# Cytoskeletal Mechanics and Cellular Mechanotransduction: A Molecular Perspective

**Hamed Hatami-Marbini and Mohammad R. K. Mofrad**

**Abstract** Cells are highly complex structures with unique physiology and biomechanical properties. A multiscale multiphysics methodology is required to properly understand the intrinsically coupled mechanobiology of the cell and describe its macroscopic response to externally applied stresses. This indeed is both a challenge and an excellent research opportunity. This chapter reviews the latest advancements in this field by bringing together the recent experimental and theoretical studies on the cytoskeletal rheology and mechanics as well as the dynamic response of the cell to environmental stimuli. The experimental observations along with computational approaches used to study the mechanical properties of the individual constituents of the cytoskeleton are first presented. Various computational models are then discussed ranging from discrete filamentous models to continuum level models developed to capture the highly dynamic and constantly changing properties of the cells to external and internal stimuli. Finally, the concept of cellular mechanotransduction is discussed as an essential function of the cell wherein the cytoskeleton plays a key role.

## 1 Introduction

Cells, the basic building blocks of all living things, are dynamic and ever changing systems composed of numerous components with distinct mechanical, chemical

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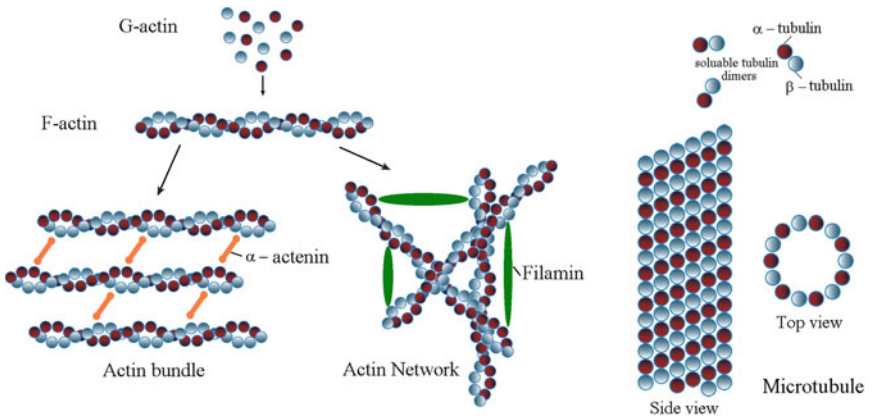


**Fig. 1** Schematic representation of an actin filament and a microtubule

and biological properties. They perform a variety of biological functions many of which directly depend on their shape and structural stiffness. Cells respond to external mechanical stimuli and interact with their surroundings by changing their morphology and biological signaling which ultimately lead to functional adaptation and/or pathological conditions. The integrity and particular shape of a cell are maintained by the interplay of cell membrane with cytoskeleton, which forms the majority of cell's mass.

Cytoskeleton is a three-dimensional dynamic network through which water, solutes and small organelles diffuse while resisting weak osmotic fluid flows and serving as scaffolds for weak contractions. Cell can exhibit a range of material properties from a viscous fluid to an elastic gel depending on the length of its filamentous constituents and the degree of their cross-linkings in the solution. It behaves elastically in response to quick deformations while showing a viscous fluid-like behavior in slow deformations. A wide range of computational and experimental investigations have been conducted to understand the rheology and mechanics of the cytoskeleton and its main constituents. Current models show remarkable disparity largely due to the varied length scales and/or biomechanical issues of interest. Therefore, there is no unified and general model describing cytoskeleton rheology yet.

The three main building blocks of the cytoskeleton are actin microfilaments (MFs), microtubules (MTs), and intermediate filaments (IFs), each of which is a polymer comprised of protein subunits. Microfilaments and microtubules are both made of a chain of globular proteins but intermediate filaments are composed of long fibrous subunits. G-actin, the globular protein subunit forming the microfilaments, consists of two domains separated by a nucleotide binding cleft. G-actins bind together through their nucleotide clefts to form the filamentous actin (F-actin) that is basically two chains loosely wrapped around other, see Fig. 1.

The polymerization of actin occurs though the hydrolysis of the Adenosine-5′-triphosphate (ATP) of the actin subunit to adenosine diphosphate (ADP) plus an inorganic phosphate. This process yields a microfilament with distinguishable polar ends; the negative end is the exposed nucleotide cleft and the positive end is the one with the buried cleft. The polymerization prominently happens at the plus end. ATP is an unstable molecule, consisting of adenosine (formed by adenine ring and a ribose sugar) and three phosphate groups, which hydrolyses to ADP and phosphate. The conversion of ATP to ADP releases energy of about 30 kJ/mol. The subunits of a microtubule are called tubulin heterodimers; $\alpha$-tubilin and $\beta$-tubilin, which bind guanine nucleotides. Fibrous proteins such as Keratin assemble to build different types of intermediate filaments. Unlike MFs and MTs, IFs do not bind to nucleotides.

The components of the cytoskeleton are constantly changing in order to accommodate cell dynamic processes such as cellular movement and division. The assembly and disassembly of microfilaments allow a cell to change its shape and to accommodate cellular movement. White blood cells are prefect examples where cells remodel microfilaments to move through capillary walls and enter damaged tissues. In order to change its shape, a cell uses various proteins such as capping, branching, and severing ones to regulate microfilament dynamics. For example, treadmilling is a dynamic process within the cell where the net rate of addition and removal of actin subunits from two ends of a microfilament are equal. The formation and morphology of actin cytoskeleton network is regulated by a variety of actin binding proteins (ABPs). ABPs are essential part of eukaryotic cells and play very important functions ranging from regulating assembly and disassembly of F-actins to controlling actin network dynamics and structure. 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 are also essential for maintenance of cell structure integrity as they interconnect different cytoskeletal elements together. They connect actin to microtubules, actin to intermediate filaments or even actin to both of them (e.g., plectin). Another group of ABPs are molecular motor protein myosins which are necessary for the cytoskeleton dynamic behavior. They bind to actin filaments and move along them to do work within the cell by generating tensile forces. Myosin converts the chemical energy of the ATP into mechanical energy to move along the cytoskeletal substrate. For example, two-headed myosin II generates tension and contraction inside the cytoskeleton, Fig. 2.

The structure of the myosin consists of two identical heavy chains and two pairs of light chains. There are three distinguishable domains in each heavy chain, the head, neck and tail. The globular head domain contains binding sites for actin and is formed due to the folding one half of a single heavy chain. The other halves of the heavy chains twist around each other and form a helical fibrous structure. A pair of light chains exists in the neck domain (the connection between the head and the tail) in order to stiffen and stabilize this domain.

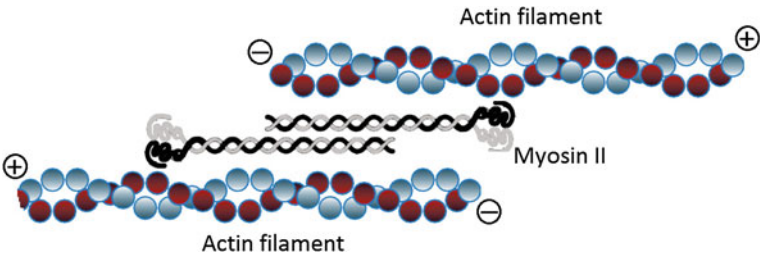


**Fig. 2** Schematic of a bipolar myosin filament interacting with two actin filaments. The ± signs indicates the polarity of actin (myosin moves toward the plus end)

The energy required for myosin movement along the actin filaments is directly linked to ATP hydrolysis. The nucleotide binding sites are at a distance of about 3.5 nm from actin binding sites. Therefore, the presence of the gamma phosphate sensor and the relay helix is essential within the myosin. Myosin distinguishes between ATP- and ADP-bound states using the gamma phosphate protein sensor. In muscle cells, the myosin head moves 5–10 nm with respect to the sarcomere due to the lever arm rotation of about 70° in each power stroke, the large sweeping movement of the myosin head. This process starts by myosin head being released from the actin because of the ATP binding to myosin. The ATP is hydrolyzed quickly to ADP plus inorganic phosphate. The myosin head rotates from the sarcomere midline and attach to the actin filament through ADP at a further location. Upon attachment, the ADP and inorganic phosphate are released. This causes the myosin lever arm to rotate and slide the myosin head toward the sarcomere midline.

Cytoskeleton is a random fibrous material undergoing constant arrangement and re-arrangement by actin cross-linking filaments such as α-actinin as well as myosin motor molecules. Therefore, the overall mechanics of the cytoskeleton is derived from both physical and mechanical properties of its microstructure. Physical properties include relative position of bio-polymer constituents, network fiber density, cross-linker types among many others. The strength, elasticity, plasticity, and creep behavior of individual filaments are examples of mechanical properties. Here, various theoretic and experimental attempts in order to analyze and understand the mechanics and micro- and macro-rheology of the cytoskeleton and its main ingredients are discussed. The structure of this chapter is as follows. First, recent experimental and computational studies to characterize the response of individual cytoskeletal filaments are reviewed. The chapter proceeds by presenting the main advances in modeling the macroscopic behavior of cells under external loads using experimental techniques as well as computational continuum- and micro-level models. The mechanics of stress fibers where motor molecules play a very important role is then discussed. Finally, the cellular mechanotransduction (a mechanism by which cells convert mechanical stimuli into chemical activities) along with its importance in physiological processes is reviewed. In subjects where

recent specialized reviews are available, the reader is referred to those references to avoid repetition.

## 2 Biophysical Properties of Actin Filaments

The linear aggregation of globular G-actin subunits with diameter of about 2–3 nm forms filamentous actin chains with diameter of about 7–9 nm and length of up to $\sim 100$ μm. The actin fibers, discovered first in 1942 [1], are polar structures and undergo constant polymerization and depolymerization which are regulated by ionic strength and the presence of capping, branching and severing proteins. The function of actin filaments derives from their mechanical properties; therefore, a proper understanding of actin properties is essential. Due to the large persistence length, the length over which the thermal bending becomes appreciable, F-actin is considered as a semiflexible polymer and is among very few of such polymers that can be visualized by video microscopy. Theoretically, it can be shown [2].

$$\langle \mathbf{t}(s) \cdot \mathbf{t}(s+r) \rangle_s = \exp(-r/2L_p)$$

where $L_p$ denotes the persistence length, $\mathbf{t}(s)$ is the tangent vector, $r$ is the distance from initial position $s$ along a filament and $\langle\rangle_s$ denote averaging over $s$. The persistence length of actin filaments are reported to be from 5 to 25 μm depending on the experimental techniques that are used to take the measurements [3–9]. For example, using fluorescence microscopy and by measuring the correlation function (defined as the dot product of tangent vectors of isolated filaments), $L_p$ is measured to be about $\sim 17$ μm [9]. The persistence length of a filament is defined as the ratio of its bending stiffness to thermal energy, i.e. $L_p = \kappa/K_BT$ where $K_B$ is Boltzmann constant, $T$ is the temperature in Kevin, $\kappa = EI$ denotes the bending rigidity, $E$ is the Young's modulus and $I$ is the second moment of inertia. Therefore, actin's $EI$ and subsequently its Young's modulus can be determined from persistence length measurements. Actin filament Young's modulus is about 1 GPa; however, they are soft and have small bending stiffness mainly because of very small cross-sectional dimensions of about few nanometers. It is noted that thermal fluctuations caused by the thermal energy introduce an entropic elasticity to the F-actin mechanics. This is a mechanism which maximizes the system entropy through increasing the number of available configurations for the filaments [10]. Several experimental and computational techniques have been used to examine the mechanical properties of actin filaments directly. For example, in order to directly measure the stiffness of $\sim 1$ μm long single actin filaments, a nano-manipulation technique is developed based on attaching filaments to microneedle and observing their deformation under a microscope [11]. In this method, the time resolution is at submillisecond and the resolution of forces and displacements are at subpiconewton and subnanometer, respectively. The elastic modulus of $\sim 1.8$ GPa was reported by modeling actin filaments as homogenous rods with cross sectional area

of $A \sim 25$ nm$^2$ based on the results of computer simulations. Moreover, the silicon-nitrade microfabricated levers have been used to stretch single actin filaments to characterize their longitudinal elasticity [12]. Single actin filaments are initially non-linear and highly compliant; however, as the tensile forces increase, they become linear and very stiff. In these experiments, no viscoelastic behavior has been observed for actin filaments and the stiffness of $\sim 34.5 \pm 3.5$ pN/nm has been found. Fitting the worm-like chain model to the results gives a persistence length of $\sim 8.75$ μm. Besides methods mentioned so far, laser traps have also been used to estimate the flexural rigidity ($EI$) of actin filaments [13]. In this approach, the ends of fluorescently labeled filament are attached to two microsphere handles which are manipulated using laser traps.

Another important factor in elasticity of actin filaments is the presence of various actin binding proteins and ionic conditions [14–16]. The F-actin elasticity, which is highly correlated with its helical microstructure, is changed due to any factor affecting its microstructure and stability. The metal ions and actin binding proteins strongly influence the functional and structural properties of G-actin monomers and subsequently F-actins. It is actually observed that actin filaments can transition from rigid to flexible due to variations in the bound divalent cations and/or the nucleotides [17, 18]. Pure actin filaments are stiffer than those decorated with myosins since myosin induces more flexible states in actins [5, 17, 19]. The above actin dynamic rigidity has been suggested for the stereocilia of the inner ear and as a possible cause of the noise induced hearing loss [20, 21].

Bound divalent cations do not have the same influence on torsional and flexural rigidities of actin filaments. The torsional motion of a single F-$Ca^{2+}$-actin and a single F-$Mg^{2+}$-actin under optical microscope shows that torsional rigidities of filaments are of the same order of magnitude as their flexural stiffnesses. While the flexural rigidity does not depend on the species of the bound divalent cation, quantitatively the F-$Ca^{2+}$-actin torsional rigidity is three times larger than that of F-$Mg^{2+}$-actin [16].

Mechanical and structural properties of actin filaments have also been studied using atomistic and molecular dynamics (MD) simulations [22–26]. From MD simulations the persistence lengths of F-ATP and F-ADP (F-actins that are composed of ATP-bound and ADP-bound G-actins) are, respectively, estimated to about 16 and 8.5 μm which agree with experimental measurements [23]. The main drawbacks of all atom MD simulations are the limited accessible time scales (on order of hundreds of nanoseconds) and length scales (of about tens of nanometer) which inhibit studying long filaments and the F-actin interactions with motor protein myosin. Coarse graining is one way to get around this problem and to increase the scales of the problem. In coarse grained models, certain features of a system are included in the model based on the properties and details obtained from its atomistic description. The crystal structure of filamentous actin is essential for these simulations; however, no high-resolution crystal structure of filamentous actin is currently available. The available molecular structures are obtained from X-ray diffraction of whole muscle and electron microscopy [27–31]. The interested reader is referred to the review done by Egelman [32] to learn more about the

low-resolution actin structural studies. The F-actin helical structure is composed of 13 actin molecule per 6 turns and a repeat of about 36 nm [33]. Holmes and colleagues solved the first crystal structure of G-actin in 1990 and proposed the F-actin atomic model, which has been widely accepted and commonly known as Holmes model [33, 34]. This is however a low resolution structure from which the detailed interaction between F-actin and actin biding proteins is described. Recently, Oda et al. obtained a high resolution fiber diffraction data by using an intense magnetic field and placing the crystal structure of G-actin molecule in the best possible orientation in the F-actin helix [35, 36]. They achieved a very good fit to the fiber diffraction pattern.

## 3 Structural Properties of Microtubules

Cytoskeleton consists of a network of microtubule filaments with important biological functions but different than those of actin filaments. They provide structural supports for extended cells like axons and cilia, control the directional migration of polarized interphase cells and transmit and generate forces, for example the mitotic spindle. Microtubules while stabilizing the structure, they serve as tracks for intracellular vesicle transport in neuronal axons. In cell division, they along with motor molecules form complex networks, the mitotic spindle, around chromosomes and separate chromosomes through their constant polymerization and depolymerization combined with the activity of a variety of microtubule-based motor proteins [37]. Microtubules are hollow cylinders with outer diameters of about 25 nm and inner diameters of about 17 nm, see Fig. 1. They are tens of nanometer to tenths of millimeter long filaments often spanning the whole cell. Microtubule Young's modulus is the same as in the F-actin; however, they are much stiffer due to their tubular structures. This large flexural rigidity is essential to enable microtubules to play their functional role, i.e., to resist the compressive and tensile forces of the motor proteins while maintaining the cell shape. They are created from the polymerization of $\alpha$- and $\beta$-subunits of tubulin which are bound to a guanosine triphosphate (GTP) molecule. Shortly after assembly the GTP is hydrolyzed to guanosine diphosphate (GDP), which remains bound at the same site and very prone to depolymerization. Therefore, microtubules are very dynamic polar structures undergoing constant depolymerization and polymerization [38]. The fast dynamics of microtubules causes considerable dispersions (beyond the usual systematic measurement errors) in their properties reported in literature. This inconsistency is usually associated to the polymerization conditions and growth velocity which is believed to yield a defective crystalline microtubule lattice [39, 40]. There has been a great research effort to elucidate the unique properties of microtubules. Here only some of main structural and physical features of microtubules are reiterated; the interested reader is referred to recent reviews illustrating the mechanics of microtubules [41–44].

The mechanical properties of individual microtubules can be determined either using an active method or a passive approach. In active approach, 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 [45–53]. For example, in hydrodynamic flow method, the flexural rigidity can be obtained from the quantitative analysis of equilibrium bending shapes of microtubules which are adhered to a glass coverslip and are subjected to hydrodynamic flow. Using motor proteins is another way to apply active forces on microtubules and measure their properties while they are interacting with the molecular motors [54–56]. Here, a portion of a microtubule filament is chemically clamped to a substrate and the conformational changes caused due to the interaction with motor molecules are measured.

Passive methodologies are those approaches in which the flexural rigidity of these filaments is derived from observation of their structural features, which are directly related to their mechanics. For example, observing conformational changes induced by thermal energy is proved to be a powerful tool to study the mechanics of biopolymers [57]. The method rests on the following two principles; (1) the amount of bending of filaments due to thermal fluctuations is directly related to their bending rigidity and (2) the result of equi-partition theorem, i.e. for a system in thermal equilibrium, each independent mode has on average an energy given by $K_BT$. Therefore, the dynamics and mechanics of microtubules can be studied from direct measurements of their shape fluctuations in three dimension using light microscope images [58, 59]. The analysis of images using automated image analysis algorithm yields the variation in curvature from which the filament bending rigidity is obtained. The accuracy of this method strongly relies on the filament tracking method, which is limited by uncertainties resulting from noise in images. This passive method has also been used to derive the properties of other biopolymers including actin filaments [9].

It is noted that passive and active experiments probe the linear response regime and nonlinear regime, respectively. The images of rapid thermally agitated bending modes only allow consideration of longest modes due to rapid decrease of signal-to-noise [57]. However, one is able to apply locally high forces along the filament length to study both the filament nonlinear response and its possible local defective structure. In the above the properties of the individual filaments were discussed; nevertheless, microtubules in vivo are surrounded by cytoskeletal network which affect their effective properties [60]. Figure 3 shows how the presence of the cytoskeletal network around the microtubule reinforces its behavior under compressive loading by prohibiting long wavelength buckling.

## 4 Intermediate Filaments

Intermediate filaments (IFs), a large family of about 60 proteins, are also present in cytoskeleton and play distinct functions in the mechanics and structure of

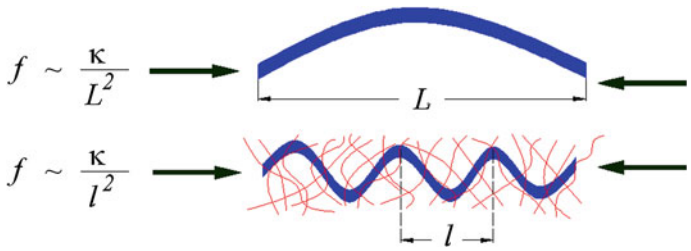


**Fig. 3** Schematic plot representing the effect of the surrounding elastic cytoskeleton on the bucking behavior of microtubules in living cells [61]

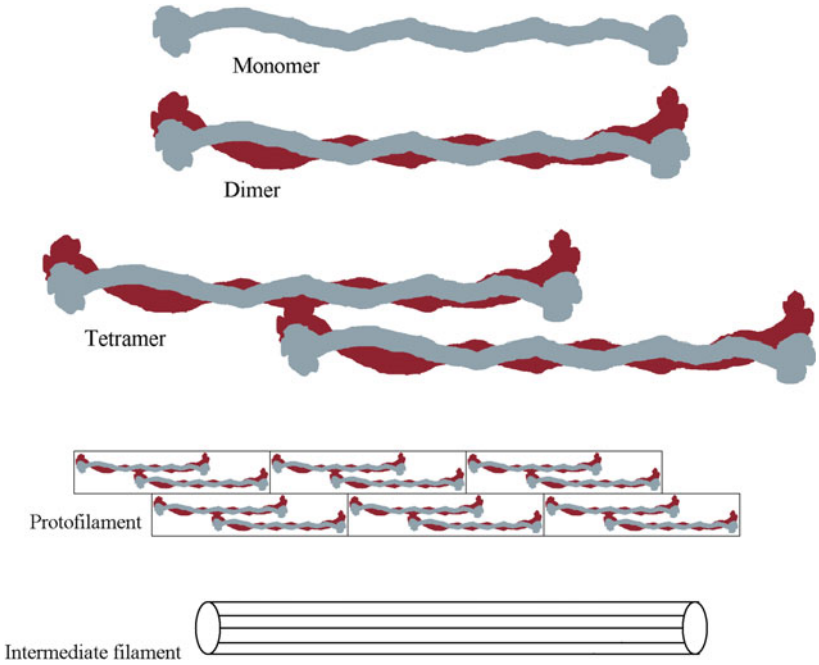


**Fig. 4** Schematic plot for the structure of an intermediate filament

cytoskeleton. The proteins found in these filaments are divided into five groups based on their gene structure, primary structure, assembly properties, and their development regulated tissue-specific expression patterns [62]. Unlike actins and microtubules, IFs are made of filamentous proteins with no enzymatic activity. Intermediate filaments are actually coiled coil bundles of protofilaments which are obtained from staggered in arrays $\alpha$-helical domains, see Fig. 4. They have no vectorial transport role because of their non-polarity and show different properties

from those observed for F-actins and microtubules. For example, they have long-term stability and little solubility in salts. It is believed that intermediate filaments provide structural stability to the cell and have a supplementary function to other components of the cytoskeleton when the cell is under mechanical stress. The IF's mechanics and structure have been the subject of different research efforts [62–66]. The atomic force microscopy was used to estimate the IF persistence length to be few micrometers and also their bending modulus of about 300 MPa by elastically deforming single filaments [63, 67]. With few exceptions, IFs are very flexible in bending with remarkable extensibility up to three-folds [66, 68].

## 5 Rheology and Mechanics of Cytoskeleton Network

In the previous sections, some of main structural and biochemical features of cytoskeleton network constituents were reviewed. The three main ingredients of the cytoskeleton, i.e., filamentous actins, microtubules and intermediate filaments, bundle together and form a dynamic network with unique properties and prominent role in a variety of essential cell functions including but not limited to adhesion, migration and mechanotransduction. The complete understanding of the behavior of this complicated structure requires investigating the properties of its individual ingredients and more importantly determining and indentifying their interactions with each other in the resulting network. This section discusses both computational and experimental models that have been proposed over the past years regarding the cytoskeletal rheology and mechanics.

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 very well developed [2]. As the persistence length becomes larger and comparable to (or larger than) the contour length, the dynamic of the filament changes. These filaments are called semiflexible and the properties of the networks formed by these fibers are less developed.

Cytoskeleton is a highly heterogeneous and consists of semiflexible filaments with interconnection lengths of the same order as fiber persistence length. It is also an active and dynamic structure undergoing constant remodeling in response to external and internal stimulants. The biophysical properties of cytoskeleton have been studied and measured in living cells; however, we are far from understanding their physical origins due to the complexity and heterogeneity of the cytoskeleton microstructure. For example, F-actin networks subjected to oscillatory shear deformation show negative normal stresses as large as shear stresses [69]. 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 [70, 71]. A reversible stress-softening behavior in compression, essential in preventing catastrophic fracture of actin networks, has also been reported [72]. Flexible and rigid polymers exhibit only monotonic stiffening in compression. The origins of these unique macroscopic behaviors are under study and there is no comprehensive theory yet.

## *5.1 Experimental Studies*

The cytoskeletal network has viscoelastic properties and shows characteristics of both elastic solids and viscous fluids. Oscillatory external stresses are used to determine its effective elastic modulus as well as the amount of stored mechanical energy. The mechanical response of actin networks is non-linear and strongly depends on the stress and experimental conditions. Depending on the length scales at which the measurements are taken, the network can show very different properties [73, 74]. The inclusion of length scale of observation is essential for analyzing and developing models for the mechanics of cytoskeleton and any other semiflexible networks [75].

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). Passive microrheology, fluorescence correlation spectroscopy and dynamic light scattering are among passive experimental measurements developed to measure the probe and measure the mechanics of actin solutions [76]. Active rheology measurements methods, where the system properties are probed by applying localized forces, include atomic force microscope, optical traps, microplates, micropipette aspiration, magnetic traps among many others [76]. These methods can be used to identify the properties of the network at various length scales. For example, while the bulk rheology of the network is estimated from two-particle microrheology method, the short-length fluctuations are isolated in one-particle microrheology [73, 77, 78].

## *5.2 Computational Studies*

The biomechanical models developed for the mechanics of a cell are either based on the continuum level and macroscopic observations (phenomenological models) or derived from its 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 [77]. These continuum-level models may only be used to describe 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, which are usually studied and characterized separately, are included in the model.

There are instances where a cell behaves at macroscopic length scales which are two to three orders of magnitudes larger than its microstructural characteristic lengths. The behavior of erythrocytes/neutrophils in micropipette aspiration and magnetocytometry induced deformations are among cases where continuum models successfully describe the overall mechanical properties [79–84]. In micropipette aspiration, the cellular elasticity and viscosity are measured by pulling on cells, such as monocytes, erythrocytes (red blood cells) and leukocytes (white blood cells). In magnetocytometry, magnetic microbeads tightly bound to the cell are used to apply controlled forces to a single cell. In these situations, the scales of observed deformation are such that a continuum model can be developed to capture cell response. 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 among solid, fluid and free ions in the cytoskeleton [85]. 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 [86]. Finally, although we have the freedom to incorporate inhomogeneous as well as 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 experimental observations and usually cannot be generalized [87].

It is clear that one of the main missing features in continuum-level models is the consideration of the fibrous and discrete structure of cytoskeleton. In discrete models, the cytoskeleton microstructure is represented by a network of randomly cross-linked fibers. A complete review of these models has been presented in [90]. Since actin filaments are heavily cross-linked on the scale of their thermal persistence length, their common modes of deformation are bending, twisting and stretching. Therefore, cytoskeleton is classified as being a semiflexible network

whose behavior is strongly non-affine. The behavior of a semiflexible network and its relationship to the mechanical properties of its constituents are much more complicated than what it is for a flexible network. The accepted models for flexible networks [2] are not appropriate to study the mechanics of semiflexible networks. There have been various research efforts to develop 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, [75, 91–96]. 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 effects. The langevin equation can be employed to describe the hydrodynamics drag force of the filaments through the solvent. The presence of cross-linker in semiflexible gels causes significant change in network properties resulting from two distinct single-filament responses, i.e., bending and stretching modes. If individual filaments only rotate and uniaxially deform under uniform loadings and the macroscopic strain distribute uniformly throughout the domain (affine deformation), the behavior of the network can be modeled by affine network models [10]. These models are not valid where the behavior is non-affine and bending motions of the filaments become important. The reader is referred to recent papers for a detailed discussion on affine versus non-affine behavior [91, 92, 95]. A new methodology has recently been proposed by Hatami-Marbini and Picu to solve boundary value problem on dense random fiber networks by determining the statistics of their behavior and accounting for all possible configurations at the desired length scale using Stochastic Finite Element Method [75]. In this methodology, the nodal displacements of the network subjected to far-field loadings are obtained by minimizing the potential energy of the entire network. Then a regular

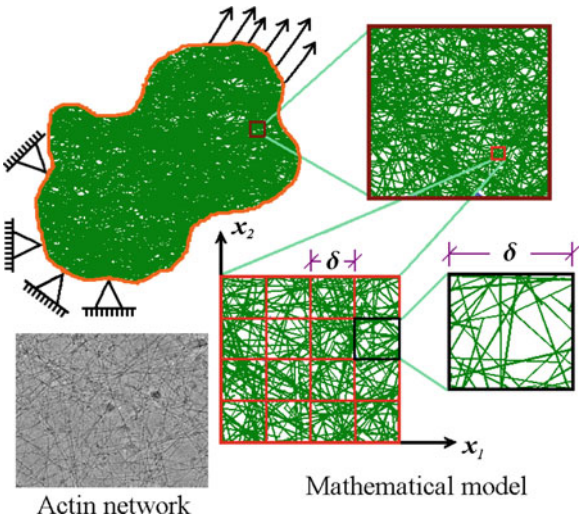


**Fig. 5** Schematic plot showing F-actin network divided into a regular mesh of characteristic dimension $\delta$. The size of *square* elements, $\delta$, denotes the length scale of observation. The plot also shows the in vitro visualization of the actin cytoskeleton [88, 89]

mesh of square elements of size $\delta$ (see Fig. 5) is overlaid on the network. Calculating the average stress and strain distribution over each square element, these elements are replaced with homogeneous continuum domains with correlated elastic moduli. Therefore, the respective equivalent continuum domain has the main features and correlations of the network microstructure. A note is in place about the fiber cross-links in cytoskeleton. In mechanical models for F-actin network, the fibers are often assumed to be permanently hinged or rigidly cross-linked. Nevertheless, these cross-links undergo constant disassociation in cytoskeleton and have variable strength depending on the ABPs' structures and properties. For instance, filamin, an approximately 150 nm long V-shape ABP, preferentially cross-link actin fibers. It is composed of $\beta$-sheets which are unfolding under load and induce a sawtooth force displacement curve for this ABP cross-link [97–101]. Therefore, the cross-links between filaments are not simple rigid structural element and are rather very important to the overall network stiffness and elasticity. This is an important detail which is conveniently ignored in many network models.

As it is 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, i.e., local microstructure and connectivity, of actin filaments which are assembled and disassembled in response to various kinds of signals resulting from cell locomotion, cell division and extension [102–104]. 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 $\alpha$-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 15 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 is greatly influenced through the contribution of forces generated by myosin so 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, see Fig. 6. 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

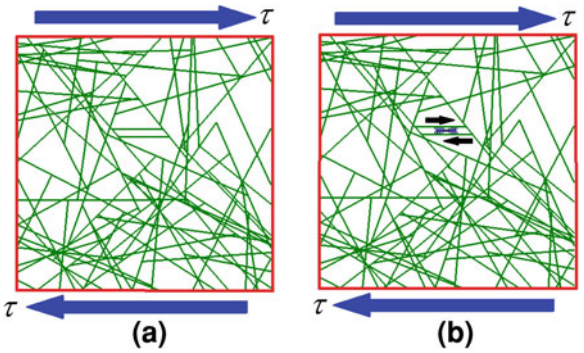


**Fig. 6** Schematic network models for **a** passive networks and **b** active networks subjected to external loadings. In active networks, myosin molecular motors control network elasticity and induce a state of pre-stress inside the network

orders of magnitude [105]; however, a mathematical and theoretic model of active network has not yet been developed.

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 the 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. However, 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 strongly 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 (called the focal adhesion), stress fibers often form [106, 107]. They also from along the direction of external forces, e.g., it is seen that stress fibers form and orient with the direction of fluid flow in the endothelial cells under shear stress [108, 109]. The mechanical properties of stress fibers can be non-uniform because of the variations in myosin spacings along the length of stress fibers. 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 cytoskeleton stress fibers to discrete sites on the nuclear envelop [110, 111]. Stress fibers enable cells to focus myosin contractility along a specific direction of resistance; therefore, cells can stiffen directionally to protect themselves against excess stress and strains in this particular direction. Moreover, cells explore the matrix rigidity and migrate in the direction of increasing rigidity through stress fibers [112]. Cell locomotion is believed to depend on and even be controlled by changes in substrate rigidity. In particular, cell response on culture surfaces is dictated by substrate rigidity: actin stress fibers are oriented along the stiffest direction of the microfabricated substrates [113].

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 [111, 114, 115]. Inclusion of this correction in the model recovers the directed and focused nature of the 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 missing 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 the cytoskeleton as well as the effects of hydrodynamic interactions on the behavior of individual filaments [114, 115].

It has been shown that the elastic modulus of stress fibers in living endothelial cells is approximately 10–15 kPa which remains constant over large strains up to 12% [116]. This stiffness is a function of myosin II since disturbing its activity by adding myosin inhibitor blebbistatin causes a 30% loss of the modulus [117]. The elastic modulus of fibroblast cells also decreases due to the application of this myosin inhibitor [118]. This further confirms the importance of the tension generated by myosin to the overall cellular stiffness. Despite considerable experimental and computational efforts to study basic physical principles of cell contractility due to myosin II motors, our understanding is still limited. It is not yet clear how contractility and pattern formation changes with microscopic parameters such as the number of myosin motors, the number of cross-linkers and the density of actin filaments. It is known that the motor activity inside the cell is a controlled process which results in formation of stress fibers in cells on flat substrates and the contractile rings during cytokinesis [119–121]. 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 have been confirmed by in vitro studies [122, 123]. In these models, the actin network is modeled as an 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 cytoskeletal 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 [124]. In other words, the 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 [125]. 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 [126].

The study of reconstituted biopolymer networks is an excellent way to model and understand the important mechanical features of the living cell cytoskeleton

[105, 123, 125]. These reconstituted networks of filamentous actin combined with myosin motors are a new class of active materials whose mechanical properties can be adjusted and tailored by enzymatic activity [127]. It has been observed that molecular motor proteins can either stiffen or weaken the F-actin solution depending on the density of F-actin cross-linkers. While the presence of actin cross-linker provides sites for mechanical anchorage and accommodates internal tension, active filament sliding occurs in their absence because of myosin activity. The protein myosin permits active control over the mechanical behavior of F-actin network solutions. For instance, the stress relaxation time of actin solutions will shorten upon addition of molecular motor myosins due to their interaction with actin filaments. The interaction of single polymer chains with surrounding polymer yields an effective viscoelastic behavior. The viscoelasticity of actin–myosin networks can be modulated using the ability of the myosin to supersede reputation with sliding motion. Myosin II replaces the thermally driven transport of individual polymers (snake-like movement described by reputation model) with active filament sliding motion. In the reputation model, a tube represents the topological constraints of neighboring polymers on the movement of a single chain [2]. A modified tube model has been proposed to model the dynamics of polar actin filaments with active, motile centers generating a longitudinal motion [128]. Molecular dynamics simulations have also been used to investigate the rheology and the structure of F-actin solutions interacting with molecular motors [129].

## 6 Cellular Mechanotransduction

In the previous sections, we focused on the mechanical properties of the cytoskeleton. It was mentioned that the highly dynamic mechanics of the cell is derived from its constantly changing cytoskeletal structure in response to external stimuli. A cell actively responds to the mechanical signals it receives from the environment. This implies that cells are capable of sensing the mechanical force and transducing these signals to the biological response. The mechanism by which a cell converts mechanical signals to biochemical signal is called cellular mechanotransduction [131]. This process has been the subject of intensive studies with the aim of understanding both its mechanism and its implications. Here, for completeness, we present a brief introduction to this phenomenon and refer the reader to the related references for a thorough discussion [130–133].

The changes in biochemical activities (e.g., changes in intracellular calcium concentration or activation of diverse signaling pathways) within the cell in response to the mechanical stress modify cell internal structure and adjust its mechanical properties. Therefore, the biological functions of a cell is highly influenced and even controlled by the mechanical forces and the way they are transduced into biochemical signals. In this process, many structures and components such as myosin motor molecules, cytoskeletal filaments, ion channels, integrin and nuclei are involved. Several cellular phenomena are found to be

influenced by mechanical stress: (1) the membrane strain controls the opening and closing rate of a stretch- or flow-sensitive ion channel, which allows influx of calcium and other ions [134–137]; (2) Hemodynamic forces caused by blood flow deform and align endothelial cells along the arterial walls to maintain a nearly constant shear stress [138–140]; (3) Chemical and mechanical cues closely regulate the assembly of focal adhesions through which cells probe the stiffness of its environment and adjust its response, e.g., cells migrate in the direction of increasing substrate stiffness [112]; (4) The external forces induce conformational changes in cytoskeletal elements which in turn alter and activate signaling pathways, e.g., applying tensile forces to cultured neurons or vascular muscle cells increases the microtubule polymerization [141, 142]. It is also seen that mechanotransduction has a central role in the functions of some of our senses such as hearing and balance, which are resulting from electrochemical responses to sound waves, pressure, and gravity [143].

Abnormality in the cellular mechanotransduction can have serious pathological consequences [130, 131, 144]. The development and maintenance of the muscle tissues and even physiological processes, e.g., the blood flow and the blood pressure, all depends on the ability of cells to sense and respond to changes in their physical environment. For example, the morphology and physiology of the heart and vasculature are affected by the pressure and shear stresses generated from the flowing blood. Since mechanotransduction regulates and influences many cellular functions including protein synthesis, secretion, adhesion, migration, and apoptosis, diseases could arise because of any inherited or acquired defects in the cellular mechanotransduction. The loss of bone mass in a microgravity environment and atherosclerosis, a condition where arterial walls thicken as the result of the build-up of fatty materials and calcification, are two examples of such diseases [145, 146]. In atherosclerosis, the fluid shear stress is disturbed at bifurcations, which in turn causes the remodeling of the vascular system. Many other diseases such as the hearing loss, the muscular dystrophy, the glaucoma, the premature aging, and even the cancer can be related to the malfunctioning of the cellular mechanotransduction.

## 7 Summary

There have been much learnt about the molecular bases of cytoskeletal mechanics during the past few decades. The mechanics of the cytoskeleton depends highly on its ever varying and dynamic microstructure. Cell maintains its stability and optimizes its response to various external and internal excitements by constant arrangement and re-arrangement of its actin filamentous reinforcements. This dynamic process requires actin cross-linking filaments such as $\alpha$-actinin as well as myosin motor molecules. In this chapter, the most important and recent experimental and theoretical studies are brought together and are concisely reviewed to shed light on the cytoskeletal rheology and, in turn, on the dynamic response of the cell. The experimental observations along with computational approaches used to

study the mechanical properties of the individual constituents of the cytoskeleton were first presented. The chapter then reviewed various computational models ranging from discrete filamentous models to continuum level models developed to capture the highly dynamic and constantly changing properties of the cells to external stimuli. It is seen that depending on the cellular phenomena under study, either type of the modeling methodology may prove to be the correct and better-suited one. Intuitively, it is expected that while continuum models better describe whole-cell deformations, the details of the filament organization and their interaction with myosin motor molecules are critical and important in cases where the scales of interest become very small, e.g., the scale of an AFM tip. Finally, in the last section, the mechanotransduction was introduced as being an essential function of the cell which controls its growth and proliferation among others. A wide spectrum of pathologies, e.g., atherosclerosis, is caused because of any disturbance in this process.

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