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Nonlinear Elastic and Inelastic Properties of Cells

Mechanical forces play an important role in various physiological processes, such as morphogenesis, cytokinesis, and migration. Thus, in order to illuminate mechanisms underlying these physiological processes, it is crucial to understand how cells deform and respond to external mechanical stimuli. During recent decades, the mechanical properties of cells have been studied extensively using diverse measurement techniques. A number of experimental studies have shown that cells are far from linear elastic materials. Cells exhibit a wide variety of nonlinear elastic and inelastic properties. Such complicated properties of cells are known to emerge from unique mechanical characteristics of cellular components. In this review, we introduce major cellular components that largely govern cell mechanical properties and provide brief explanations of several experimental techniques used for rheological measurements of cell mechanics. Then, we discuss the representative nonlinear elastic and inelastic properties of cells. Finally, continuum and discrete computational models of cell mechanics, which model both nonlinear elastic and inelastic properties of cells, will be described. [DOI: 10.1115/1.4046863]

Keywords: inelastic, nonlinear elastic, cell mechanics, viscoelasticity, cytoskeleton

1 Introduction

Mechanical forces play an indispensable role in a myriad of physiological processes, such as morphogenesis, cytokinesis, and migration. In those processes, cells adhere to each other or are surrounded by an extracellular matrix (ECM) [1]. Cells exert forces on their surrounding environment, but they are also subjected to forces transmitted from the surrounding environment. Thus, the balance and transmission of forces are of great importance for determining the shape, stability, and adaptability of cells in the processes [2,3]. The mechanical properties of cells highly affect how forces are balanced and transmitted in the cells. Therefore, understanding the mechanical properties of cells is crucial for illuminating intrinsic mechanisms of the physiological processes regulated by forces.

To better understand the mechanics of cells with highly heterogeneous intracellular structures, researchers in the past often treated cells as homogenous materials with certain rheological properties. The most naïve assumption popularly employed in traditional research is that cells can be simplified into isotropic, linear elastic materials. With such simplification, constitutive laws with two independent parameters can relate stress to strain. For example, Young's modulus and the Poisson's ratio relate all stress components with any strain component. In addition, responses of purely elastic material do not show dependence on timescales or loading rates. For example, regardless of how fast a strain applied to a material is increased from zero level, stress acting on the material is identical if the current strain is the same. This drastic assumption made it very easy to understand the force balance between cells and tissue in biological systems because their deformation is always reversible and dependent on neither time nor strain. Based on the assumption, several theoretical models were suggested, such as the Hertz model for cell indentation [4] and Johnson–Kendall–Roberts theory for cell spreading [5]. However, a number of experimental studies using various measurement techniques showed that cells exhibit much more complicated behaviors than those of linear elastic materials; cells are able to

show a wide range of nonlinear elastic properties, such as stiffening [6], softening [7], and superelasticity [8], as well as inelastic properties, such as viscoelasticity [9–11], viscoplasticity [12], and poroelasticity [13].

Such nonlinear elastic and inelastic properties of cells play an important role in various cellular processes. For example, when cells infiltrate into size-limiting physical barriers during transmigration and three-dimensional (3D) cell migration, very high deformability of cells is crucial [14], which is not feasible if a cell body behaves as a linearly elastic material. However, cells in other circumstances need to be rigid enough to maintain their shapes and support external loads [15]. This implies that cells have the ability to behave as a material with distinct properties depending on conditions or dynamically modulate their mechanical properties to a great extent. It was indeed shown that cells are capable of enhancing their fluidity when they collectively migrate during morphogenesis, cancer cell migration, and wound healing [16]. Variable mechanical adaptability of cells is known to be of great importance for mechanosensing and mechanotransduction [17]. Such variations in the mechanical properties of cells are partially regulated by modulation of gene expression arising in response to external mechanical signals transmitted to a nucleus through the cytoskeleton [17].

The complicated properties of cells emerge from unique characteristics of cellular components, such as the membrane, cytosol, cytoskeleton, and nucleus [18–21]. Thus, understanding the dynamics and mechanics of the cellular components is prerequisite for illuminating the molecular origins of the complicated mechanical properties of cells. Researchers have employed various in vitro experiments and models to study the characteristics of each cellular component [22,23]. In particular, it was shown that the cytoskeleton and nuclear membranes, consisting of biopolymeric structures, exhibit a wide variety of mechanical responses that are far from responses of linear elastic materials, which potentially contributes significantly to the mechanical properties of cells.

In this review, we first provide a brief background on major cellular components that highly contribute to cell mechanical properties as well as explanations of diverse experimental techniques employed to probe rheological properties. Then, we introduce representative nonlinear elastic and inelastic properties of cells,

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Manuscript received December 8, 2019; final manuscript received March 27, 2020; published online August 31, 2020. Assoc. Editor: Anna Grosberg.

Fig. 1 Cellular structure with various intracellular constituents. A plasma membrane, nuclear components (lamin, nuclear membrane, and chromatin), and the cytoskeleton (actin filament, intermediate filament, and microtubule) are shown in a schematic diagram. For a better visual representation, some of the components are drawn on a larger scale than their real sizes.

including viscoelasticity, viscoplasticity, stiffening, softening, superelasticity, and poroelasticity. Finally, computational models of cells designed for investigating the nonlinear elastic and inelastic properties of cells are briefly introduced. We also discuss possible combinations of forthcoming experiments and models to address potential gaps in understanding cell mechanics during biological processes.

2 Cellular Structures

A cell is comprised of numerous components, many of which affect the mechanical properties of cells directly or indirectly. Among those, the critical cellular structures determining cell mechanical properties are known to be a nucleus and the cytoskeleton consisting of three major types of biopolymers: actin filament (F-actin), intermediate filament (IF), and microtubule (MT). Thus, during recent decades, the structural, mechanical, and dynamic properties of the cytoskeleton and the nucleus have been extensively investigated in experiments. In this section, we briefly explained some of the key features of the cellular structures.

2.1 Actin Cytoskeleton. The actin cytoskeleton plays an important role in diverse cellular functions, such as migration, cytokinesis, and morphogenesis [24]. The actin cytoskeleton is a polymeric structure consisting of F-actin and various types of actin-binding proteins (Fig. 1) [25]. F-actin is a filamentous structure with 5–9 nm in diameter formed by self-assembly of actin monomers (G-actin) [26]. F-actin has a double helical structure with polarity, and the two ends of F-actin are called barbed and pointed ends. F-actin is a semiflexible polymer with a persistence length of $\sim 9 \mu\text{m}$ comparable to its typical contour length [27]. F-actin is unable to resist compressive forces due to its low bending stiffness, whereas it can resist tensile forces strongly. Several types of actin cross-linking proteins (ACPs) connect pairs of F-actins, forming higher-order structures, such as networks and bundles [24]. Tightly crosslinked bundles called filopodia are capable of forming finger-like protrusions by pushing a cell membrane hard since the effective persistence length of the bundles is much longer. In addition, Arp2/3 complex consisting of seven subunit proteins forms a dense and branched network in the lamellipodia regions near a cell membrane [28]. Due to the short length and high density of F-actins, the branched network is able to induce sheet-like protrusions by overcoming compressive loads from the

membrane. Myosin motor proteins walk toward the barbed end of F-actin by converting chemical energy stored in adenosine triphosphate to mechanical energy, inducing contractile forces in the actin cytoskeleton [29].

2.2 Microtubules. Microtubules usually span a cell radius with one end attached to the centrosome and the other end located near the cell membrane (Fig. 1) [30]. However, during cytokinesis, MTs form the mitotic spindle to facilitate segregation of chromosomes to two daughter cells [31]. At the initial stage of cell migration, MTs induce polarization of a cell body into leading and trailing edges [32]. MT is a biopolymer with a tubular structure composed of a globular protein called tubulin [33]. The outer and inner diameters are $\sim 24 \text{ nm}$ and $\sim 12 \text{ nm}$, respectively [34]. The basic building block of MTs is a dimer formed by α - and β -tubulins. MTs have much longer persistence length than F-actin (1–5 mm), so MTs can resist larger compressive forces [35]. Considering the long persistence length, MTs in most types of cells whose diameter is much smaller than the persistence length are expected to be quite straight with minimal curvatures. However, MTs in cells tend to exhibit much higher curvatures than their intrinsic curvature [36]. MTs coexist with networks consisting of F-actin and IFs, and they are physically linked to each other via proteins that can bind to different types of cytoskeletal polymers simultaneously or feel each other via volume exclusion [37,38]. These interactions reduce the length of a unit that can undergo buckling from the contour length of MTs to the length of shorter segments between adjacent points pinched by F-actin or IFs. This results in the enhanced ability of MTs to resist compressive forces exerted by extracellular spaces.

2.3 Intermediate Filament Networks. Intermediate filaments are filamentous proteins prevalent in the cytoplasm and the nuclear envelope (Fig. 1). There are several types of proteins categorized as IFs. Historically, they were found after F-actin and MT and named “intermediate” filaments because their diameter (8–10 nm) was between the diameters of F-actin and MT [39]. The most abundant type of IFs is keratin often found in epithelial tissues [40]. Vimentin exists in mesenchymal, endothelial, and hematopoietic cells [41]. Desmin is found in striated, smooth, and cardiac muscle cells [42]. Neurofilament exists in the cytoplasm of neural cells [43]. The structural building unit of IFs is a tetramer formed by lateral binding of a pair of dimers via ionic bonds and hydrophobic interactions [44]. The hierarchical structure of IFs is much more complicated than the double helical structure of F-actin and the tubular structure of MTs. The persistence length of IFs is typically one order of magnitude smaller than that of F-actin [45], making it more flexible than F-actin, but IFs are still categorized as semiflexible polymers. In addition, IFs can be highly extended by tensile forces via either a transition from α -helix to β -sheet structures occurring in IF dimers or sliding between tetramers before stiffening [23,46]. Such high extensibility without a rupture is a unique property of IFs compared to F-actin and MT [47], enabling IFs to serve as a major load-bearing component that maintains the structural integrity of cells [23].

2.4 Nucleus. A cell nucleus is encapsulated by a semipermeable nuclear envelope which consists of outer and inner membranes (Fig. 1) [48]. The inner membrane serves as a scaffolding structure that maintains the mechanical integrity of the nucleus under high stress [48]. The inner membrane is mainly composed of a dense fibrillar network of class V IF called lamin [49]. There are two types of lamins: lamin A/C and lamin B [48]. It was shown that lamin A/C is a major component that contributes to nuclear mechanical properties [50]. Lamin interacts with chromatin within the nucleus and the cytoskeleton located outside the nucleus. A protein called the linker of nucleoskeleton and cytoskeleton complex connects a lamin network to the cytoskeleton [51]. Since the cytoskeleton is also linked to the extracellular

matrix, mechanical signals applied to a cell membrane in an extracellular environment can be transmitted to chromatin via the cytoskeleton and the lamin network, which is often called mechanotransduction in cells [51]. The mechanotransduction plays a very important role for cells to adapt to highly volatile environments via the regulation of gene expressions affected by mechanical signals [48].

3 Rheological Measurement Techniques

During the past decades, various measurement techniques have been developed for evaluating the mechanical properties of cells. Measurement methods can be classified into two categories, depending on whether they measure properties of a whole cell or a local part of the cell. In the whole-cell measurements, a large fraction of a cell directly feels applied mechanical signals, whereas only a small portion of the cell experiences the mechanical signals in local measurements. However, a few techniques

support both local and whole-cell measurement modes. In this section, we briefly introduce representative measurement methods (Fig. 2).

In a typical experimental setup for whole-cell stretching called the simple uni-axial stretching geometry [10], a cell adheres to two plates (Fig. 2(a)). One plate is very rigid, whereas the other is more flexible with variable rigidity. The stiffness of the flexible plate is calibrated to use it as a nanonewton force sensor. The force applied to the cell can be estimated from the deflection of the flexible plate; the force is directly proportional to the deflection. By controlling the deflection with a precision of < 200 nm, this device was commonly employed to apply constant forces or stress to a cell and measure the response of the cell [52].

Atomic force microscopy (AFM) has also been used for stretching a whole cell in a few studies by making a large portion of the cell adhere directly to the AFM cantilever without any tip (Fig. 2(b)). AFM is able to control applied force or the deflection of its cantilever with high precision via a piezoelectric material and a

Fig. 2 Rheological measurement techniques designed for estimating cell mechanical properties. (a) Whole-cell uni-axial stretching: a cell adheres to a very rigid plate (top) and a more flexible plate (bottom). The rigid plate is brought down toward a cell to form adhesion then moves gradually upward to stretch the cell. A force applied to the cell is calculated using the deflection of the flexible plate whose stiffness is known. (b) AFM: in AFM, force or displacement can be controlled at high resolution via a piezoelectric element, a deflection detector, and an electronic feedback loop to estimate mechanical properties of cells. Although AFM can be used with only a cantilever for measuring whole cell properties (top), AFM is usually employed for measuring local cell properties via either a sharp (middle) or spherical (bottom) tip. (c) Micropipette aspiration: A fraction of a cell located closely to a narrow micropipette tip (top) is sucked into the micropipette due to a pressure difference (bottom). By measuring aspiration length over time, mechanical properties, such as elastic modulus and viscosity, can be estimated. (d) Optical tweezers: the optical tweezers use a laser-beam trap (yellow) to control the force and displacement of micron-sized dielectric particles or beads (small sphere) with very high resolution. (e) Magnetic tweezers: the magnetic tweezers employ ferromagnetic, ferrimagnetic, paramagnetic, or superparamagnetic probes (small sphere) inserted into the cell or attached on a cell membrane. The probes then undergo various types of motions in response to applied magnetic fields, such as translation and rotation. (f) Traction force microscopy: cells are placed on an optically transparent substrate with fluorescent particles embedded (small spheres with arrows). Cells adhere and then generate contractile forces to the substrate. Using known stiffness of the substrate and displacements of the particles, traction stress exerted by cells can be estimated. (g) Deoxyribonucleic acid (DNA) tension sensor: as a cell exerts force to the tension sensor, a fluorophore (top circle on the spring) moves away from a quencher (bottom circle on the spring), resulting in the emission of lights that can be detected by fluorescence microscopy.

feedback loop [53]. AFM can measure forces of the order of a few piconewton (pN) and a vertical displacement shorter than 0.1 nm. AFM has also been widely used for measuring local mechanical properties of cells. To apply mechanical signals to only a small part of the cell, the end of the AFM cantilever is attached to a spherical or cone-shaped tip that is much smaller compared to the cell size (Fig. 2(b)) [54]. By compressing a cell membrane by the tip, a local response of the cell body can be probed.

In addition, for measuring properties of whole cells, several research groups employed micropipette aspiration in which a pressure difference makes a large portion of a cell sucked into a micropipette (Fig. 2(c)). Observations on how much and fast a cell body is sucked into the micropipette provides information about the mechanical properties of the cell body [55,56]. In general, with higher pressure difference or with lower stiffness of cells, the portion sucked into the micropipette becomes larger. The Young–Laplace equation was popularly used with the micropipette aspiration to estimate line tension acting along the cell membrane and cortex underlying the membrane [57]. Using similar principles, microfluidic devices were used for measuring cell mechanical properties. In these devices, cells are transported and pushed into channels narrower than their diameter by fluid flow [58–60]. The noticeable advantage of the microfluidic devices is the capability of high-throughput measurement unlike the micropipette aspiration, meaning that mechanical properties of a large number of cells can be automatically measured.

In addition, optical tweezers using optical traps have been employed in several experiments for measuring cell mechanical properties. The optical stretcher uses optical traps formed by two opposing laser beams to stretch a whole cell and evaluate the response of the cell [61]. The optical stretcher has been popularly used for measuring the mechanical properties of human red blood cells because the red blood cells with high deformability can be manipulated relatively easily by forces exerted from the optical trap [62–64]. Unlike uni-axial stretching geometry [10], AFM, and micropipette aspiration, the optical stretcher does not require physical contact to cells, enabling contact-free rheological measurements. Optical tweezers have been employed more often as micromanipulators. In optical tweezers using a single laser-beam trap, force and displacement of micron-sized dielectric particles can be controlled via feedback with resolutions of nanometer and subpiconewton (Fig. 2(d)) [65]. In several studies, cell stretch experiments were conducted by displacing beads attached to the opposite sides of a cell membrane via the optical tweezers [66,67]. The optical tweezers were also used to apply forces or displacements to subcellular structures by modulating beads embedded within cells [66,68].

For local property measurements, magnetic tweezers using ferromagnetic [9,69], ferrimagnetic [70], paramagnetic [71], or superparamagnetic probes [71,72] have been widely employed in numerous traditional experimental studies. While ferromagnetic and ferrimagnetic probes retain magnetism even after removal of magnetic fields, paramagnetic and superparamagnetic probes lose magnetism. Since ferromagnetic particles tend to aggregate without magnetic fields [73], paramagnetic and superparamagnetic probes have been employed more popularly for the magnetic tweezers. In measurements using magnetic tweezers, small probes are inserted into a cell or attached on a cell membrane to match the length scale of cells. Then, the probes undergo various types of motions in response to applied magnetic fields (Fig. 2(e)). More recently, rotational magnetic spectroscopy working based on controllable motions of micro-actuators was developed [74]. In several studies, magnetic wires with various sizes within a cell were rotated at constant angular speed or back-and-forth oscillatory rotation [74]. The 3D trajectories of the wires were analyzed from images obtained via microscopy in order to estimate local mechanical properties of the cell.

Recently, in order to evaluate the mechanical properties of intracellular spaces, many research groups employed microrheology technique that can measure rheological properties of a

medium using the trajectory of a micron-sized tracer. Initially, one-point microrheology using trajectories of individual particles was widely used. However, substantial differences between rheological properties measured by the one-point microrheology and those evaluated by other measurement tools were reported in several studies [75]. In particular, the one-point microrheology based on thermal equilibrium satisfying the fluctuation–dissipation theorem significantly underestimated the stiffness of active systems in a nonequilibrium state, such as the actin cytoskeleton with myosin motors [76]. To resolve the intrinsic issue of the one-point microrheology, researchers developed two-point microrheology using a correlation between pairs of particles [77]. However, active microrheology based on the optical tweezers or the magnetic tweezers tends to be used for measuring local cell mechanical properties more commonly in recent days.

Measurement of traction stress exerted by cells can be a way to evaluate cell mechanical properties. Traction force microscopy (TFM) is a technique that measures traction stress exerted by cells on an optically transparent substrate with fluorescent particles embedded (Fig. 2(f)) [78,79]. Unlike other measurement techniques, TFM measures stress generated by cells instead of a response to applied mechanical cues. Using known constitutive relations for material properties of the substrate, it is possible to estimate traction stress by calculating strain using the displacements of the fluorescent particles. TFM can be classified into two-dimensional (2D) and 3D. While the 2D TFM only measures in-plane traction stress, the 3D TFM measures both in-plane and out-of-plane traction stress. Tension sensor modules are an alternative method for measuring cell traction forces. DNA is widely used as a tension sensor [80–83]. The DNA sensor has a hairpin structure with a fluorophore and a quencher which reduces fluorescence intensity (Fig. 2(g)). Two ends of the tension sensor are attached to the adhesion proteins on the cell surface, such as integrins, and a substrate. When an adhesion protein is under tension, the hairpin structure can be unfolded, leading to an increase in the distance between a fluorophore and a quencher. This leads to higher fluorescence intensity that can be detected by fluorescence microscopy [83].

To select appropriate rheological measurement tools for cell mechanical properties, one should carefully consider the range, resolution, and rate of forces and deformation induced by the tools. Scales of forces and deformations involved with various measurement methods, such as AFM, TFM, and optical tweezer as well as biological structures including cells, cytoskeleton, and motor proteins are explained in detail in previous studies [84,85]. In some of the studies, more than one measurement method was combined to measure mechanical properties with different scales simultaneously. For example, recently, micropipette aspiration was coupled with optical traps and tension sensor to measure the variation of tension along the cell membrane at micron scales [86].

These aforementioned rheological measurement techniques helped us understand the origins of the mechanical properties and rheological behaviors of single and collective cells across a wide range of scales. More thorough reviews of rheological measurement techniques can be found in previous review articles [87–91]. To correlate the intrinsic mechanical properties of cells with intracellular structures more precisely, more efforts in advanced combinations of state-of-the-art techniques are required. High- or super-resolution live cell imaging of the cytoskeleton and nuclear structures can be integrated with devices that can apply or measure forces in order to further investigate the origins of the mechanical properties and responses of cells. Future hybrid studies merging these techniques could bring prominent benefits to clinical tool development.

4 Nonlinear Elastic and Inelastic Properties of Cells and Their Origins

A number of experiments have shown that cells are far from a linear elastic material, which is not surprising considering that a

Table 1 Properties of biopolymers and their contributions to cell mechanical properties

Biopolymer	Properties	Contributions
F-actin	<ul style="list-style-type: none"> • Formed by self-assembly of G-actin [26] • Semiflexible polymer (typical contour length is comparable to its persistence length) [27] • Undergo rapid turnover in cells [92] 	<ul style="list-style-type: none"> • Stiffening: via strain-stiffening of F-actins and networks [93] • Superelasticity: via dilution of actomyosin cortex [8] • Viscoelasticity: via turnover of cross-linkers and F-actins [94–97] • Poroelasticity [98]
Intermediate filaments (IFs)	<ul style="list-style-type: none"> • IFs refer to several types of filaments whose diameter is larger than F-actin but smaller than microtubule (e.g., keratin, vimentin, neurofilament, and lamin) [26] • Persistence length of IFs is shorter than that of F-actin [45] • High extensibility [47] 	<ul style="list-style-type: none"> • Stiffening: via strain-stiffening of IFs • Viscoelasticity: via viscoelastic behaviors of vimentin IF [46,99] • Viscoplasticity: via deficiency of keratin IF [100] • Poroelasticity [101]
Microtubules	<ul style="list-style-type: none"> • Formed by self-assembly of dimers with α- and β-tubulins [26] • Rigid polymer (persistence length is much longer than its typical contour length) [35] • Highly dynamic in cells with rapid growth and shrinkage • Resist compressive forces [35] 	<ul style="list-style-type: none"> • Viscoelasticity: via dynamic behaviors of microtubules [102,103]
Nuclear lamin	<ul style="list-style-type: none"> • Type V IF with two major categories: lamin A/C and lamin B [48] • Interact with inner nuclear membrane to form nuclear lamina which serves as a scaffold of the nucleus [48] 	<ul style="list-style-type: none"> • Cell stiffening: via nucleus stiffening induced by a transition of lamin A into a load-bearing element at high extension [104] • Superelasticity: via high extensibility of a lamin network initially in a compressed form [105] • Viscoelasticity: via viscous behaviors of lamin A and elastic behaviors of lamin B [106]
Chromatin	<ul style="list-style-type: none"> • Composed of DNA and proteins 	<ul style="list-style-type: none"> • Viscoplasticity: via plastic deformation of a cell nucleus due to alignment of chromatin fibers [107]

large fraction of a cell body is water and that intracellular structures are very heterogeneous and complex. Nevertheless, cells were assumed to behave as a linear elastic material in many traditional studies. This assumption is valid under certain conditions, but it is a too drastic simplification of the cell under general conditions. Complicated mechanical properties of cells are emergent properties from various cellular components. Some of the cellular components show nonlinear responses or undergo dynamic turnover and thus make greater contributions to the mechanical properties of cells deviating from linear elasticity, than others. The cytoskeleton and nucleus briefly explained earlier are known as critical regulators of the cell mechanical property. In this section, we discuss nonlinear elastic and inelastic properties of cells and how they are related to the properties and dynamics of the cytoskeleton and nucleus, which are also summarized in Table 1.

4.1 Nonlinear Elasticity. Linear elastic materials obey Hooke's law, showing a linear stress–strain relationship. Theories describing behaviors of linear elastic materials were established very well. However, even most of the industrial materials show such linear elasticity only at very small strains. Cells also exhibit highly nonlinear elastic responses, meaning that force (or stress) exerted on a cell is not always directly proportional to deformation (or strain).

4.1.1 Stiffening. A representative nonlinear elastic response of cells that has been widely observed is stiffening. A cell becomes much stiffer if strain or stress applied to the cell increases beyond a critical level. Fernandez et al. showed strain-induced stiffening of fibroblasts by stretching it using the uniaxial stretching geometry based on microplates (Figs. 3(a)–3(c)) [108]. Another study showed local stiffening of fibroblasts induced by stress, using the magnetic tweezers [109]. Different types of cells, human alveolar epithelial cells and mice myoblast cells, also showed stiffening in response to a constant force applied by beads trapped in optical tweezers [15].

Cell stiffening may originate from various cellular components. One of the origins is the nonlinear extensional response of F-actin, intermediate filaments, and their networks. Since F-actin and intermediate filaments have relatively low persistence length [35,110], they tend to thermally fluctuate actively in water, resulting in their curvy native form. If these filaments are extended by tensile forces, their end-to-end distance becomes closer to their contour length. Then, entropy, which is proportional to the number of possible configurations of the filaments, decreases, leading to resistance to an extension called the entropic spring effect [93]. Due to this additional resistance, the force–extension curve of these filaments is highly nonlinear. Strain-induced stiffening at the network level has been shown in studies using reconstituted

networks and computational models [111–114]. F-actins are deformed largely in a bending mode at low strain, resulting in relatively low network stiffness. By contrast, as strain substantially increases, a larger portion of F-actins undergo extension, leading to much higher network stiffness. As a result, a stress–strain relationship for the network becomes highly nonlinear. *in vitro* experiments showed that the application of stress to a network resulted in the network stiffening if stress is greater than a critical level (Fig. 3(d)) [115,116]. Recent *in vitro* experiments demonstrated that IF networks also exhibit similar stiffening due to a transition from the bending mode to the extension mode at high strain [117].

Note that filaments in a network can be extended better by external mechanical cues if they are connected to other filaments relatively well. IFs are often crosslinked by divalent ions in reconstituted networks. F-actins are commonly connected by cross-linking proteins that can bind to two F-actins simultaneously. It was shown that poorly crosslinked F-actins in a network deform in the bending mode even at high strain, so the network does not undergo significant stiffening. However, it is also possible that a network stiffens due to other factors. An *in vitro* study showed that MTs embedded in a crosslinked actin network can facilitate the extension of F-actins by suppressing their bending deformation, leading to network stiffening [118]. It was also shown that attraction between hydrophobic tails of IFs leads to strain-stiffening of IF networks [117].

These *in vitro* experiments and computational models have provided insights into understanding the microscopic origins of

stiffening behaviors. However, there are more complications in the cytoskeleton within cells. Although F-actins in reconstituted networks experience bending deformation at low strains, it is likely that F-actins in the *in vivo* actin cytoskeleton are already in the extension mode even without any cell deformation. Myosin II motors are known to generate tensile forces by pulling actin filaments, which induces prestress in actin structures within cells, such as stress fibers and cell cortex [119,120]. Thus, in regions with high myosin concentration, F-actins are likely to be extended to some extent in the absence of any external stimulus, leading to relatively high network stiffness [121]. In addition, unlike reconstituted actin networks, more F-actins can be recruited to regions with high stress in cells [15]. It was shown that more actin is recruited around an optically trapped bead exerting local forces, resulting in an increase in local stiffness [15].

Cell stiffening emerging at very high deformation can be attributed to a cell nucleus which is known to be stiffer than any other intracellular component [105,122]. It was shown that a cell nucleus exhibits strain-stiffening; an isolated nucleus becomes much stiffer when the nucleus is stretched by micropipettes [104]. While relatively softer chromatin plays a dominant role in resisting deformation at extension smaller than $3\ \mu\text{m}$, lamin A consisting of IFs becomes a load-bearing element at high extension level, leading to significant stiffening of the nucleus.

In sum, cell stiffening is an emergent response from combined effects of the actin cytoskeleton, IF networks, the nucleus, and possibly other intracellular structures. The ability of cells to increase their stiffness can be useful for resisting large external

Fig. 3 Nonlinear elastic properties of cells: stiffening and superelasticity. (a)–(c) Cell stiffening measured in whole-cell stretching experiments. (a) A fibroblast under large stretch induced by two microplates. (b) The length of a cell is increased with oscillation, and force in response to a change in length is measured. (c) A stress–strain relationship calculated from (b). The inset shows a differential modulus as a function of the force. The cell exhibits higher stiffness with larger stress. Reprinted with permission from American Physical Society © 2008 [108]. (d) Stiffening of crosslinked actin networks. As shown in the inset, a finite amount of stress was first applied to the network via a bulk rheometer as prestress (σ_{ext}), and then small oscillatory stress ($\delta\sigma_{\text{ext}}$) was superimposed to measure a differential modulus (K'). K' indicating instantaneous stiffness is relatively constant at low prestress, but it increases significantly if the prestress becomes larger than the critical level (σ_{crit}). In blue, green, and red cases, the ratio of myosin concentration to actin concentration is 0.02, 0.005, and 0.001, respectively. Open triangles represent a passive network without any motor. All cases have the same amount of filamin A as F-actin crosslinkers. Adopted and modified with permission from PNAS © 2009 [115]. (e) and (f) Superelastic behaviors of an epithelial dome induced by a pressure difference, ΔP . Surface tension acting on the dome (σ) increases progressively as areal strain increases. With a further increase in strain, the surface tension reaches a plateau, meaning superelasticity. Reprinted with permission from Springer Nature © 2018 [8].

forces or stress in order to maintain their structural integrity. However, cells do not always show stiffening as discussed in Sec. 4.1.2; emergence of cell stiffening depends on the rate, duration, and nature of mechanical signals and the type of cells.

4.1.2 Superelasticity. Superelasticity was often observed in metal alloys, such as Nickel Titanium alloys [123]. During a superelastic response, strain increases substantially with a minimal change in stress, and the deformation is reversible. The superelasticity is attributed to microscopic material instabilities resulting from strain-softening [8]. Interestingly, superelasticity was recently observed in cells. A study used 3D traction microscopy to show that when epithelial cells forming a lumen-like dome structure are pressurized, they exhibit very large reversible deformation with a tensional plateau (Figs. 3(e) and 3(f)) [8,124]. It means that the surface tension acting along the epithelial cells did not change significantly while deformation changes over a wide range. The study found that within the tensional plateau, some cells increased their area up to 1000% of the original area. The study demonstrated that the superelasticity observed in the epithelial cells originates from a transition of actin cortex from an elastic state to a fluid-like state induced by tension above a critical level. As the actin cortex is stretched, it becomes thinner and diluted because the amount of cortical constituents is limited. The study also demonstrated that IFs prevent the epithelial cells from undergoing unlimited deformation by behaving as springs that resist tension strongly only at very high deformation level as well as enable the cells to recover their initial shape when the applied tension is reduced. The ability of cells to maintain a constant force

regardless of deformation level was shown in another study although cells were not deformed to a very large extent [125]. In this study, the authors stretched fibroblasts slowly using the AFM cantilever without any tip and observed that forces exerted by fibroblasts do not change significantly. Such a tendency named tensional homeostasis was lost when α -actinin, one of the most common actin cross-linking proteins, was overexpressed, implying that the ability to maintain the constant force is involved with the actin cytoskeleton.

A cell nucleus is also known to exhibit very large deformability due to the interesting mechanical property of a lamin network constituting nuclear membranes. A study using isolated *Xenopus* oocyte nuclei showed that the lamin network exists in a compressed form, and it can expand up to nearly twice in terms of surface area [105]. The study suggested that such high extensibility reminiscent of superelasticity helps cells migrate through size-limiting environments without structural damage on the nuclear membranes.

It is interesting that cells are capable of exhibiting both stiffening and superelasticity in response to mechanical stimuli. The loading rate of stimuli seems to be the most important factor determining how cells respond. In the study showing the superelasticity of cells, it was suggested that superelasticity only occurs at a long timescale during which cells are stretched with their own rate [124], explaining why previous experiments with rapid application of forces increasing within seconds or minutes have not shown the superelastic behaviors of cells. In addition, the tensional homeostasis was not observed when fibroblasts were stretched rapidly [125]. However, it remains unclear what defines

Fig. 4 General responses of viscoelastic materials and lumped parameter models. (a) If a viscoelastic material is subjected to constant strain, stress acting on the material gradually decreases over time, which is called stress relaxation. (b) If constant stress is applied to a viscoelastic material, strain of the material gradually increases over time, which is called creep. (c) and (d) If oscillatory stress is applied to an elastic material, strain is in phase with stress. By contrast, if the material is viscoelastic, there is a phase delay between stress and strain. (e)–(g) Representative lumped parameter models for describing responses of viscoelastic materials: Maxwell model, Kelvin–Voigt model, and standard linear solid model. Configuration and the numbers of elastic springs and viscous dashpots in the lumped parameter models can be varied to fit experimental data better.

critical loading rates below which cells can maintain stress or forces during an increase in strain or deformation.

As discussed above, cell behaviors can highly vary depending on a loading rate. Therefore, it is important for the mechanical testing of cells to use measurement tools supporting the relevant range of loading rates. A previous study summarized the bandwidths of various measurement methods including AFM, micropipette aspiration, and optical tweezer [84]. The dependence on the loading rate is even more important for the viscoelastic properties of cells that will be discussed below.

4.2 Viscoelasticity. A difference between elastic and viscoelastic materials is the dependence of the response of a material on time or frequency. Elastic materials show instantaneous responses to applied mechanical signals, whereas viscoelastic materials exhibit time-varying behaviors, such as stress relaxation in response to constant strain and creep behavior in response to constant stress (Figs. 4(a) and 4(b)). In addition, when oscillatory signals are applied, elastic materials always show in-phase responses that are independent of the frequency of the applied signals while viscoelastic materials show frequency-dependent responses with phase delay (Figs. 4(c) and 4(d)).

Numerous experimental studies have shown that cells can exhibit both elastic and viscous properties. To understand the viscoelastic behaviors of cells, lumped parameter models have

been used popularly (Figs. 4(e)–4(g)). Basic components of the lumped parameter models are springs and dashpots, which represent elastic and viscous parts of a system of interest, respectively. A certain number of springs and dashpots are connected in series or in parallel in the lumped parameter models. The simplest models are Maxwell and Kelvin–Voigt models consisting of one spring and one dashpot connected in series and in parallel, respectively. While the Maxwell model describes force/stress relaxation relatively well, the Kelvin–Voigt model describes a creep behavior better. Due to the limited ability of these models to recapitulate various viscoelastic behaviors, the standard linear solid model with two springs and one dashpot has been used more often. More advanced lumped parameter models have been used in many research studies to fit experimental data and provide insights into the origins of the viscoelastic behaviors.

A vast amount of studies measured the viscoelastic response of a whole cell to a change in deformation or force applied via techniques, such as microplates, micropipettes, microfluidic devices, and AFM without a tip. In an earlier study using a piezocontrolled microplate micromanipulation system, the authors estimated the elastic moduli and apparent viscosity of chick fibroblasts by fitting cell responses to compression and stretch with the standard linear solid model [10]. In a more recent study, a more advanced microplate-based rheometer called the uni-axial stretching rheometer was developed for investigating the creep response of

Fig. 5 Viscoelastic behaviors of cells and the cytoskeleton. (a) Creep response of mice myoblast cells to a constant force applied by a uni-axial stretching rheometer. During force application, the displacement of the bead initially jumps and then gradually increases, showing creep. Cells exhibit a rupture at high strain. Reprinted with permission from Elsevier © 2005 [126]. (b) and (c) Frequency-dependent shear moduli of mouse fibroblast cells measured by multifrequency force modulation AFM. (b) Measurements were performed in regions above (central square) and away from (surrounding squares) a cell nucleus. (c) The curves represent storage modulus (G' , filled circles) and loss modulus (G'' , open circles) measured in central and surrounding square regions in (b), respectively. G' and G'' depend on the frequency and locations. Reprinted with permission from AIP Publishing © 2015 [128]. (d) and (e) Measurement of mechanical properties of chondrosarcoma cell lines using an AFM. (d) Stress relaxation of a cell in response to constant strain applied by AFM indentation. A solid line represents fitting via a thin-layer viscoelastic model based on the Hertz model that assumes an infinitely hard sphere indenting a deformable and flat substrate. (e) Mechanical properties of three kinds of chondrosarcoma cell lines with different metastatic potential and invasiveness (JJ012, FS090, and 105KC) seeded for 2 days. Relaxed modulus (E_R), instantaneous modulus (E_0), apparent viscosity (μ), and Young's modulus (E_Y) are estimated by fitting stress relaxation data with the thin-layer viscoelastic model. Adopted and modified with permission from Elsevier © 2007 [132]. (f) G' (closed symbols) and G'' (open symbols) of actin networks crosslinked by fascin. G' and G'' measured with various fascin density collapse into a single curve after normalization. G'' shows a minimum at a frequency that corresponds to the unbinding rate of fascin. Reprinted with permission from the American Physical Society © 2007 [96].

myoblasts to constant stretching forces (Fig. 5(a)), and it was found that the response is fitted well by a power law [126]. Interestingly, another study using the magnetic twisting cytometer demonstrated that the creep behavior locally measured in human airway smooth muscle cells also shows the power-law behavior [127]. This implies that there is neither distinct molecular relaxation time which is often used to characterize an exponential decay nor a time constant that can characterize the creep responses.

Micropipette aspiration was also used to estimate the instantaneous and relaxed moduli and apparent viscosity of chondrocytes isolated from different locations of cartilage. They found that chondrocytes harvested from farther locations from the surface of cartilage tend to have smaller elastic moduli and apparent viscosity [56]. These mechanical properties were also compared with values obtained using AFM indentation with a spherical tip and the standard linear solid model. The elastic moduli were comparable, but the viscosity measured using AFM was lower. Such a difference in the estimated viscosity may be attributed to what those measurement techniques probed in cells. Micropipette aspiration results in larger-scale cell deformation than AFM indentation with a tip, so it may reflect the mechanical properties of intracellular structures located away from the cell membrane, unlike AFM measurement.

Another study using AFM also showed mechanical heterogeneity of intracellular space. Frequency-dependent shear moduli of mouse fibroblasts were measured in a location near or away from a cell nucleus (Fig. 5(b)) [128]. In the region near the nucleus, shear storage modulus (G') was much higher, and shear loss

modulus (G'') was slightly larger, which is attributed to the contribution of a stiffer nucleus (Fig. 5(c)). Note that G' measured in living cells is typically much higher than G'' at low frequencies, whereas G'' is higher than or comparable to G' at high frequencies. This implies that cells behave in an elastic manner at long timescales but act in a viscoelastic material at short timescales.

To probe mechanically heterogeneous intracellular environments, various kinds of microrheology have been used. A study using wire-based rotational magnetic spectroscopy demonstrated that cytoplasm of fibroblasts and human cancer cells behave like a viscoelastic fluid rather than an elastic gel [74]. In addition, optical tweezers that can rotate particles trapped within a cell were employed to measure intracellular viscosity [68]. The static and dynamic viscosities were determined based on the rotational velocity or rotational displacement of the particles. It was found that the cytoplasm behaves as a viscous fluid at the tested range of frequencies, which is consistent with previous observations [129]. Interestingly, the intracellular viscosity was negatively correlated with the rotation frequency of the particle, which is in line with the frequency dependence of shear moduli of cells mentioned above [127].

Viscoelastic properties of cells also depend on their type and state. One study used optical tweezers to measure the elastic and viscous moduli of fibroblasts, neurons, and astrocytes. It was shown that fibroblasts show the highest stiffness with the most solid-like behaviors, whereas astrocytes exhibit very viscous responses [67]. A study used the micropipette aspiration to show that dedifferentiated chondrocytes have larger stiffness and

Fig. 6 Viscoplasticity and poroelasticity of cells. (a)–(c) Viscoplasticity of mouse embryonic fibroblasts measured by magnetic tweezers and beads bound to cells. (a) Cell response to an applied force can be fitted well by a power law, but it does not follow the power-law prediction (i + ii) corresponding to the combination of a response to an ongoing force (i) and a response to a counterforce applied in the opposite direction (ii). This implies that part of the cell deformation is nonreversible (i.e., plastic). (b) A cell response (black) can be fitted well by the combination of a viscoelastic response (blue, 2) and plastic deformation (red, 1). (c) The plastic deformation estimated via curve fitting shows an increase during force application but remains relatively constant during relaxation periods. Reprinted with permission from Springer Nature © 2016 [147]. (d) Poroelastic properties of mouse embryonic fibroblasts. Optical tweezers were used to displace and hold a bead embedded in the fibroblasts to evaluate force relaxation. Fibroblasts with wild type (WT), null vimentin (Vim $-/-$), only vimentin (Ghost cell), and overexpressed vimentin (OverE) were employed. At short timescales ($t < 0.05$ s), force relaxation curves exhibit an exponential decay, indicating poroelastic behaviors, whereas force relaxation curves exhibit a power-law decay at longer timescales ($t > 0.05$ s), indicative of viscoelastic behaviors. Fibroblasts only with vimentin do not exhibit viscoelastic behaviors even at longer timescales, implying the important role of vimentin for cell poroelasticity. Reprinted with permission from Ref. [101]. (e) Poroelasticity of cell nuclei. Responses of nuclei isolated from HEK-293 cells to AFM indentation with various loading rates. This study showed that the rate-dependent responses are attributed to poroelasticity of the cell nuclei. (inset) Peak forces as a function of the loading rate. Reprinted with permission from AIP Publishing © 2016 [159].

slightly higher viscosity than freshly isolated chondrocytes. The study claimed that the higher elastic stiffness is attributed to stronger adhesions between the membrane and actin cortex originating from an increased organization of the cortical cytoskeleton [56].

In addition, disease states of cells highly affect cell viscoelasticity. One study showed differences between the mechanical properties of fibroblast cells and HeLa cells [66]. While fibroblasts showed typical viscoelastic behaviors with a power-law behavior at lower frequencies, HeLa cells exhibited viscoelasticity that is very close to soft glassy materials [70,130]. Several studies have investigated a relationship between malignancy and viscoelasticity of tumors and cancerous cells. By analyzing the movement of cells driven by fluid flow in a confining microchannel via the standard linear solid model, researchers found that the most malignant breast cancer cell line has a lower relaxed modulus and higher viscosity than those measured in normal breast cells [59], consistent with previous observations [60,131]. In addition, stress relaxation measurement performed on chondrosarcoma cells showed that tumorigenicity representing the ability of cells to produce tumors is associated with a decrease in both elastic modulus and apparent viscosity of the cell (Figs. 5(d) and 5(e)) [132]. More recently, there was an attempt to extract the viscoelastic properties of benign and cancerous cell lines using AFM and also correlate changes in viscoelastic properties of cells with tumorigenesis [133]. The authors found that cancer cells tend to be softer (lower Young's modulus) and more viscous (higher power-law exponent) than benign cells. Another study also reported that elastic modulus and relaxation time estimated from the relaxation of forces applied to cancer cells by AFM differ significantly from those of benign cells [134].

The cytoskeleton is known to be a major factor that determines cell viscoelasticity. The importance of the cytoskeleton is evidenced by an experimental study demonstrating that adhesions between the cytoskeleton and the AFM tip can affect viscoelastic properties [54]. The spherical tip attached to the top of a Chinese Hamster Ovary cell via adhesion proteins called intercellular adhesion molecule 1 was displaced upward to induce local stretching and then characterize a force–displacement curve. The authors showed that local viscoelastic properties of cells can substantially vary depending on whether or not the adhesion proteins can be physically linked to the cytoskeleton. With mutated intercellular adhesion molecule 1 that cannot form links to the cytoskeleton, elastic modulus and viscosity estimated by fitting the curve with viscoelastic models showed lower values. The role of the cytoskeleton as the main regulator of cell viscoelasticity is also demonstrated by showing a variation in cell properties induced by drugs that perturb the structure and dynamics of the cytoskeleton. In one study using microfluidic devices, hydrodynamic forces were applied to fibroblasts and tumor-initiating cells with low confinement. For both types of cells, shear modulus and fluid parameter showed a strong dependence on the softening and stiffening of the cytoskeleton induced by agents [58]; with softer cytoskeleton, shear modulus tends to be lower, whereas the fluid parameter is slightly higher, indicative of the important role of the cytoskeleton for the viscoelastic property of the cells. Another study used laser-tracking microrheology to trace endogenous granules within kidney epithelial cells as probes for measuring local properties [135]. The study showed that perturbation of the actin cytoskeleton using drugs, such as latrunculin A or cytochalasin D, significantly softens and fluidizes the lamella. Mechanical and dynamic behaviors of F-actins lead to elastic energy dissipation and stress relaxation in the cytoskeleton, which can drive the viscoelastic properties of cells. Several computational models have shown that turnover of F-actins facilitated by various modes of actin dynamics can substantially reduce and stabilize network stress [136–139]. An in vitro study demonstrated that a branched actin network formed by Arp2/3 shows reversible softening when the network is compressed via a mechanical load applied by the AFM cantilever [22]. The authors of the study hypothesized that the reversible softening originates from buckling of F-actins

induced by compressive forces from the load. Furthermore, a computational model showed that fragmentation of F-actins facilitated by buckling can also result in significant stress relaxation in actin networks [140].

Transient natures of crosslinks formed between F-actins are also one of the main sources of elastic energy dissipation and stress relaxation in the actin cytoskeleton. Most of the ACPs reversibly bind to F-actins; ACPs unbind from F-actins faster (slip bonds) or slower (catch bonds) in response to higher applied forces [141,142]. Theoretical and computational models demonstrated that ACP unbinding leads to substantial stress relaxation and softening in the actin cytoskeleton [143,144]. Viscoelastic behaviors of the actin cytoskeleton that depend on the frequency of applied mechanical cues were explained by the timescales of ACP unbinding (Fig. 5(f)) [94–96]. Consequently, viscoelastic properties of cells are affected by dynamic behaviors of ACPs. For example, it was demonstrated that a point mutation on ACPs that varies their binding kinetics results in a change in cell viscoelastic properties [97].

Energy dissipation and stress relaxation can also occur via conformational changes in cytoskeletal proteins subjected to loading. It was shown that a single vimentin filament under extension exhibits a viscoelastic behavior by dissipating energy through folding and unfolding of α -helices in the vimentin monomers [46,99], which can lead to viscoelastic behaviors of networks consisting of vimentin filaments. It is likely that various viscoelastic properties observed in in vitro IF networks composed of keratin and desmin also originate from similar molecular conformation changes although further investigations are needed [23,145]. Direct contributions of the viscoelastic behaviors of single IFs to cell mechanical properties remain to be investigated more.

Microtubules showing highly dynamic behaviors are known to contribute significantly to cell viscoelasticity. For example, several studies showed that perturbation in MT dynamics induced by drugs affects cell viscoelastic properties. For example, stabilization of MTs via taxol enhanced viscous energy loss in smooth muscle cells measured by the micropipette pulling [102]. Myeloid cells stretched by the microfluidic optical stretcher showed less relaxation with taxol treatment due to enhanced bundling and polarization of MTs [103]. In addition, it was observed that the deformation of Michigan Cancer Foundation 7 epithelial cells is proportional to taxol concentration [146].

It was also shown that a nucleus exhibits viscoelastic behaviors. One study employed micropipette aspiration to evaluate viscoelastic responses of a nucleus [106]. By changing the stoichiometry of Lamin A and B, the authors found that lamin A acts as a viscous fluid, whereas lamin B acts as an elastic material. Since a nucleus and the intracellular cytoskeleton have distinct mechanical properties, a majority of previous studies attempted to measure cytoskeletal mechanics in intracellular regions away from the nucleus.

In sum, cells exhibit both solid-like and fluid-like behaviors called viscoelastic responses, such as stress relaxation, creep, and the frequency-dependent shear moduli. Viscoelastic properties of cells can vary depending on disease states, differentiation, and cell types. Structural changes and dynamic behaviors of the cytoskeleton and the cell nucleus make great contributions to viscoelastic properties of cells. Thus, distinct viscoelastic properties observed in cells are likely to originate mainly from differences in characteristics of the two cellular constituents. Despite previous experimental efforts, investigations with more advanced measurement techniques are required for illuminating the molecular origins of cell viscoelasticity.

4.3 Viscoplasticity. If an external mechanical load that caused deformation of a material is released, the material usually shows recovery toward its initial shape. If the material is purely elastic, it instantaneously returns to its initial state after the release of the mechanical load. However, the recovery process of cell

shapes after the release often occurs neither instantaneously nor completely; it takes substantial time for deformation to reach plateau level corresponding to permanent deformation [147]. This is typically observed in viscoelastic materials. However, if the recovery takes place in a purely viscoelastic manner, the time course and magnitude of curves during loading and recovery phases can be fit well by Boltzmann superposition of two power-law creep responses. A study showed that this is not the case for deformation of cells caused by magnetic tweezers (Fig. 6(a)) [147]. Only when an additional plastic component is added, the curves could be fit well (Figs. 6(b) and 6(c)). Such an inelastic behavior is called viscoplasticity.

The role of viscoplasticity is of particular importance in plant cells. Plant cells undergo significant viscoplastic deformation during anisotropic growth, so it has been understood relatively well how plant cells deform in an irreversible manner due to turgor pressure without the loss of structural integrity [148–150].

Viscoplastic deformation of animal cells has received less attention. However, it is known that the viscoplasticity of cells is crucial for embryogenesis because cell shapes keep changing and need to be stabilized as embryogenesis proceeds [151]. Origins of the viscoplasticity have been investigated using individual animal cells in recent studies. It was shown that the plastic deformation of fibroblasts found by magnetic tweezers mentioned above scales linearly with applied force, implying that viscoplasticity may originate from force-dependent dynamic behaviors of cellular components, such as breakage and slippage of crosslinked cytoskeletal filaments or bond ruptures [147,152]. Another study showed that beads embedded in the cytoplasm of Dictyostelium cells exhibit viscoplastic motions when they are subjected to forces exerted by colloidal magnetic tweezers [153]. It was also suggested that the apparent viscosity calculated from the viscoplastic motions is a measure of the breakage rate of bonds within cytoplasmatic networks.

Fig. 7 Continuum models designed for investigating cell mechanical properties. (a) and (b) Simulation of magnetocytometry performed on a cell monolayer. (a) A FEM-based model was used to predict the displacement of a bead attached on the cell monolayer. The cell monolayer is composed of membrane and cytoskeleton which are modeled as the Maxwell model with distinct parameters. Only the portion of the bead that contacts the cell is simulated. In this example, the cell subjected to a force ($F=500 \text{ pN}$) applied to the bead. (b) Bead displacement (top, black curves) induced by an oscillatory force (bottom) in the model. An increase in the bead displacement indicates the creep response of the cell monolayer. Experimental results (top, gray curves) from magnetocytometry performed on NIH 3T3 fibroblasts also show similar creep behaviors. Reprinted with permission from Elsevier © 2003 [167]. (c) and (d) Simulation of nano-indentation performed on fibroblasts. (c) A model for a spherical indenter and a cell based on the FEM. Only a portion of the cell and the indenter are simulated in the model to reduce computational cost. (d) Indentation forces measured in the simulation (solid lines) show moderate stiffening and are consistent with experiments (circles). Loading and unloading curves do not correspond to each other, resulting in a hysteresis loop that is indicative of the viscoelastic nature of fibroblasts in the model. Reprinted with permission from Ref. [171].

Cell resilience represents the ability of cells to recover their initial shapes, so higher resilience means lower viscoplasticity. One of the most important regulators for the cell resilience is IFs. It was shown that disruption of IFs makes cells more vulnerable to mechanical stress because such cells cannot recover their shape well after deformation. A well-known example is a disorder called epidermolysis bullosa simplex caused by a mutation in keratin IFs. The basal layer of keratinocyte cells with this disorder is likely to fracture when the skin is exposed to physical trauma [154]. Recently, the effects of the mutation in keratin IFs on cell mechanical properties were investigated using AFM and magnetic tweezers [155]. It was found from creep tests that keratin-deficient keratocytes are significantly softened with higher viscosity and show significantly larger plastic deformation. Considering remarkable extensibility and resilience of IF networks [100], viscoplasticity of cells may depend highly on properties of IF networks as seen in this example.

A cell nucleus also exhibits viscoplasticity. It was shown that nuclei of human embryonic stem cells exhibit irreversible deformation, whereas differentiated cells show reversible deformation [107]. The authors suggested that the plastic deformation of a cell nucleus is attributed to the irreversible alignment of chromatin fibers within the nucleus. Another study demonstrated that HeLa cells with a spectrin-deficient nucleus show an irreversible change in their shapes after unconfined compression, whereas cells with an intact nucleus show more reversible deformation [156], which implies the important role of spectrins in the resilience of the cell nucleus.

In sum, plastic deformation for viscoplasticity of animal cells is induced by subsequent force-dependent dynamic events occurring on filaments or bonds within cytoskeletal networks. Considering IF networks play a negative role in viscoplasticity by increasing cell resilience, the actin cytoskeleton is likely to be responsible for irreversible deformation. Indeed, a computational model showed that crosslinked F-actin networks exhibit substantial plastic deformation after they are deformed by shear strain, due to force-dependent unbinding of actin cross-linking proteins [144]. Since F-actins in cells turn over rapidly, the actin cytoskeleton may be able to maintain its structural integrity up to certain strain level by repairing itself. However, stress is relaxed by F-actin turnover and crosslinker unbinding, so the extended networks cannot recover to their initial state by themselves. Further investigations are necessary for a better understanding of how the actin cytoskeleton contributes to viscoplastic responses.

4.4 Poroelasticity. Deformation of a porous elastic material immersed in fluid induces fluid flow within pores and thus interactions between the solid material and fluid flow. Poroelasticity plays a particularly important role when a material is compressed by an applied load. Since water efflux does not occur instantaneously, resistance to abrupt compressive stress can be stronger than that without fluid, which can be inferred from a difference between a fluid-saturated sponge and a dry one. However, as the water gradually leaves the porous material over time, the resistance is reduced toward plateau level, resulting in relaxation of stress or force. The response of a poroelastic material depends on a poroelastic diffusion coefficient determined by Young's modulus of the solid part, pore size, and viscosity of the fluid.

It is well-known that many parts of a cell body can be considered a porous medium. All cellular components are immersed in water, and many of them have dense network structures with small pores that can potentially lead to poroelastic effects. The poroelasticity of cells has been suggested in several studies. For instance, in one study, cancer cells subjected to external heterogeneous osmotic stress showed cellular responses independent of their metabolic conditions, indicating that short-time responses of cells are physical rather than chemical [157]. The authors demonstrated that the physical cellular responses can be explained better by a theoretical framework called the fluid-filled sponge model

that combines mechanics and hydraulics with the poroelastic description of the cytoplasm. Another study demonstrated that relaxation of forces developed by large, rapid local cell deformation induced by AFM indentation can be highly affected by water flow in porous cytoplasm [98]. The force–relaxation curve could be varied by changing the timescale of water movement, which reflects pore size, between 0.1 and 10 s. Another study investigated the propagation of stress induced by AFM indentation over adherent cells. The stress was measured by tracking particles embedded within the cells. The study found that cells located farther away from the indentation site show smaller displacement and longer equilibration time, which can be explained only by a poroelastic model [158].

Several studies investigated how cell poroelasticity is regulated. A study using AFM found that the actin cytoskeleton and myosin II play a major role in cell poroelasticity, whereas MTs and IFs are much less important [98]. However, a recent *in vivo* study used optical tweezers and microbeads to show the importance of IFs for poroelasticity of mouse fibroblasts (Fig. 6(d)) [101]. The study used three types of cells: (i) wild type, (ii) a cell without vimentin IF networks, and (iii) a ghost cell only with vimentin IF networks in the absence of other cytoskeletal structures. When constant strain is applied, all three types of cells showed an exponential decay of stress at the beginning, indicating they are a poroelastic material. At later times, wild-type cells and vimentin-deficient cells switched to a power-law decay of stress indicating a viscoelastic behavior, whereas ghost cells retained the exponential decay. The results suggested that vimentin IF networks in cells facilitate cell poroelasticity [101]. Likewise, there is still a controversy over the relative importance of each cytoskeletal network. Based on the small pore size of IF networks, it is likely that IF networks indeed play an important role in poroelasticity of cells as demonstrated in those studies. However, it is not clear how MTs contribute to poroelasticity because MTs do not form dense networks in cells. It is probable that MTs indirectly affect poroelasticity by physically interacting with other cytoskeletal networks. To define the exact role of MTs in cell poroelasticity, further investigations are necessary.

A cell nucleus also exhibits a poroelastic behavior. Theoretical analysis with the finite element method (FEM) showed that an isolated cell nucleus indented by AFM exhibits time-dependent mechanics that is better described by poroelasticity rather than viscoelasticity (Fig. 6(e)) [159]. It was hypothesized that the nucleus poroelasticity may arise from a dense nucleus matrix composed of fibers and fluid contained within the nucleus. It is possible that a poroelastic nucleus makes a partial contribution to cell poroelasticity if the cells are deformed significantly. The contribution of the nucleus to cell poroelasticity remains to be investigated.

Poroelasticity has been a popular model to describe the resistance of soft tissues to compression. Only recently, the poroelasticity model was applied to explain various cellular behaviors, such as cell migration [160,161], cell responses to an oscillatory pressure [162], and bleb formation [163–165]. It was also suggested that poroelastic parameters may serve as biomarkers for cancer diagnosis and therapies [166]. However, the poroelastic model needs to be used for cell mechanics with caution. Unlike soft tissues, many parts of intracellular spaces do not consist of dense networks. Therefore, it is expected that poroelastic effects would emerge only in specific parts of cells or arise more under certain conditions.

5 Computational Models

Although a wide variety of experiments have probed the mechanical properties of cells and subcellular structures, it is still challenging to understand how emergent properties at the cell scale originate from the mechanical properties of subcellular structures. Computational models can help correlate mechanics at the subcellular level to that at the cell level and uncover the

molecular origins of the mechanical properties of cells by recreating a cell-like structure consisting of important subcellular structures. In this section, we will review the discrete and continuum models designed to investigate cell mechanics.

5.1 Continuum Models. In continuum models, intracellular spaces are treated as a material with continuous mass rather than as discrete particles. FEM has been extensively used in a number of studies as a continuum-based approach to characterize the local or global cell mechanics. FEM models are suitable for probing behaviors of cells emerging in response to various mechanical signals.

One of the earlier studies used FEM to simulate a magnetic bead attached to a cell monolayer in order to mimic measurement of viscoelastic properties of cells via micromanipulation applied by the magnetocytometry (Fig. 7(a)) [167]. The membrane, cortex, and cytoskeleton are modeled as Maxwell viscoelastic materials. The bead subjected to time-varying forces in simulations showed a displacement curve and a temporal variation consistent with a linear viscoelastic behavior predicted by the Maxwell model (Fig. 7(b)). When sinusoidal forces were applied to the bead, the maximal displacement of the bead decreased over cycles, indicating the viscoelastic nature of cells. In addition, it was observed that stress and strain patterns were highly localized near the bead, suggesting that the effects of mechanical stimulation applied by the magnetocytometry are confined only to a local region of the cell. It was also found that the shear modulus and relaxation time of the cytoskeleton are more critical regulators of the viscoelastic responses of cells than the membrane and cortex. A similar study simulated interactions between cells and a bead manipulated by magnetic tweezers, using FEM [168]. Adherent

epithelial cells were described as a nonlinear elastic material. The authors showed that a relationship between applied torque and bead rotation/displacement is quite linear up to the rotation angle of 15 deg. Above the critical rotation angle, geometrical nonlinearities become very important for the spatial distribution of strain on cells. In another study with almost identical approaches, the authors investigated influences of various geometrical parameters, such as bead diameter, bead embedding, and cell thickness, on the cellular response to applied mechanical signals. For example, it was found that the thickness of a cell located below the rotating bead was negatively correlated with the nonlinearity of the response [169]. In addition, a viscoelastic FEM model of magnetocytometry was developed to investigate force transmission during mechanical sensing of vascular endothelial cells [170]. The model accounts for focal adhesion contact sites near the basal surface of a cell to evaluate the spatial distribution of shear stress in the cell. It was found that shear stress is transmitted to the focal adhesion sites in a nonuniform manner, and local focal adhesion sites with concentrated stress are translocated more significantly [170].

Recently, a FEM model was employed to simulate various nano-indentation experiments of fibroblast cells with cyclic, stress relaxation, and creep tests (Fig. 7(c)) [171]. This study showed viscoelastic behaviors of fibroblasts arising in response to those mechanical signals, consistent with experimental observations (Fig. 7(d)). In addition, micropipette aspiration experiments with chondrocytes were simulated by a nonlinear FEM model [172]. By comparing aspiration length in response to an increase in aspiration pressure between simulations and experiments, the authors found that the creep response of cells can be attributed to intrinsic viscoelasticity of cytoplasm rather than cell poroelasticity.

Fig. 8 Discrete models of cells. (a)–(c) A cell model composed of subcellular elements. (a) Subcellular elements for the cell model. Cortical cytoskeleton (i) and nuclear envelope (ii) are modeled as triangulated networks. Actin filaments (iv), intermediate filaments (v), and microtubules (vi) are modeled as filamentous structures. (b) Simulation of nano-indentation using the cell model. (c) A force-indentation relationship measured in the simulation shown in (b). Both cells exhibit stiffening as indentation depth increases. A cell with a nucleus starts showing the stiffening from lower indentation depth. Reprinted with permission from American Physical Society © 2017 [177]. (d) and (e) A cell model based on the DPD. (d) The model consists of a nucleus envelope, the cytoskeleton, and a membrane. The nucleus envelope and the membrane are modeled as elastic triangular meshes. The cytoskeleton is modeled as elastic elements interconnected by crosslinks. (e) Simulation of a cell passing through a narrow microfluidic channel shows that velocity of the passage (V) depends on cell viscosity (η). Reprinted with permission from Ref. [182].

In another study, a FEM model of an osteoblastic cell attached to an elastomeric membrane was developed [173]. The model accounts for actin stress fibers by incorporating a few linear elastic cables that can support only tensile loads. The elastomeric membrane was subjected to cyclic strain at various frequencies with low amplitude. Results in the study indicate that stress and strain that cells undergo are significantly amplified when the cells were subjected to high-frequency strain applied to the elastomeric membrane. This observation implies that the frequency of applied mechanical stimulation plays an important role in cells; the enhanced stress and strain observed within the cytoplasm and nucleus in the study may be indicative of a mechanism by which high-frequency external mechanical signals stimulate a response within cells.

Computational models using continuum-based approaches have also been widely used for simulating cell dynamics with the assumption of poroelastic material properties. In the models, all points are assumed to be occupied by both solid and fluid particles. For instance, in one study, the authors developed one-dimensional and 2D poroelastic models with consideration of cytoskeletal network remodeling and with contraction for simulating the crawling motion of epidermal keratocyte [161]. Motility of the cell in the FEM-based model showed biphasic dependence on a frictional coefficient that represents viscous forces acting due to adhesion between the cytoskeleton and a substrate underlying the cell. Interestingly, a cell in this poroelastic model shows an oscillatory motion under a specific condition, which was observed in crawling epidermal keratocyte in a previous experiment [174]. The authors in this study suggested that the oscillation is caused by phase differences emerging in poroelastic and frictional systems.

In another study, a poroelastic cell was modeled using the immersed boundary method instead of FEM [175]. The immersed boundary method is suitable for modeling a cell-like structure because it can simulate the motion of elastic structures immersed in viscous fluids. The authors in this study compared the migration of a poroelastic cell model with that of a purely elastic model. The migration speed of poroelastic cells is substantially larger than that of the elastic cell. This could be attributed to the ability of fully poroelastic cells to generate much larger traction stress than elastic cells. Although the poroelastic models and theories can capture sufficient experimental observations, they need to be improved further to reflect more advanced structures with biological and physiological relevance.

Although they have been popularly used due to their advantages, continuum models have shown lack of ability to recapitulate rate- or frequency-dependent mechanical properties because they depend mostly on lumped parameter models consisting of springs and dashpots. Although the lumped parameter models can reproduce simple viscoelastic responses, such as creep and stress relaxation, they are unable to recapitulate more complicated viscoelastic responses, such as storage and loss shear moduli. Thus, continuum models based on the lumped parameter models are incapable of simulating complicated responses. In addition, it is hard to describe heterogeneous structures and distributions of intracellular structures using continuum approaches. To overcome these limitations, discrete models and hybrid models that combine discrete and continuum approaches have been developed as explained below.

5.2 Discrete Models. In discrete models of cells, each subcellular component, such as the cytoskeleton and nucleus, is modeled as an explicit element, and interactions between elements are imposed using physical laws or phenomenological rules. The discrete models are useful particularly for investigating the contributions of each subcellular component to the mechanical behavior of cells. However, the computational cost of simulations using discrete models tend to be higher than other models.

In one study, a cell model was built with a plasma membrane, a nuclear envelope, and F-actins [176]. In this model, the membrane

and the nuclear envelope were modeled as triangular meshes whose nodes are interconnected by springs. Interaction potential was applied between nodes of the membrane and those of the nuclear envelope to resist a change in the distance between the nuclear envelope and the membrane. F-actins were modeled as linear elements whose length is maintained by a spring constant. F-actins undergo a change in their spring energy as the cell is deformed. The whole-cell stretching was simulated by applying a tensile load to this cell-like structure, and deformations of the constituents were calculated via energy minimization. In this study, the authors showed F-actins are aligned in a direction relatively parallel to the tensile load, resulting in the nonlinear force-extension behavior of cells.

Another study used a similar approach based on energy minimization to simulate a cell-like structure [177]. In this model, IFs, MTs, and adhesions between a cell and a surrounding environment were incorporated (Fig. 8(a)). By simulating nano-indentation with different levels of adhesions, the study found that cell stiffness increases with higher traction forces and with the existence of the nucleus (Figs. 8(b) and 8(c)). In another model, a cell membrane and a nuclear envelope were modeled as neo-Hookean membranes, whereas F-actins and crosslinkers were modeled as randomly distributed linear Hookean springs connected to the membranes [178]. The authors could predict nuclear strain as a function of connectivity of the cytoskeleton during simulations of micropipette pulling. These modeling approaches based on energy minimization are suitable for measurements of mechanical quantities of static cells. However, these models cannot account for dynamic behaviors of subcellular structures which affect cell viscoelasticity, such as turnover of F-actins.

To investigate dynamic cellular structures, researchers in several studies have employed Brownian dynamics (BD) and dissipative particle dynamics (DPD). For example, the subcellular element model was developed based on BD by dividing a cell volume into approximately ~ 1000 subvolumes [179]. Each subvolume is represented by a node that experiences viscous drag and thermal forces as well as interactions with neighboring nodes. For simulating a single cell, the authors used two types of nodes representing cytoplasm and a membrane. The interactions between neighboring nodes consist of repulsive and attractive forces in a form similar to the Morse potential commonly used to describe interatomic forces in a diatomic molecule [180]. Using the model, they simulated the whole-cell stretching for probing a creep response and also the microbead rheology for measuring frequency-dependent shear storage modulus (G') and loss modulus (G''). Although this study showed viscoelastic behaviors of the cell-like structure qualitatively similar to experimental observations, there are still discrepancies due to the limitation of the simple interaction potential imposed between neighboring nodes.

Like BD, subcellular structures in DPD can be represented by particle elements that interact with each other via repulsive, random, and dissipative forces [181]. Compared to BD, DPD is better for simulating a cell-like structure, because it explicitly accounts for a viscous medium using coarse-grained fluid particles. Explicit consideration of the viscous medium enables DPD to describe the flow of cytoplasm induced by cell deformation, which cannot be done in BD easily. In one study, a cell-scale model was developed with nuclear envelope, chromatin, cytoskeleton, and membrane (Fig. 8(d)) [182]. As in the model mentioned above, the nuclear envelope and membrane were modeled as triangular meshes comprised of DPD particles connected by springs. The cytoskeletal polymers and the chromatin were modeled as linear structures consisting of serially connected DPD particles. Cross-links formed between the cytoskeletal polymers are represented by springs. By simulating micropipette aspiration and microfluidic devices, the authors showed that properties of the cytoskeleton and nucleus and cell viscosity play a crucial role for a cell mechanical behavior. With stiffer cytoskeleton and nucleus and higher cell viscosity, it took more time for the cell-like structure to pass through size-limiting obstacles (Fig. 8(e)).

The discrete models introduced above tend to have high computational cost compared to continuum models. Thus, as explained above, simplification of structures and dynamics of cellular structures to some extent is inevitable. For example, heterogeneous spatial distributions of cytoskeletal structures in cells were neglected in many of the previous models. In addition, the rapid turnover of F-actins, force-dependent unbinding of cross-linking proteins, and myosin activities have not been incorporated properly in previous models. To capture realistic rheological behaviors and properties of cells, discrete cell-scale models need to account for these features of cells in more detail. Considering the significant enhancement of computing powers and parallel processing schemes, it is anticipated that discrete models will gradually evolve over time in such a direction.

To overcome the limitations of both discrete and continuum models, researchers have attempted to develop hybrid models that combine continuum and discrete models. For example, in one study, FEM models of cytoplasm, cortex, and nucleus were combined with discrete models of F-actins and MTs [183]. By simulating cell indentation induced by a spherical tip of AFM, the authors showed that cortex and MT are major contributors to cell incompressibility. Although these attempts are novel and meaningful, several issues need to be resolved to simulate the mechanical properties and behaviors of cells in a rigorous manner.

6 Summary and Conclusions

In this review article, we reviewed the nonlinear elastic and inelastic mechanical properties of cells. First, a brief summary about critical regulators of cell mechanical properties—the cytoskeleton and the nucleus—was provided, and several rheological measurement techniques that have been used for cell mechanics were delineated. Then, we introduced representative nonlinear elastic and inelastic properties of cells—stiffening, superelasticity, viscoelasticity, viscoplasticity, and poroelasticity—and explained how the mechanical and dynamic properties of the cytoskeleton and the cell nucleus potentially affect those cell mechanical properties. Finally, we introduced discrete and continuum models developed for studying the mechanical properties of cells.

Due to these efforts using experimental techniques and computational models, we now have a much better understanding of complicated cell mechanical properties than a few decades ago. However, there are still a few ongoing issues and controversies. First, the mechanical properties of cells often show significant variations, depending on which measurement method is used. A wide range of the mechanical properties of cells has been reported in experiments using different techniques although they intended to measure the same properties. For example, frequency-dependent shear moduli estimated by one-point passive microrheology, which used to be employed in many studies, were found to be much smaller than those measured by the bulk rheology. To avoid the underestimation issue, researchers have developed two-point passive microrheology and active microrheology as an alternative tool for measuring local properties of cells. However, in general, the origins of the large differences in the magnitudes of cell properties reported in experimental studies have not been explained well.

Second, a universal framework that can capture various aspects of cell mechanical properties does not exist yet. As explained above, diverse theories in mechanics have been adopted to explain nonlinear elastic and inelastic mechanical properties of cells. However, each theory can describe only a specific type of mechanical properties. For example, most of the poroelasticity models assume that solid parts of a material are purely linear elastic. Thus, those models may not be able to capture viscoelasticity, viscoplasticity, or superelasticity. Development of a universal theory that is flexible enough to incorporate any type of assumptions will be beneficial for a better understanding of the complicated mechanical properties of cells.

Third, using current rheological measurement techniques, it is not easy to evaluate the mechanical properties of cells embedded within ECMs. Under physiological conditions, most of the cells are surrounded by ECMs. It is well-known that cells adapt to extracellular environments and change their mechanical properties accordingly. Thus, properties of cells measured on 2D substrates or without ECMs could be quite different from those within 3D ECMs. For example, the actin cytoskeleton in cells within 3D micro-environments exhibits distinct structures and spatial distributions compared to those observed in cells on substrates, implying that they may have different mechanical properties. Nevertheless, since measurement devices cannot make direct contact to cells within 3D ECMs, the bulk mechanical properties of cells are very hard to measure. Recently, optical tweezers were employed to estimate the stiffness of breast cancer cells in ECMs, but the measured stiffness represents only local parts of cells [184]. There is still a long way to go until we clearly understand the molecular origins of cell mechanical properties. However, a synergy between continuous advances in experimental techniques and those in computational models will help us illuminate where emergent mechanical properties of cells originate.

Acknowledgment

W. J. and J. L. wrote and revised the paper and prepared all figures. T. K. supervised the writing process and revised the paper. O. C. participated in the revision process.

Funding Data

- National Institutes of Health (1R01GM126256; Funder ID: 10.13039/100000002).

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