

Rheology of the Cytoskeleton

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living cells, mechanics, actin filaments, molecular mechanics

Abstract

The cytoskeleton is the primary internal structure of the cell, providing its structural integrity. The rheology and mechanics of the cytoskeleton, therefore, are key to the cell's ability to accomplish its diverse functions in health and disease. Although the importance of the cytoskeleton is well established, the relationship between the microstructural details and the macroscopic rheological behavior of the cytoskeleton remains elusive. A wide range of computational and phenomenological models as well as experimental techniques have been proposed over the past two decades to describe the cytoskeleton, giving rise to several, often contradictory, theories for describing its rheology. This concise review attempts to bring together the key experimental methods and theoretical and computational models regarding cytoskeletal rheology and mechanics.

1. INTRODUCTION

Cells are the basic functional units of life. To perform their various functions, they undergo or control a wide range of intra- and extracellular activities, most of which involve mechanical phenomena or may be guided by the forces experienced by the cell.

Cells are living, dynamic, changing systems with the capability to alter their mechanical properties in response to external stimuli. Many of their biological functions, for which mechanics is central, are active processes for which the mechanics and biology are intrinsically linked. In many cases, the cell's ability to perform its function depends on its shape, which is maintained through structural stiffness and rheology. The internal structure of the cell, the cytoskeleton, along with the cell membrane provide the structural integrity to maintain the particular shape needed by the cell to accomplish its function, although the specific components of the structure are highly variable and diverse. Many cells migrate, certainly during development as the organism grows, but also at maturity in health (e.g., angiogenesis, wound repair, disinfection) and disease (e.g., cancer metastasis, atherosclerosis). Hence, the role of cytoskeletal mechanics and rheology is important in migration. Cells can sense and respond to externally applied mechanical forces and transduce them into a cascade of biochemical events that affect their behavior in health and disease. Although the detailed mechanisms of mechanotransduction remain elusive, the consequences of force applied to cells are well documented (e.g., see Davies 1995, 2002; Dewey et al. 1981; Gimbrone et al. 2000; Hamill & Martinac 2001; Lehoux & Tedgui 2003; Malek & Izumo 1994; McCormick et al. 2003; Mofrad & Kamm 2009).

A wide range of computational and phenomenological models as well as experimental techniques have been proposed over the past two decades to describe the cytoskeleton. These experimental observations along with computational and theoretical approaches have given rise to several, often contradictory, theories for describing the rheology of the cytoskeleton. This article presents a concise review that brings together the key experimental methods and theoretical and computational models regarding cytoskeletal rheology and mechanics.

2. CYTOSKELETON: ITS STRUCTURE AND COMPOSITION

The cytoskeleton is primarily responsible for the structural integrity and stiffness exhibited by a cell. It comprises a system of highly entangled protein filaments that permeate the microfluidic space of the cytosol. This fibrous matrix plays a prominent role in a variety of essential cell functions, ranging from migration to adhesion to mechanotransduction (the phenomena involved in a cell's sensing and responding to mechanical stimuli). The major components of the cytoskeletal network are actin filaments, intermediate filaments, and microtubules and their cross-linking proteins. These protein filaments are collectively responsible for the main structural properties and motilities of the cell.

Actin filaments form by the polymerization of globular, monomeric actin (G-actin) into a twisted strand of filamentous actin (F-actin) 7–9 nm in diameter with structural polarity, having a barbed end and a pointed end. Monomers consist of 375 amino acids with a molecular weight of 43 kDa. ATP can bind to the barbed end, which allows for monomer addition and filament growth, whereas depolymerization occurs preferentially at the pointed ends. Filament growth and organization are regulated by many factors, including ionic concentrations and a variety of capping, binding, branching, and severing proteins. From actin filaments, tertiary structures such as fiber bundles (termed stress fibers) or a three-dimensional lattice-like network (**Figure 1**) can be formed through the action of various actin-binding proteins (ABPs). Some examples of ABPs are α -actinin, instrumental in the formation of stress fibers or bundles of actin filaments, and filamin, which connects filaments into a three-dimensional space-filling matrix with filaments joined at nearly a right

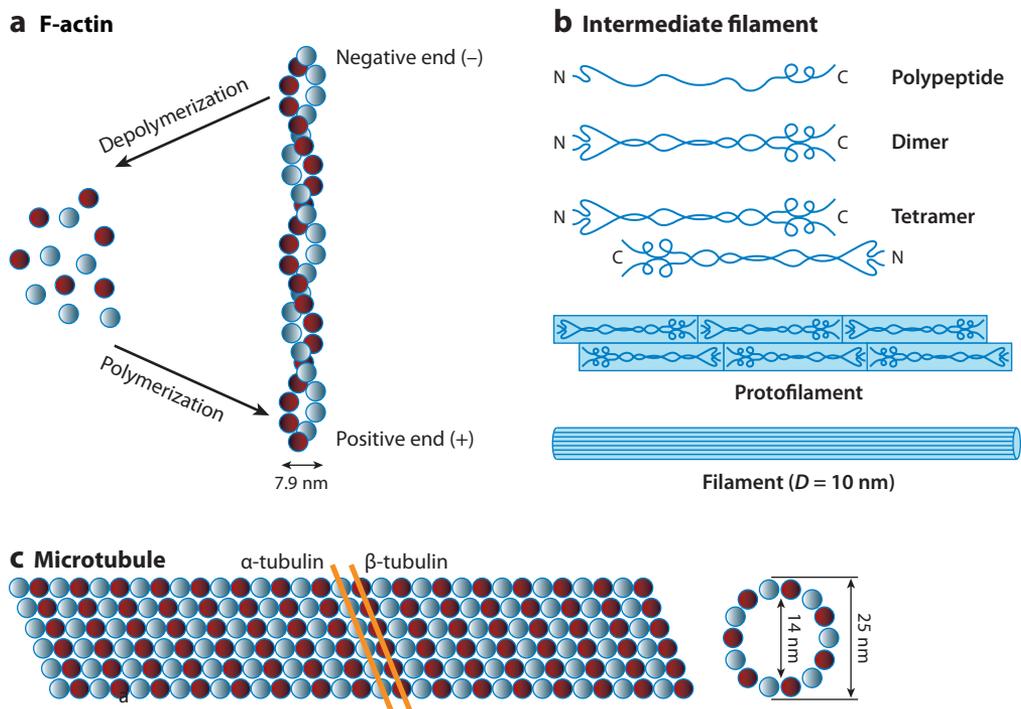


Figure 1

Primary constituents of the cytoskeleton: (a) actin filaments, (b) intermediate filaments, and (c) microtubules.

angle. Recent rheological studies of reconstituted actin gels containing various concentrations of ABPs (Tseng et al. 2004) have illustrated the rich complexities of such simple systems but have also provided new insights into the nature of such matrices. Stress fibers are known to form between points in the cell at which actin myosin contractility is resisted. Typically, these are hot spots of protein activity known as focal adhesions, where the actin cytoskeleton is anchored to *trans*-membrane protein integrins, which are in turn anchored to the matrix proteins. The focal adhesion also includes a cascade of proteins that relay biochemical signals, regulate its strengthening, and organize the cytoskeleton for the stress-fiber growth (e.g., cross-linking actin and recruiting myosin) (Burrige et al. 1988, Katoh et al. 1995, Kolahi & Mofrad 2008, Lee et al. 2007, Romer et al. 2006).

Actin is thought to be the primary structural component of most cells, constituting up to 10% of all the protein in most cells. It responds rapidly and dramatically to external forces and plays a key role in cell migration. Actin filaments have been measured by a variety of techniques (Higuchi & Goldman 1995, Tsuda et al. 1996, Yasuda et al. 1996). These filaments are stiff, having a persistence length of several micrometers and an effective Young's modulus, determined from bending stiffness and radius, of $1\text{--}3 \times 10^9$ Pa, comparable with that of polystyrene (3×10^9 Pa) and nearly equal to that of bone (9×10^9 Pa).

Microtubules constitute another major component of the cytoskeleton. These are polymerized filaments constructed from monomers of α - and β -tubulin in a helical arrangement, both 55-kDa polypeptides, that organize into a small, hollow cylinder (Figure 1c). The filaments have an outer diameter of approximately 25 nm and exhibit a high bending stiffness, even greater than that of an actin filament, with a persistence length of approximately 6 mm (i.e., three orders of magnitude longer than the length scale of most cells) (Gittes et al. 1993). Tubular structures tend to be more

resistant to bending than solid cylinders with the same amount of material per unit length, which, combined with the larger radius, accounts for the high bending stiffness of microtubules despite having an effective Young's modulus similar to that of actin.

Microtubules are highly dynamic, even more so than actin, undergoing constant polymerization and depolymerization, so that their half-lives are typically only a few minutes (Mitchison & Kirschner 1984). Growth is asymmetric, as with actin, with polymerization typically occurring rapidly at one end and more slowly at the other, and turnover is generally quite rapid.

Intermediate filaments constitute a superfamily of proteins containing more than 50 different members. They have in common a structure consisting of a central α -helical domain of over 300 residues that forms a coiled coil. The dimers then assemble into a staggered array to form tetramers that connect end to end, forming protofilaments (**Figure 1b**). These in turn bundle into ropelike structures, each containing approximately eight protofilaments with a persistence length of approximately 1 μm (Mucke et al. 2004). Intermediate filaments differ from microfilaments and microtubules in terms of their long-term stability and high resistance to solubility in salts. Also, unlike the polymerization of other cytoskeletal filaments, filament formation occurs without GTP or ATP hydrolysis.

Although the protein filaments mentioned above (actin filaments, microtubules, and intermediate filaments) are the primary protein constituents of the cytoskeleton, numerous other linking proteins (ABPs constituting one family) exist within the cytoskeletal network that influence the strength and integrity of the cytoskeleton. Any of these constitutes a pathway for transmitting force through the cytoskeletal network.

Although the importance of the cytoskeleton is well established, the relationship 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. As no single method has emerged as clearly superior, this might reflect the need for approaches not yet envisaged. Below I present a spectrum of views on current approaches to modeling and measurements in cytoskeletal rheology.

3. EXPERIMENTAL TECHNIQUES TO STUDY CYTOSKELETAL RHEOLOGY

A variety of experimental techniques have been developed for studying the mechanical properties of the cytoskeleton, such as its viscoelastic properties and its diffusion parameters. The cytoskeleton is highly heterogeneous and has an intricate yet diverse structure. Coupled with a small linear response regime and an active character (continuously remodeling and undergoing biochemical changes), the heterogeneous structure of the cytoskeleton prevents simple measurement of its rheology. The methods developed to study rheology attempt to circumvent these challenges in different and unique ways. Some monitor passive Brownian movements without directly engaging the cell mechanically, whereas others measure responses to the direct application of external force. For a detailed account and history of these experimental techniques, the reader is referred to an excellent review by Janmey & Schmidt (2006). The following section summarizes the most impactful experimental methods for studying cellular mechanical properties.

3.1. Passive Measurement Methods

Different microrheology measurement approaches have been developed to capture and characterize the rheology of the cytoskeleton. These methods can be largely divided into two broad classes: active techniques involving the application of forces and passive techniques that examine the motion of inherent or introduced particles due to thermal fluctuations.

3.1.1. Passive microrheology. One approach to measuring the microrheology of the cytoskeleton is to monitor the displacement of a probe owing to thermal fluctuations, referred to as passive microrheology. The technique does not apply external forces, but rather monitors microscopic probes undergoing thermal fluctuations characteristic of their environment (Addas et al. 2004, Lau et al. 2003, MacKintosh & Schmidt 1999, Mason et al. 1997, Schmidt et al. 2000, Schnurr et al. 1997, Yap & Kamm 2005). Micrometer-sized beads are embedded into the cytoskeleton and monitored using either video recordings and particle tracking (Crocker & Grier 1996) or laser beam interferometry (Denk & Webb 1990, Gittes & Schmidt 1998, Pralle et al. 1999). Original passive microrheology experiments utilized a method that monitored the movement of a single particle. This method was unable to account for the particle's active (nonthermal) movement or its own effects on its environment. Later, passive microrheology experiments utilized a method that calculated the cross-correlation between the individual movements of two particles (Crocker et al. 2000, Levine & Lubensky 2000). Jonas et al. (2008a,b) recently developed a new fluorescence laser-tracking microrheometer to measure cytoskeletal rheology using fluorescent microspheres as tracer particles. This novel technique offers nanometer spatial resolution over a frequency range extending from 1 Hz to 50 kHz.

3.1.2. Dynamic light scattering. For samples with a homogeneous solution of proteins or particles, the viscoelastic properties can be explored using a dynamic light-scattering experiment (Berne & Pecora 1990). In this technique, a laser beam is passed through the sample, and a detector collects light scattered by the sample. Using the measured scatter, one can calculate the average mean-squared displacement of the particles. This method is not suited for the study of the cellular environment directly because of the heterogeneity of the cytoskeleton.

3.1.3. Fluorescence correlation spectroscopy. Based on the principles of dynamic light scattering, a method has been developed that is suitable for use in measuring diffusion and viscoelasticity within a cell. Fluorescence correlation spectroscopy uses a laser beam focused on a small volume within the cell and photon detectors to record fluctuations in fluorescence resulting from the movement of fluorescent molecules into and out of the volume (Hess et al. 2002, Webb 2001). The method is well suited for the study of small particles.

3.2. Active Measurement Methods

Unlike the passive measurement approaches, the active microrheology estimation methods incorporate some type of force application typically localized at the site of the interrogation. A summary of prominent techniques for active rheology measurement follows.

3.2.1. Glass needles. The glass needle method was designed originally for experimentation with neuronal extension and uses a thin glass needle to apply nanonewton or smaller forces. The thin glass rod or needle has been calibrated by a series of precalibrated thin rods, each slightly larger than the previous one. One larger rod is originally calibrated using a known weight, and this rod is used to calibrate a rod slightly smaller than itself. Each rod is then used to calibrate the rod smaller than itself sequentially until the thin glass rod is calibrated (Heidemann et al. 1999).

3.2.2. Cell poker. The cell poker is a device designed to indent the cell using an oscillating glass needle tip. The cell is suspended from a coverslip on top of the glass needle. The glass needle is attached to a wire needle fastened to a piezoelectric actuator. As the actuator oscillates the glass needle vertically, and the glass needle makes indentations on the cell, the applied force is measured

by the difference in displacement between the wire needle and the glass needle. This difference results from the bending of the glass needle, which has been calibrated using a hanging weight, and thus the force resulting in its deformation can be calculated. The cell poker can produce forces less than 10 nN and could also reveal local deformation in different parts of the cell (Daily et al. 1984, Janmey & Schmidt 2006).

3.2.3. Atomic force microscope. The atomic force microscope (AFM) can be used essentially as a high-resolution cell poker. Originally, studies of cellular mechanics used AFM's sharp micro-fabricated tips with radii on the order of 10 nm to apply both pulling and pushing forces on the cellular surface (MacKintosh & Schmidt 1999). The heterogeneous structure of the cytoskeleton prevented the accurate measurement of cellular properties using the thin microtips, so more recent AFM studies of cellular mechanics have utilized polystyrene beads as tips, allowing for more control of spatial resolution (Chaudhuri et al. 2007, Mahaffy et al. 2004).

3.2.4. Micropipette aspiration. A micropipette can be used to measure the mechanical properties of cells. Suction can be applied within the micropipette and can be used to apply pulling forces on cells both in solution and attached to a surface. The method allows for the measurement of cellular elasticity and viscosity, as well as mechanical tension in the cellular membrane. Micropipette aspiration has been used to study the mechanical properties of monocytes, red blood cells, leukocytes, and erythrocytes (Discher et al. 1998, Dong et al. 1988, Hochmuth 2000, Richelme et al. 2000).

3.2.5. Microplate. The microplate method has been developed and used to measure the mechanical properties of surface-adherent cells. In this method, the cell is grown on a rigid microplate coated with fibronectin, and a flexible microplate is placed on top of the cell. The rigid plate is then moved to produce compression, extension, or shear. Measurement of the corresponding deflections in the flexible microplate allows for the accurate measure of the stress imposed on the cell (Thoumine et al. 1999). This method has been used to study fibroblasts and has shown the elastic modulus to be approximately 1 kPa (Caille et al. 2002, Thoumine & Ott 1997).

3.2.6. Parallel-plate flow chambers. Vascular endothelial cells, osteocytes, and several other cell types are physiologically exposed to fluid flow and shear stress (Barbee et al. 1995, Burger & Klein-Nulend 1999, Weinbaum et al. 2003). To study these cells' response to fluid flow, one can use a technique in which a cell monolayer is coated onto the surface of a chamber surface. Fluid is then allowed to flow through the chamber and over the surface. A cellular probe can then measure the cell's response. The method has revealed cellular sites with significant strain. It is unclear if these are sites of cellular mechanosensing or sites with little strain.

3.2.7. Optical trap. A flourishing technique that has recently led to many molecular-scale insights into the cytoskeleton is the optical tweezer or trap. A laser beam passed through a high-aperture objective lens can spatially trap a particle if 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 (Ashkin 1997, Lang et al. 2003, Svoboda & Block 1994). To study the microrheology of a cell, one uses a micrometer-sized silica or latex bead as the trapped particle. The trapped particle can be attached to the cell's surface and used to apply local forces on the cell. This method produces forces lower than 100 pN and can be used to measure the cell's linear response (Peterman et al. 2003). The optical-trap method provides several advantages, including the ability to measure the mechanical properties of cells not mechanically accessible, the ability to apply forces less than 100 pN, and the detection of strain with time steps as small as 10 ms (Gittes & Schmidt 1998,

Pralle et al. 1999). Newer optical-trap methods have been developed to trap individual cells and thereafter apply stretching forces on them (Guck et al. 2000, 2001). Two opposing laser beams trap a cell and then stretch it, recording its strain response. Optical traps were recently used in a clever experimental assay system to measure the rupture force of a complex formed by an ABP (namely, filamin or α -actinin) linking two quasiparallel actin filaments (Ferrer et al. 2008). ABPs regulate the assembly of actin filaments (F-actin) into networks and bundles that provide the structural integrity of the cell. Two ABPs, filamin and α -actinin, have been used extensively to model the mechanical properties of actin networks grown in vitro; however, there is a lack of understanding as to how the molecular interactions between ABPs and F-actin regulate the dynamic properties of the cytoskeleton (Ferrer et al. 2008, Kolahi & Mofrad 2008; J. Golji, R. Collins & M.R.K. Mofrad, submitted manuscript).

3.2.8. Magnetic traps. Paramagnetic and ferromagnetic microbeads can be manipulated by magnetic-field gradients and used to apply large forces both on the surface of cells and in the intracellular environment. Magnetic-field gradients applied to paramagnetic beads can generate pulling forces (Bausch et al. 1998, Karcher et al. 2003, Charras & Horton 2002, Keller et al. 2001), whereas their application to ferromagnetic particles can generate torsional forces (Fabry et al. 2001, Valberg & Butler 1987, Valberg & Feldman 1987, Wang et al. 1993). For use on the cellular surface, the magnetic beads are coated with fibronectin and attached to the surface. For use in measurements inside the cell, the magnetic beads are either forced through the cellular matrix or engulfed by phagocytic cells (Janmey & Schmidt 2006). Forces up to 10 nN (Karcher et al. 2003, Vonna et al. 2003) can be generated using paramagnetic beads, and forces of several piconewtons (Trepap et al. 2003, 2007) can be generated using ferromagnetic beads.

4. CYTOSKELETAL MODELS

A wide range of computational models exist for cytoskeletal rheology and mechanics, ranging from continuum to discrete descriptions of the cytoskeleton (Lim et al. 2006, Mofrad & Kamm 2006). Numerous experimental techniques have also been developed to quantify cytoskeletal mechanics. As described above, these experimental techniques often involve a mechanical perturbation to the cell in the form of either an imposed deformation or force and the observation of the static and dynamic response of the cell. These experimental measurements, along with new computational approaches, have given rise to several theories describing the mechanics of living cells, modeling the cytoskeleton as a simple mechanical elastic, viscoelastic, or poroviscoelastic continuum (Guilak et al. 2006), porous gel (Pollack 2006), or soft glassy material (SGM) (Fabry et al. 2001, Trepap et al. 2007); using discrete descriptions such as a tensegrity (tension-integrity) network model that incorporates discrete structural elements that bear compression (Ingber 2008, Stamenović 2006, Sultan et al. 2004); and more recently modeling based on actin filaments (Bindschadler et al. 2004, Kim et al. 2008, McGrath & Dewey 2006). With such remarkable disparity among these models (largely owing to the relevant scales and biomechanical issues of interest), it may appear to the uninitiated that various authors are describing entirely different structures. Yet, depending on the test conditions or length scale of the measurement, identical cells may be viewed quite differently, as either a continuum or a matrix with fine microstructure, as fluidlike or elastic, or as a static structure or one with dynamically changing properties. A major challenge in cytoskeletal rheology and mechanics, therefore, is how to relate experimental observations to theoretical and phenomenological models.

Cells can be modeled as continuum media if the smallest length scale of interest is significantly larger than the dimensions of the microstructure (Bausch et al. 1998, Evans & Yeung 1989,

Fabry et al. 2001, Fung & Liu 1993, Schmid-Schönbein et al. 1995, Theret et al. 1988). For example, when considering whole-cell deformations, the length scale of interest is at least one or two orders of magnitude larger than the distance between the cell's microstructural elements (cytoskeletal filaments), and, as such, a continuum description may be appropriate. For instance, the micropipette aspiration of erythrocytes or neutrophils (Discher et al. 1998, Drury & Dembo 2001, Hochmuth 2000) has been captured successfully by continuum viscoelastic models. Another example is cell deformation in magnetic trap cytometry: Because the bead size and the resulting deformation in such experiments are much larger than the mesh size of the cytoskeletal network, one does not need to worry about the heterogeneous distribution of filamentous proteins in the cytoskeleton, allowing the successful application of the continuum viscoelastic model (Karcher et al. 2003, Mijailovich et al. 2002).

In a continuum model, there are no constraints in terms of the isotropy or homogeneity of properties, as these can be incorporated easily to the extent to which they are known. Predictions of the continuum model, however, are only as good as the constitutive law (stress-strain relation) on which they are based. This could range from a simple linear elasticity model to a description that captures the viscoelastic behavior of an SGM (see below). Accordingly, the continuum model tells us nothing about the microstructure, other than what might be inferred indirectly based on the ability of one constitutive law or another to capture the observed cellular strain. It is important that modelers recognize this limitation. In essence, continuum mechanics is a coarse-graining approach that replaces the contributions of the cytoskeleton's discrete stress fibers to the local microscopic stress-strain relationship with averaged constitutive laws that apply at a macroscopic scale. This in turn leads to continuous stress-strain relationships and deformation descriptions that are applicable to the whole cell or cellular compartments. Depending on the dynamic timescale of interest, such continuum descriptions can be elastic or viscoelastic with appropriate complexity. To the extent that such continuum models can capture stress and strain patterns within the cytoskeleton, they can help us relate biological influences of various types of force application and dynamics under different geometrical configurations of the cell. The utility and limits of such continuum models have been demonstrated by contrasting computational results against experimental data obtained using various techniques (as described above) probing single cells, such as micropipette aspiration (Discher et al. 1998, Dong & Skalak 1992, Drury & Dembo 2001, Sato et al. 1996, Guilak et al. 2000, Theret et al. 1988, Vaziri & Mofrad 2007, Yeung & Evans 1989), AFM (Charras et al. 2001, Parekh et al. 2005, Vaziri et al. 2006), magnetocytometry (Karcher et al. 2003, Mijailovich et al. 2002), and optical tweezers [stretching erythrocytes (Bao & Suresh 2003, Mills et al. 2004)].

In addition to helping interpret experiments, continuum models are also used to evaluate strains and stresses under physiological or pathophysiological conditions [e.g., for endothelium of blood vessels (Fung & Liu 1993)]. For example, microcirculation studies have examined the passage of blood cells through a narrow capillary (e.g., for neutrophils, see Bathe et al. 2002; for erythrocytes, see Barthes-Biesel 1996), using finite-element models to predict changes in cell shape and the cell's transit time through capillaries. In the case of neutrophils, these inputs are crucial in understanding their high concentration in capillaries, neutrophil margination, and individual neutrophil activation preceding their departure from the blood circulation to reach infection sites. Neutrophil concentration depends indeed on transit time, and activation has been shown experimentally to depend on the timescale of shape changes (Yap & Kamm 2005). Similarly, continuum models can shed light on blood cells' dysfunctional microrheology that arises from changes in cell shape or mechanical properties [e.g., the time-dependent stiffening of erythrocytes infected by malaria parasites (Mills et al. 2004) and in the force microscopy of nonadherent cells in an attempt to examine the distinct deformability in leukemia cells (Rosenbluth et al. 2006)].

The creation of multiphase constitutive laws has been motivated by the cytoskeleton's complex structure, whose biomechanical and biochemical properties depend on interactions among the varying concentrations of water, charged or uncharged macromolecules, ions, and other molecular components contained within the cytoplasm (Guilak et al. 2006). These multiphase models for the cytoskeleton represent interactions among solid, fluid, and (in some cases) ionic phases of cells. Such models successfully explore the contributions of different physical mechanisms responsible for cytoskeletal phenomena such as cell viscoelasticity. The disadvantage of the multiphase models is that the added complexity in the modeling and parameter sets requires additional experimental tests to determine these parameters. The application of such biphasic or triphasic continuum-based approaches has been combined with other biomechanical models (e.g., tensegrity models, see below) to examine the interactions of the different constitutive phases in controlling cell mechanics (Guilak et al. 2006).

One major limitation of the continuum models, in general, is that they exclude small Brownian motions due to thermal fluctuations of the cytoskeleton, which correspond to fluctuations of the network nodes in a continuum model and have been shown to play a key role in cell mechanics (Mogilner & Oster 1996). Another limitation is that they imply the use of a limited number of time constants to characterize the cell's behavior. However, the cytoskeleton has been shown to exhibit behaviors with power-law rheology, implying an infinite number of timescales (Desprat et al. 2005, Fabry et al. 2001). Modeling the cell with no intrinsic time constant has successfully captured this behavior (e.g., Djordjević et al. 2003), although this type of model cannot and does not aim at predicting or describing force or strain distribution within the cell. The use of continuum models for the prediction of intracellular stress and strain patterns is therefore limited to cell material models involving a finite number of time constants consistent with the timescale of the experimental technique used to probe these cells.

Fabry et al. (2001) performed both static (step strain) and dynamic tests (sinusoidal strains), using magnetic twisting cytometry to measure the relaxation modulus, $G(t)$, the frequency dependence of the storage modulus, $G'(\omega)$, and the loss modulus, $G''(\omega)$, of the cytoskeleton (**Figure 2**). Similar tests were performed to examine the behavior of the cytoskeleton under the effect of different drugs (see **Figure 3**). G' was observed to increase with increasing frequency, ω , according to a power law $\sim \omega^x$, where $x = 1.20$ under control conditions. G'' also increases with increasing frequency and follows the same power law in the range of 0.01–10 Hz. Above 10 Hz, however, the same power-law behavior is not seen. Similar experiments were performed by manipulating the cells with various drugs to create contraction or relaxation in the cytoskeleton, again observing identical qualitative properties (**Figure 3**). G' increased with increasing frequency as a power law $\sim \omega^x$; G'' also increased with increasing frequency with the same power law and same exponent up to frequencies of 10 Hz. Above 10 Hz, the behavior changed in a manner similar to that observed

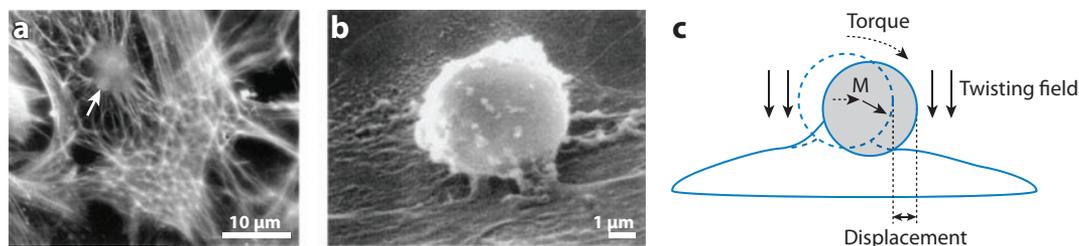


Figure 2

(a, b) Beads attached to the cytoskeleton. (c) The application of a magnetic field and the displacement of the bead. Figure reproduced with permission from Fabry et al. 2003.

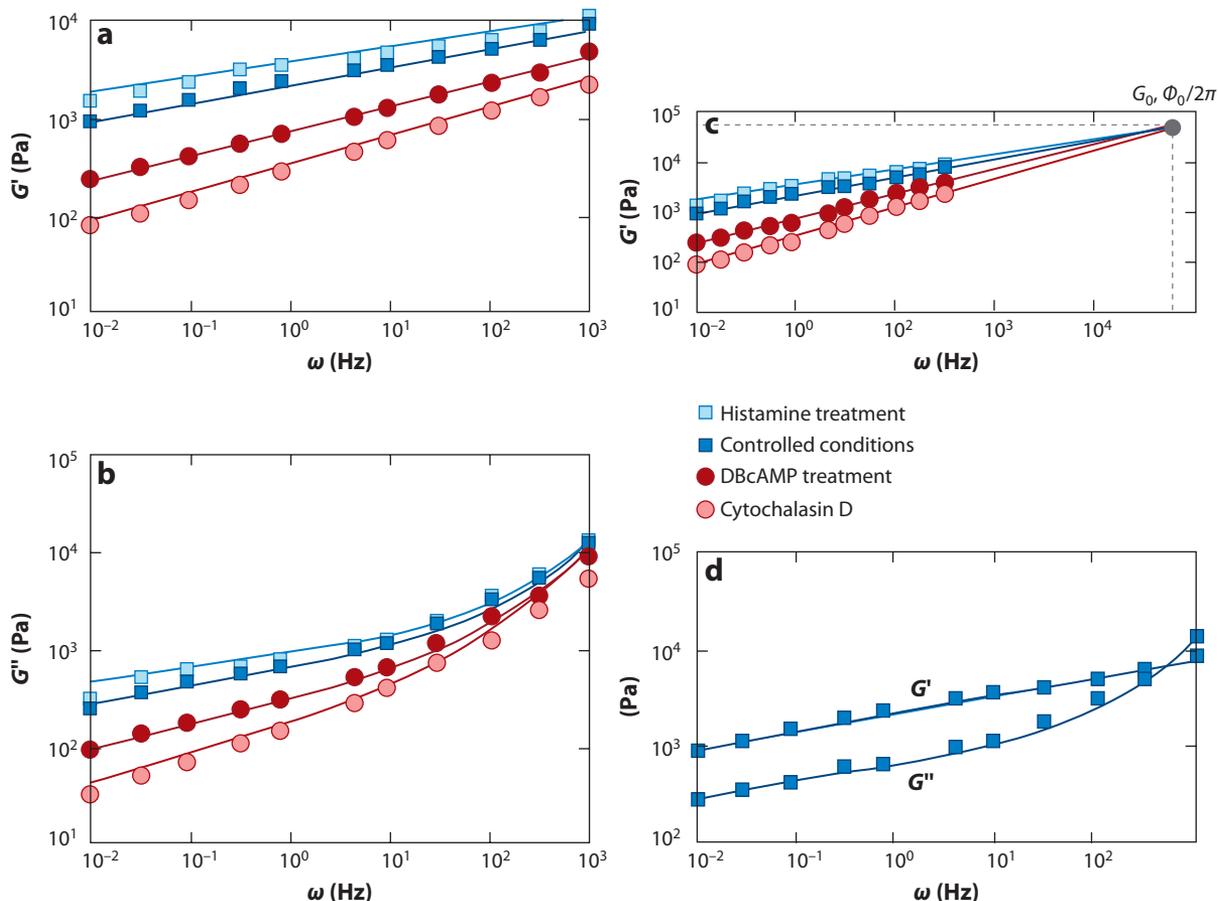


Figure 3

(a) G' and (b) G'' as a function of frequency ω for different drug treatments: under controlled conditions (dark blue squares), treatment with histamine (light blue squares), treatment with DBCAMP (red circles), and treatment with cytochalasin D (pink circles). (c) Extrapolation of solid line cross-over. (d) Dynamic moduli under control conditions. Figure reproduced with permission from Fabry et al. 2003.

in the control. Inspired by the similarity between the experimental data on cells and those reported on SGMs, Fabry et al. (2003) proposed that the cytoskeleton is an SGM that can be modeled by the soft glassy rheology model (SGR) developed by Sollich and colleagues (Sollich et al. 1997). SGMs compose a class of materials that include emulsions, slurries, and pastes. They are viscoelastic in nature and possess the following general properties: (a) They are soft in the sense that they possess mechanical moduli in the pascal-to-kilopascal range, (b) their loss tangent $\tan(\delta) = G''(\omega) / G'(\omega)$ is nearly constant for a wide range of frequencies, (c) the frequency dependencies of these moduli are weak power laws of the frequency of the applied load, and (d) under certain conditions they display aging behavior.

The abstract system properties that characterize the dynamics of these SGMs are structural disorder and metastability (Cloitre et al. 2000, Ramos & Cipelletti 2001, Sollich et al. 1997, Weeks & Weitz 2002). Bouchaud (1992) presented the first theoretical study of structural disorder and metastable configurations in spin glasses. The theory considers the conformational energy landscape of a finite disordered system to be extremely rough, with many local minima that

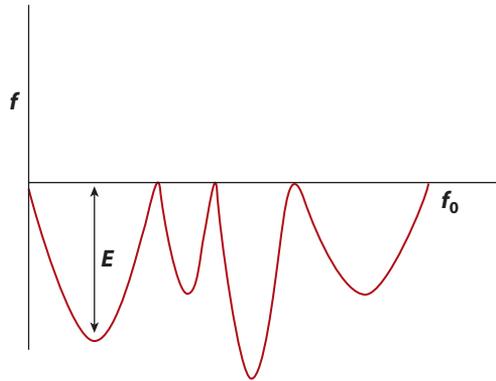


Figure 4

Energy landscape, showing different metastable states. Here, f_0 is the energy level below which the states are disconnected. It is the minimum energy required to hop between any two states. It is assumed here that the dynamics between the traps is very fast and that the probability to find the system between two metastable states is negligible. The depth of a trap is $E = f_0 - f$, $f > 0$. See Mandadapu et al. (2008) for more details.

Figure adapted from Bouchaud 1992.

correspond to metastable configurations or states. The local minima are surrounded by high energy barriers. Thus, the states are considered to be traps in which the system is held for certain periods of time τ . A schematic of the energy landscape is shown in **Figure 4**. Inspired by this, Sollich (Sollich 1998, Sollich et al. 1997) proposed the SGR model for SGMs. This model considers the material to consist of a large number of elements that are trapped by their neighbors. An individual element sees an energy landscape of traps of various depths and, when activated, hops from its current trap to another trap. The activation in glasses is generally thermal in nature. However, Sollich claims that in SGMs, thermal activation is very small compared to the typical trap depths. Thus, he considers that the activation results from interaction between elements (i.e., rearrangements somewhere in the material can cause rearrangements elsewhere). This phenomenon is represented by an effective noise temperature x . To describe material deformation and flow, Sollich incorporated strain degrees of freedom for the elements. By using this model with simple numerical simulations, we can see power laws for the storage modulus and loss modulus.

An interesting regime is the range in which x lies between 1 and 2. In this range, G' and G'' have a constant ratio and both vary as $\sim \omega^{(x-1)}$ (**Figures 5 and 6**), which is similar to the results from magnetic twisting cytometry. Despite some of the above similarities between the results from the SGR model and the experiments on the cytoskeleton, some questions still need to be addressed before applying the model to the cytoskeleton. Mandadapu et al. (2008) present a critical review of the SGR model and its application to cytoskeletal rheology.

Continuum models of the cytoskeletal rheology have mostly focused on capturing the passive dynamics of the cytoskeleton. Current models do not account for active biology (i.e., they cannot predict deformations and stresses experienced as a direct consequence of the cell's biochemical responses to mechanical load). However, by contrasting the predicted purely mechanical cell response to experimental observations, one could isolate phenomena involving active biology (e.g., cell contraction or migration) from the passive mechanical response of the cell. Another limitation of continuum models stems from the lack of description of cytoskeletal fibers. As a first milestone, continuum models must be able to incorporate an understanding of the cytoskeletal stress field owing to the contractile machinery offered by the actin-myosin interaction that leads to the formation of stress fibers (see **Figure 7**). The growth and initiation of stress fibers may require systematic ordering at the focal adhesions, but the direction and strengthening of the stress fibers

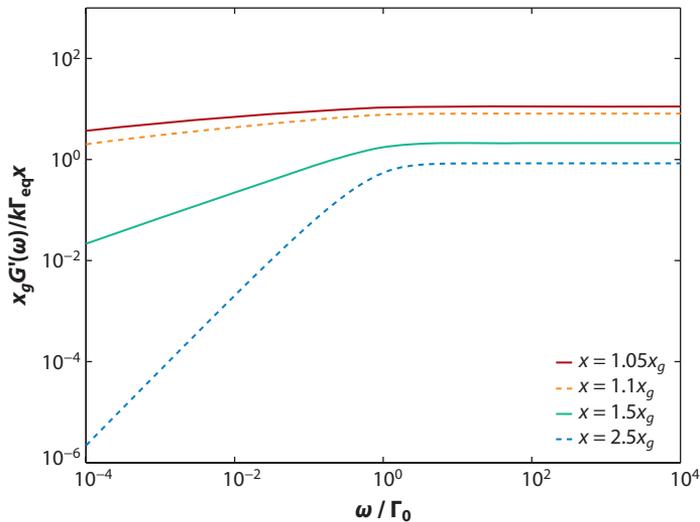


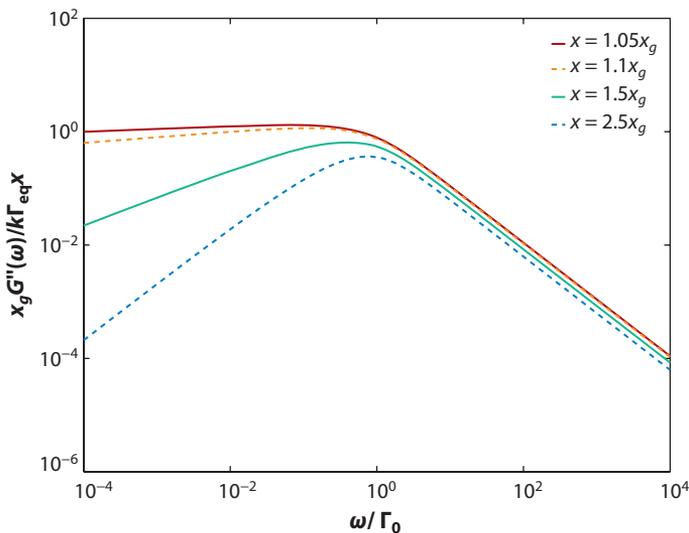
Figure 5

Variation of storage modulus $[G'(\omega)]$ with frequency ω for $x/x_g = 1.05, 1.1, 1.5,$ and 2.5 . Here, both the storage modulus and the excitation frequency are normalized using the characteristic parameters for the soft glassy material element, as follows: x_g is the system's glass transition temperature, x is the effective noise temperature, $P_{eq}(E)$ is a stationary normalizable probability distribution for finding a cytoskeletal (abstract) element of stiffness k in a trap of energy depth E and a certain local strain, and Γ_0 denotes the attempt rate for the system's escape from its trap of depth E . Assuming the element is linearly elastic with an elastic constant k , and a canonical-like distribution at effective noise temperature x , the system can escape from its trap of depth E , with a rate of $\Gamma_0 e^{-\frac{E - \frac{1}{2} k l^2}{x}}$ per unit time (see Mandadapu et al. 2008 for further details).

are known to be controlled by the stress field within the cell. For instance, stress fibers in a migrating cell continually realign in the direction of migration (Wang 1984). The stress field within the cell is influenced by the force of myosin contraction, the deformability of the actin cytoskeleton, and the constraints on the deformability of the cell owing to matrix attachments and external force.

Figure 6

Variation of loss modulus $[G''(\omega)]$ with frequency ω for $x/x_g = 1.05, 1.1, 1.5,$ and 2.5 . See **Figure 5's** caption for further details.



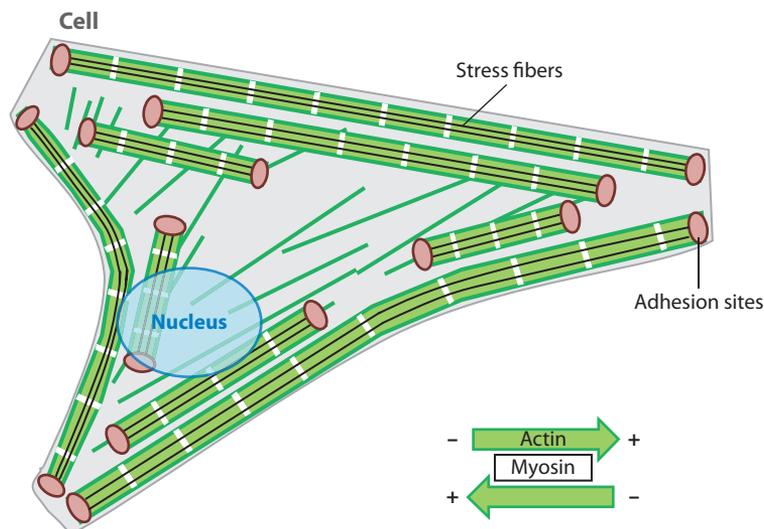


Figure 7

A schematic of contractile machinery offered by the actin-myosin interaction leading to the formation of stress fibers.

The interplay between these factors and the resulting formation of stress fibers provides the basis for a cell's response to its force environs. For instance, the cell selectively strengthens stress fibers along the direction of maximum resistance to myosin contractility. This not only allows the cell to stiffen itself in a particular direction to protect against excess stress and strains, but it also allows the cell to scout the matrix rigidity and migrate toward the stiffest direction (Lo et al. 2000, Saez et al. 2007). Changes in stress-fiber patterns affect cell shape and orientation (Flavahan et al. 2005, Kumar et al. 2006, Noria et al. 2004). Finally, the bandlike nature of stress-fiber contractility allows the cell to target the force through large distances in a precise and undissipated manner (Blumenfeld 2006, Chicurel et al. 1998, Hu et al. 2003, Maniotis et al. 1997).

New ideas were recently introduced for understanding the cytoskeletal stress field resulting from actin-myosin contractility that leads to the formation of stress fibers as bundles of actin filaments between matrix constraints (P.L. Chandran, C.B. Wolf & M.R.K. Mofrad, submitted manuscript). The authors used the context of recent continuum-based modeling of cells contracting under simple and well-defined matrix attachments (Deshpande et al. 2007). A continuum approach to modeling stress-fiber formation is true to the idea that stress fibers arise from an underlying much-finer network of actin filaments, which maintains a continuity of stress with it. However, a continuum-based approach does not capture the bandlike propagation of contractile stress that is characteristic of stress fibers and that accounts for the directed force transfer and directed stiffening of cells. The actin-myosin interaction extended to a disordered cytoskeletal network requires that one additionally accounts for a perpendicular compression force along the stress fiber (P.L. Chandran, C.B. Wolf & M.R.K. Mofrad, submitted manuscript). The perpendicular compression force arises from the zippering-in action of actin filaments by myosin, which results in the myosin contraction force being transmitted by the bending actin filament to the network in directions away from myosin alignment. To investigate the effect of the perpendicular compressive force, Chandran et al. (P.L. Chandran, C.B. Wolf & M.R.K. Mofrad, submitted manuscript) proposed a minimalistic model of cell contractility that captures the zippering action

in a coarse-grained sense. Although based on continuum ideas, this model can reproduce stress channeling and its transmission along edges in the constrained cells.

In contrast to continuum models, the discrete cytoskeletal models (Boey et al. 1998, Satcher & Dewey 1996, Stamenović et al. 1996) consider discrete stress-bearing elements of the cytoskeleton that do not fill the space. The cytoskeleton is considered as a combination of a large number of these discrete elements. The behavior of each discrete element is subject to conditions of mechanical equilibrium and geometrical compatibility at every node. Similar to continuum models, the discrete models of the cytoskeleton can range from simple to complex, multimodular and multi-compartmental (Stamenović 2006). Inger and colleagues (see reviews in Ingber 2003, Stamenović 2006) proposed one such discrete model of the cytoskeleton that is based on a building system known as tensegrity architecture. The key premise of the cellular tensegrity model is that the cytoskeleton carries pre-existing tensile stress that offers shape and structural stability (Stamenović 2006). This cytoskeletal prestress, generated primarily by the actin-myosin contractile apparatus and actin polymerization forces, is balanced partly by forces that arise at focal adhesions to the extracellular matrix and partly by compression-bearing microtubules. This model is consistent with the observed relationship between cell stiffness and the cytoskeletal contractile stress (Wang et al. 2001, 2002).

Recently, owing to advances in experimental technologies (scanning force microscopy, high-resolution microscopy, and optical tweezers) and computational modeling techniques (all-atom molecular dynamics simulation methods), the single-molecule properties of many key ingredients of the cytoskeleton have been studied in exquisite detail. Several *in vitro* experiments have aimed at understanding some of the unique mechanical properties of solutions and networks of cytoskeletal filaments (Chaudhuri et al. 2007, MacKintosh 2006). In parallel with these experiments, many new computational and theoretical models have emerged that attempt to shed light on the rheological properties of the cytoskeletal networks and the regulation of cytoskeletal rheology (e.g., cross-linking and bundling proteins) (e.g., see Kim et al. 2008, Kolahi & Mofrad 2008, Zaman & Mofrad 2004; J. Golji, R. Collins & M.R.K. Mofrad, submitted manuscript). Cytoskeletal networks exhibit unique rheological properties, namely dynamic and nonlinear response (e.g., the shear modulus may elevate by an order of magnitude in response to modest strain levels of $\sim 10\%$) (Janmey et al. 1994, MacKintosh 2006). Semi-flexible polymer network models have been introduced recently to describe some of the actin cytoskeleton.

The three broadly important cytoskeletal filaments (i.e., actin, microtubules, and intermediate filaments) are all classified as semiflexible because the length over which their contour angles correlate (i.e., the persistence length) is on the order of the lengths typically found in the cytoskeleton. Consequently, unlike in typical flexible polymers, the entropic contribution to the free energy is very small for these filaments, and the enthalpic or strain energy contribution plays a larger role. A popular conception of the free-energy state of a semiflexible filament is Kratky & Porod's (1949) worm model. This model likens a semiflexible filament to a continuously flexible and isotropic rod, with the bending at any point governed by Euler beam mechanics. The filament's persistence length, which captures the competition between flexural rigidity and thermal energy in determining the conformation or shape of the filament, can be determined from the decorrelation of the filament's tangent angles along its contour (Howard 2001). Networks of semiflexible filaments show mechanical properties that differ from flexible polymer networks. Networks of actin filaments show a reversible stress softening in compression (Chaudhuri et al. 2007), whereas any known stress softening in flexible polymer networks results from failure and yielding and is not reversible. Moreover, networks of semiflexible polymers show negative normal stresses in shear that tend to pull the shear plates inward (Janmey et al. 2007). Networks of flexible polymers, conversely, show positive normal stresses in shear or push the shear plates

outward. Also, networks of semiflexible filaments show nonlinear shear stiffening much larger than that observed with networks of flexible polymers (Storm et al. 2005). It is unclear whether the anomalies can be explained within the worm model description of a network filament as a uniform flexible rod or if one needs to account for additional complexities due to nonhomogeneous protein microstructure (Ben-Avraham & Tirion 1995). The underlying filament deformations that lead to such a network behavior are also unclear (Wen et al. 2007, 2008). One approach to understanding the microstructural basis of the above mechanics, as well as to avoid the mathematical complexities with analytically solving semiflexible behavior, is the dynamic simulation of the filaments and their networks. A force-balance-based dynamic simulation of the filament networks has multiple advantages as an approach to understanding their anomalous mechanics. However, this kind of simulation has difficulty capturing filament hydrodynamics and bending mechanics in a computationally efficient and physically consistent manner. P.L. Chandran & M.R.K. Mofrad (manuscript submitted) recently introduced a new approach to dynamically simulate the Brownian dynamics of semiflexible filaments. The key strategy is to idealize the semiflexible filament as a string of continuous and flexible rods and idealize the Brownian forces on it as Einsteinian-like point normal and tangential forces. This approach can capture the dynamics of a semiflexible filament from its rigid, to semirigid, to semiflexible limit.

Kim et al. (2008) recently introduced a Brownian dynamics simulation model in which actin monomers polymerize and become cross-linked by two types of cross-linking molecules that form either parallel filament bundles (representative of α -actinins) or perpendicular cross-links (representative of filamins). They analyzed the effects of various system parameters on the growth, morphology, and rheology of the resulting network. Their model generated simulated cytoskeletal networks that exhibit structural and rheological patterns (at least qualitatively) similar to those observed experimentally using synthetic cross-linked gels (Kim et al. 2008) (see **Figure 8**). This model offers a platform that can be used to replicate cytoskeletal measurements, that may provide new insight into the power-law scaling of the cytoskeletal rheology, and that can also shed light on how different measurement techniques can lead to vastly different rheological properties.

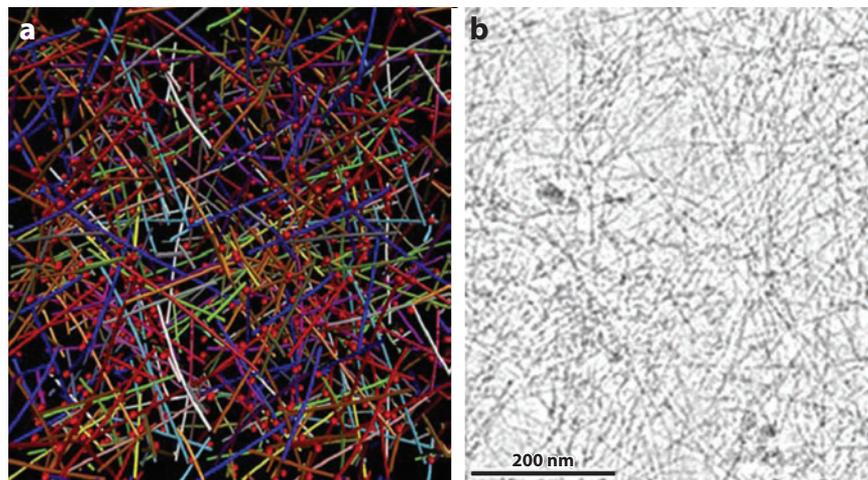


Figure 8

(a) Simulated (Kim et al. 2008) versus (b) in vitro visualization (Resch et al. 2002) of the actin cytoskeleton by cryo-electron microscopy.

5. CLOSURE

This review aims to bring together the key experimental methods and theoretical and computational models regarding cytoskeletal rheology and mechanics. A wide range of computational and phenomenological models are described, ranging from continuum models to discrete, actin filament-based models. The article also concisely reviews numerous experimental techniques, all of which aim to shed light on the cytoskeletal rheology by exerting some sort of pointed perturbation and examining the static and dynamic response of the cell. These experimental observations along with computational approaches to the cell have given rise to several, often contradictory, theories for describing cytoskeletal rheology: a model of the cytoskeleton as a simple mechanical elastic, viscoelastic, or poroviscoelastic continuum; an SGM model; a tensegrity (tension-integrity) network incorporating discrete structural elements that bear compression; and filament-based models beginning with polymerizing and depolymerizing actin monomers.

Experimental data are accumulating, and promising methods have been proposed to describe cell rheology. It is important to determine what constitutive law best fits the observed structural behavior. Whereas a linear elastic or even linear viscoelastic material description is sufficient to mimic certain observations, other more complex descriptions almost certainly will be needed to encompass a range of excitation frequencies and large deformations. Although there appears to be some degree of convergence regarding the values and frequency dependence of viscoelastic parameters for the cytoskeleton, the results obtained remain somewhat dependent on the method used to probe the cell.

A unique aspect of the cytoskeleton is that active as well as passive characteristics need to be considered. Cells are highly dynamic in that their cytoskeletal structures are constantly changing in response to a variety of external stimuli, especially external forces. Consequently, each time we probe a cell to measure its mechanical properties, we alter those same properties. One exception to this statement is the use of the Brownian motions of intracellular structures to infer stiffness, but these measurements are still being refined and, as currently implemented, are subject to some degree of uncertainty. Nonetheless, this offers an important opportunity for further research toward the refinement and wider use of these noninvasive methods to better capture the dynamic, active, and remodeling nature of the cytoskeleton.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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