational models

The biomechanical models developed for the mechanics of a cell are either based on the continuum level and macroscopic observations (phenomenological models) or based on the cell nano-structure (micromechanics models). In the former, it is assumed that cell behaves as a continuum material for which a constitutive model based on the experimental observations can be chosen to describe its behavior. The cytoskeleton has been modeled as a simple viscoelastic continuum, porous gel and soft glassy material. These continuum-level models may only be used to describe the cell behavior if the length scale of interest is much larger than the dimensions of the cell microstructure. In micromechanics methods, the main microstructural features of the system are included in the model.

There are instances where a cell behaves at macroscopic length scales which are two to three orders of magnitude larger than its microstructural characteristic lengths. The behavior of rythrocytes/neutrophils in micropipette aspiration and magnetocytometry-induced deformations are among cases where continuum models successfully describe the overall mechanical properties[38,39,57,60-62]. The cell environment consists of ionic water solution and charged macromolecules that are influencing its biomechanical and biochemical properties. For example, it is observed that the cell volume changes under osmotic loadings due to changes in ion concentrations. While many of continuum level models neglect the interactions of these different phases and consider the cell as a single-phase material, multi-phasic models have been developed to take into account the interactions between solid, fluid and

free ions in the cytoskeleton[63]. Most of the work in this area as well as the first multi-phasic model have been developed in order to study the articular cartilage and chodrocyte cells. Few notes are in place about the range of applicability and accuracy of continuum-level modeling of the cell behavior. In these models, the small but important Brownian motions of cytoskeleton due to thermal fluctuations are neglected. Moreover, the infinite number of time scales, a characteristic feature of power-law rheology of the cell response, is replaced with a limited number of time constants[43]. Finally, although we have the freedom to incorporate inhomogeneous and isotropic properties for the representative continuum domain through selecting proper constitutive laws (i.e. force displacement relation), these models often cannot be related to and derived from the cell microstructure. Therefore, the continuum-level methods are highly specific to the experimental observations and usually cannot be generalized[64].

In continuum-level models, the fibrous and discrete structure of the cytoskeleton is missing. In discrete models, a network of randomly cross-linked fibers is used to represent the cytoskeleton microstructure. A complete review of these models is given in [65, 97]. Since cytoskeletal filaments are heavily cross-linked on the scale of their thermal persistence length, they store energy in bending, twisting and stretching modes of deformation. Cytoskeleton is a semiflexible network whose behavior is non-affine. The models for flexible networks[1] are unable to provide a thorough description for the mechanical behavior of semiflexible networks. Over past decades, extensive efforts have been devoted to developing a unified and general model to describe the elasticity and unique features of semiflexible gels such as large shear moduli, nonlinear response, and power-law scaling of the cytoskeletal rheology,[66-72]. The cytoskeletal filaments resist bending and exhibit thermally induced fluctuations due to Brownian forces. A filamentous protein is therefore considered as an elastic rod with bending and stretching rigidities resulting from both elastic and entropic response. The langevin equation describes the hydrodynamics drag force of the filaments through the solvent.

Affine network models are effective in modeling the behavior of networks whose individual filaments only rotate and uniaxially deform under uniform loadings, i.e. the macroscopic strain distributes uniformly (affine deformation). These models are not valid where the behavior is non-affine and bending deformations of filaments become important[66,67,70,73]. As discussed in reference [65], boundary value problem on dense random fiber networks can be solved using Stochastic Finite Element Method[72].

As stated earlier, cytoskeleton is an active biological system responding to external stimuli through actin-myosin interactions which lead to formation of stress fibers. The interplay of multiple phenomena including myosin contraction, actin network elasticity, the internal and external constraints imposed on the cytoskeleton creates the stress distribution inside the cell. The plethora of actin binding proteins controls the organization (e.g., local microstructure and connectivity) of actin filaments. These filaments are as-

sembled and disassembled in response to various kinds of signals resulting from cell locomotion, cell division and extension[74-76]. The mechanical stability of the cell is due to dynamic arranging and rearranging of cytoskeletal filaments via crosslinking molecules. For instance, Arp2/3 complex forms 70° branching networks, fimbrin and α-actinin put F-actins in parallel bundles, and filamin and spectrin form three-dimensional actin networks. Filamin and spectrin are protein complexes consisting of multiple actin-binding domains which arrange actin filaments into dense meshworks. Other types of ABPs are those which are used as a physical support or scaffold and are less directly involved in regulating actin structure. Myosins are a member of this category of ABPs.

There are over fifteen different types of myosins using actin filaments to move their specific cargo inside the cell. Myosins are molecular motors which produce movement through ATP hydrolysis and can generate forces on order of pico-Newtons inside the actin network or bundles. The mechanical properties and structure of cytoskeleton are greatly influenced by the contribution of forces generated by myosins. Thus, their effect in cell mechanics cannot be overlooked. The effect of motor molecules may be included in the filamentous network models for the cytoskeleton. In these models, internal forces exerted by myosin motor molecules induce a state of pre-stress condition inside the system. These networks, whose elasticity is controlled by myosin, are often called active networks and their behavior is different than that of passive networks. It has been shown experimentally that these active myosin stresses stiffen the network by two orders of magnitude[77]; however, a mathematical and theoretic model of active network has not yet been developed (Fig.5.2).

The stress field in the cytoskeletal network depends on the contractile machinery called stress fibers. They are formed by the actin-myosin interactions and are characterized by repeating units of myosin proteins. The movement of myosin motors in the stress fibers causes actin filaments to contract and slide past one another. The dynamics of stress fibers has not yet been well understood and is currently under study. It is known that their elasticity is a function of myosin spacings and changes over time. Cellular functions such as wound healing, proliferation, shape stability, and apoptosis depend heavily on how the force is acted upon the cell. Forces transmit into and out of the cell cytoskeleton through these bundles at the basal surface, where it interacts with the surrounding extracellular matrix, and where cytoskeletal contractility is resisted. At these interaction sites (focal adhesion), stress fibers often form[78,79]. They also form along the direction of external forces, e.g. it is found that stress fibers orient along the direction of fluid flow in endothelial cells under shear stress[80,81]. The mechanical properties of stress fibers can be non-uniform due to the variations in myosin spacings along stress fiber length. Moreover, the direction and strength of stress fibers depend on stress field within the cell. Forces are transferred and propagated directly and in a band-like structure from the cytoskeleton stress fibers to the discrete sites on the nuclear envelop[82,83]. As stress fibers enable the cell

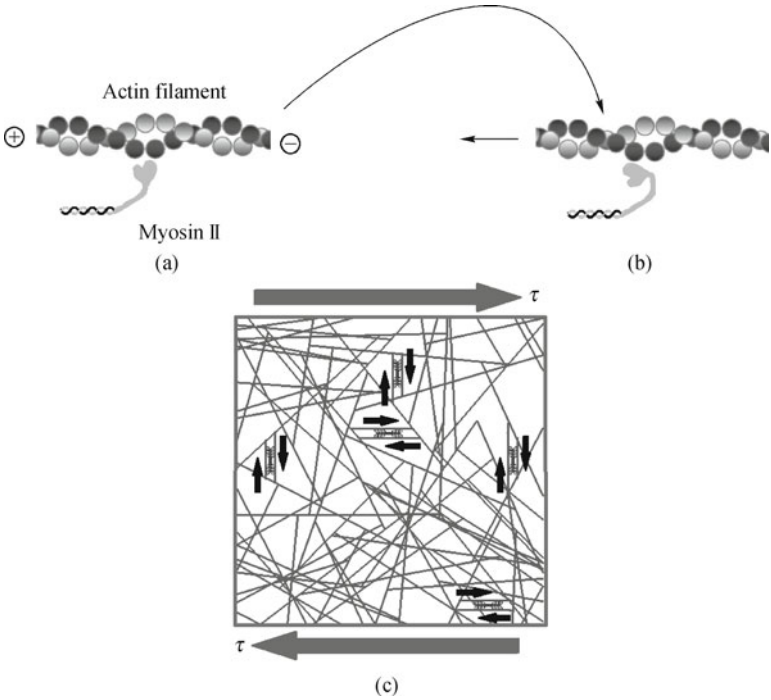

Fig. 5.2 Schematic of a bipolar myosin filament interacting with two actin filaments. The +/− signs indicate the polarity of actin; myosin moves toward the plus end. (a) Myosin head attaches to the actin; high energy configuration. (b) The myosin head bends and pulls on the actin filament; power stroke. (c) Active networks subjected to external loadings. In active networks, myosin molecular motors control the network elasticity and induce a state of pre-stress inside the network (color plot in the book end).

to focus myosin contractility along a specific direction of resistance, the cell can stiffen directionally to protect itself against excess stress and strains in this particular direction. Moreover, the cell explores the matrix rigidity and migrates in the direction of increasing rigidity through stress fibers[84]. Cell locomotion is believed to depend on and even be controlled by changes in substrate rigidity. Particularly, cell response on culture surfaces is dictated by substrate rigidity; actin stress fibers are oriented along the stiffest direction of the microfabricated substrates[85].

In literature, stress fibers have often been simulated by continuum models. These models predict the diffusion of stress away from the points of matrix attachment, as opposed to the directed and focused stress propagation observed in experiments. A recent study, however, showed that in translating the discrete microscale actin-myosin interaction to the continuum scale, these models are missing the perpendicular component of the myosin force acting on the actin filaments[83]. Inclusion of this correction in the model

recovers the directed and focused nature of stress fibers. Moreover, the stress fiber arrangements for simple cell-matrix attachments predicted by this model match those observed in experimental studies. It is noted that key aspects of the interactions are missed in coarse graining and in approximating discrete macromolecular interactions by a continuum model. Therefore, a multi-scale approach retaining the discrete nature of the macromolecular interactions and keeping material properties in the discrete macromolecular scale is required. Such a model requires taking into account the discrete nature of cytoskeleton as well as the effects of hydrodynamic interaction on the behavior of individual filaments[8,9].

It has been shown that elastic modulus of stress fibers in living endothelial cells is approximately 10–15 kPa which remains constant over large strains up to 12%[86]. This stiffness is a function of myosin II since disturbing its activity by adding myosin inhibitor blebbistatin causes a 30% loss of the modulus[87]. The elastic modulus of fibroblast cells also decreases due to the application of this myosin inhibitor[88]. This further confirms the importance of the tension generated by myosin to the overall cellular stiffness. Despite considerable experimental and computational efforts devoted to study basic physical principles of cell contractility due to myosin II motors, our understanding of this process is still limited. It is not yet clear how contractility and pattern formation changes with microscopic parameters such as number of myosin motors, number of cross-linkers and density of actin filaments. It is known that the motor activity inside the cell is a controlled process which results in the formation of stress fibers in cells on flat substrates and the contractile rings during cytokinesis[89-91]. The contractile rings, which are composed of actin, myosin II, septins and GTP-binding hetero-oligomers, generate a furrow which partitions one cell into two. There have been some efforts to describe these rings using continuum-level hydrodynamics models whose accuracy has been confirmed by in vitro studies[92,93]. In these models, the actin network is modeled as active viscoelastic polar gel which is forced out of its equilibrium state because of the ATP hydrolysis. These studies suggested that the assembly and disassembly of cystoskeletal structures can be tuned via varying the concentration of local myosin II. Furthermore, F-actin cross-linkers such as filamin A and α-actinin must be present for the contraction of F-actin networks induced by myosin II at physiological ATP concentrations[94]. In other words, myosin cannot generate large forces if actin filaments are not cross-linked. The dependence of contractility on the number of cross-linkers and myosin motors per actin filament has been studied by constructing well-controlled model system of purified actin, myosin, and α-actinin[95]. The results of recent theoretical studies show that the force generated by myosin is not sufficiently large for ring contraction during cytokinesis unless the actin filaments are heavily cross-linked[96].

5.5 Conclusion

In this chapter, current experimental and computational methods are concisely reviewed to shed some light on the cytoskeletal rheology. Living cells exhibit physical properties similar to those of a liquid or a solid depending on properties of external stimuli; the transition between these two states is among unresolved problems of the field. Understanding and characterizing the underlying molecular events involved in the distinctive response of cells are among major goals of cell mechanics research. Despite tremendous progress in the cytoskeletal mechanics and rheology over the past decades, there is still a long way to go before developing a thorough understanding for the basis of cell individual and collective mechanical behavior. Recent advancement in nano-mechanics and imaging has brought a wealth of information about the molecular composition and properties of cytoskeletal constituents. Furthermore, the ever improving microrheometry-based experiments have expanded our understanding for the overall macroscopic behavior of cells. Nevertheless, the key question remaining to be answered is how these unique properties are emerged from cell generic microstructural features.

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