Computational modeling of single-cell mechanics and cytoskeletal mechanobiology

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Cellular cytoskeletal mechanics plays a major role in many aspects of human health from organ development to wound healing, tissue homeostasis and cancer metastasis. We summarize the state-of-the-art techniques for mathematically modeling cellular stiffness and mechanics and the cytoskeletal components and factors that regulate them. We highlight key experiments that have assisted model parameterization and compare the advantages of different models that have been used to recapitulate these experiments. An overview of feed-forward mechanisms from signaling to cytoskeleton remodeling is provided, followed by a discussion of the rapidly growing niche of encapsulating feedback mechanisms from cytoskeletal and cell mechanics to signaling. We discuss broad areas of advancement that could accelerate research and understanding of cellular mechanobiology. A precise understanding of the molecular mechanisms that affect cell and tissue mechanics and function will underpin innovations in medical device technologies of the future. © 2017 The Authors. *WIREs Systems Biology and Medicine* published by Wiley Periodicals, Inc.

INTRODUCTION

It is well established that cellular mechanics plays a significant role in cellular and tissue biology, from tissue and organ development to wound healing and cancer cell metastasis and migration. Significant research has been conducted to develop an integrated understanding of cellular mechanics and biology. However, as with any complex system, these

advances have only scratched the surface of a complete understanding of cellular mechanobiology.

In this on-going pursuit of a comprehensive picture of the cell, mathematical models play a dual role: (1) as *in silico* hypothesis test models—not too dissimilar from experimental animal models—to discover new mechanisms from the experimental data that would otherwise have limited power in providing insights on the integrative biology of cell behavior; (2) as re-usable and extensible repositories to integrate research findings from multiple and disparate reductionist experiments.

There are many excellent reviews that have covered all aspects of modeling cell and cytoskeletal mechanics. Lim et al.[1] provide an elegant summary of continuum-based models of the mechanical stiffness of cells. Reviews such as that of Sun et al.[2] and others[3,4] discuss models in the context of cell migration. There are also reviews of specific aspects of cell mechanics such as the cytoskeleton,[5–7] or actin

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protrusion,[8] or cell signaling in cell shape and cell motility[9] for example.

In light of these reviews and the advancements in our understanding of cell mechanics, the main aims of this review are as follows: (1) to provide an updated review of continuum and particle-based models of cell and cytoskeletal mechanics, from bulk stiffness to cytoskeletal protein contributions and from actin protrusion to cell adhesion (thus advancing on Lim et al.[1] and taking a broader perspective on cell mechanics, not just stiffness or just motility); and (2) to discuss our current understanding of cell signaling in relation to cell migration and cytoskeletal mechanics. In particular, this review will focus on how the field is moving towards understanding the feedback from mechanics to signaling. Finally, (3) we highlight key experimental results that have been or can be used to constrain and/or parameterize models of single cell and cytoskeletal mechanics. Throughout the sections we discuss drawbacks, advantages and challenges in the different modeling approaches that one can adopt to simulate different experimental observations of cell mechanics.

The structure of this review is as follows. We first provide a brief overview of the mathematical frameworks that are employed when simulating cell mechanics and associated signaling. We then discuss measurements and associated models of bulk single cell properties, followed by the contribution of the cytoskeletal network and its constituent cytoskeletal proteins. The contribution of the external surrounding environment in determining the emergent mechanical behavior of single cells is then discussed. We further review our current understanding of how biochemical signaling processes modulate cell and cytoskeletal mechanical behavior. We conclude with a discussion of what we believe are key areas of advancement that the community could target to further our understanding of cellular mechanobiology.

MATHEMATICAL TECHNIQUES FOR MODELING MECHANICS AND SIGNALING IN CELLULAR MECHANOBIOLOGY

An animal cell is a composite of soft biopolymers that is enclosed by a hydrophobic phospholipid bilayer. In *Simulation Equations for Modelling Cellular Mechanics* section we will discuss current computational approaches that are in use to capture the mechanical deformation of the cell. These approaches can be categorized into one of two broad approaches: (1) methods based on continuum approximations that discretize the cell into subregions on which Newton's equations are applied; or (2) methods that discretize the cell into collections of particles that mechanically interact with each other on a point-to-point basis following Newton's laws. The stiffness and mechanical behavior of a cell is regulated by the quantity, spatial organization and interactions of different cytoskeletal proteins that make up the cell. These parameters are tight regulated by a myriad of interconnected biochemical processes. These processes are often described and depicted as signal pathway maps. In *Simulation Equations for Modelling the Regulation of Cytoskeletal Mechanics by Signalling Pathways* section we outline the most common mathematical techniques that are used to simulate these processes in the context of cell mechanics and mechanobiology.

Simulation Equations for Modeling Cellular Mechanics

Figure 1 highlights three models published in literature that use three different techniques to describe cell mechanics at three different spatial scales. These methods will be discussed in the following sections.

Nonlinear Continuum Mechanics Simulation Equations

Continuum models seek to describe the bulk behavior of cellular materials without requiring a description of the molecular details of those materials. Nonlinear continuum mechanics theory is a natural fit for simulating bulk mechanics of cells undergoing large deformations. The theory is based on the principles of conservation of mass, conservation of linear momentum and conservation of angular momentum, described by canonical Eqs. (1), (2) and (3):

$$\int_{V_o} \rho_o dV_o = \int_V \rho dV, \tag{1}$$

$$\frac{d}{dt}\int_V \rho v_k dV = \int_S \tau_k dS + \int_V \rho b_k dV, \tag{2}$$

$$\frac{d}{dt}\int_V \epsilon_{ijk} x_j \rho v_k dV = \int_S \epsilon_{ijk} x_j \tau_k dS + \int_V \epsilon_{ijk} x_j \rho b_k dV, \tag{3}$$

where ρ_o and ρ represent the mass density in the undeformed and deformed geometries respectively; V_o and V represent the volume of the material in the undeformed and deformed geometries; v_k represents the velocity component of a moving body, τ_k is the
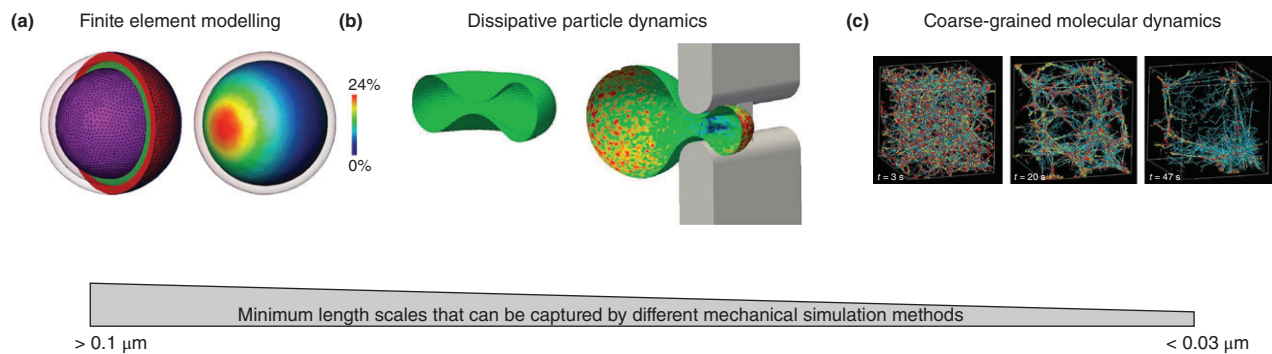
FIGURE 1 | Mechanical modelling techniques across spatial scales. Each panel shows the unloaded, initial geometry, and simulated deformation of the cell or cytoskeleton. (a) a continuum mechanics model of an actin cytoskeleton cortex (red), intermediate vimentin filaments (green) and the nucleus (Reprinted with permission from Ref 10. Copyright 2011 Elsevier Ltd); (b) a dissipative particle dynamics simulation of a red blood cell passing through a narrow slit (Reprinted with permission from Ref 11. Copyright 2016 National Academy of Sciences); (c) a coarse-grained brownian dynamics simulation of a small portion of the cell actin cytoskeleton to study the role of individual cytoskeletal proteins to the emergent mechanical behaviour of the cytoskeleton. The image shows snapshots of the cytoskeleton at different time points (Reprinted with permission from Ref 12. Copyright 2016 Nature Publishing Group).

traction stress vector component, b_k is the body force component, and t represents time. In Eq. (3) ϵ_{ijk} represents the permutation symbol in tensor notation and x_j represents the distance of each particle in the continuum from a point of reference about which the angular momentum is calculated. The consequence of Eq. (3) is that it imposes a symmetry condition on the stress tensor. Equation (1) is also converted into a constraint in simulations that assume volume is conserved. This leaves Eq. (2) as the primary nonlinear equation that must be solved computationally. The finite element method[13] is the most popular method to numerically solve these equations.

Equations (1) and (2) represent the cell as a nonlinear elastic/viscoelastic solid. The quasi-static approximation is routinely used to simulate deformations at iteratively increasing loads, thus removing the velocity and acceleration variables in the equations. In simulations of cell mechanics in contexts such as cell migration,[14] the mass of the cell is small enough to neglect inertial forces (note, however, that a recent study showed that gravity affects some larger cells that are >10 μm in diameter[15]). However, a viscous component is typically introduced to simulate intracellular drag forces induced by a viscous cytoskeleton. Another valid extension of the representation of the cell is as a poroelastic system[16–19] consisting of polymer networks whose pores are filled by a viscous fluid. This representation of the cell is backed up by structural images of the cytoskeleton,[20] confocal images of fluid flow in migrating cells[21] and more recent experimental measurements of poroelastic behavior.[22] Recently, more generalized versions of Eqs. (1) and (2), termed

virtual power equations, have been employed.[23] Virtual power equations reformulate the force equilibrium equations above in terms of velocities and power and enable the study of the mechanics of materials with evolving microstructure—such as a cell undergoing cytoskeletal remodeling.

Rather than track the individual polymers that make up the cytoskeleton, the continuum representation models regional variations in cytoskeletal distributions, which can be used to represent, for example, stiffness[10] (see Figure 1(a)). Multi-scale approaches can also be implemented whereby a constitutive equation is derived from the mechanics of a network microstructure[24] but these methods have not been validated or adopted extensively (more details in *Measurements and Models of the Bulk Mechanical Properties of Cells*).

A major advantage of the continuum mechanics approach is that many of its parameters are physically measureable (such as stiffness and viscosity). Additionally, model predictions of deformation of the entire cell can be readily compared against microscopy images of cell shape. This approach incurs relatively little computational cost when compared to particle-based methods. A disadvantage of this approach is that finite element implementations give rise to complications when simulating significant changes to cell shape; large deformations can distort element shapes and introduce numerical instabilities.

Particle-Based Mechanics Simulation Equations

In these methods, a cell is represented by a collection of points, where each particle is subject to

elastic, viscous/dissipative and stochastic forces (Figure 1(b) and (c)). These forces cause the particles to move in accordance with Newton's laws of motion:

$$m_i \frac{d^2 \boldsymbol{r}_i}{dt^2} = \left(\sum_i F_i^C + F_i^D + F_i^R \sqrt{dt} \right), \qquad (4)$$

where m_i represents the mass of each particle, $\boldsymbol{r}_i$ is the position vectors of each particle, F_i^C is the elastic, conservative force that arises between particles, F_i^D represents the dissipative forces acting on each particle and F_i^R represents the stochastic, random forces that exert influence on each particle. There are several variations to Eq. (4) that depend on the assumptions made when representing the cell. Dissipative particle dynamics (DPD)[25] treats the cell as a complex fluid where each particle can be considered as a particle of that fluid or a collection of physical particles of the fluid, depending on the spatial scale of interest. Smoothed particle hydrodynamics (SPH) represents length-scales similar to continuum models,[26] but with the advantage of being free of mesh topology constraints. Unlike DPD, SPH is derived from the Navier–Stokes equations and therefore the parameters have direct physical meaning.

In the broad sense of the term, coarse-grained molecular dynamics (also termed Brownian dynamics) simulations[27–29] are also particle-based methods that have been successfully utilized to study the mechanical interactions between the proteins that compose the cytoskeleton.[30–32] A key difference between DPD and coarse-grained Brownian dynamics (BD) models is that the equations for DPD are more rigorously developed for the treatment of hydrodynamic properties than Brownian dynamics models.[33] Readers can delve deeper into the theory and utility of different particle-based methods by reading an excellent review by Ye et al.,[26] albeit in the context of simulations of red blood cells.

An important advantage of the particle-based methods is that points within a topology can move with more freedom—subject to mechanical equilibrium constraints—than those in mesh-based methods. Mesh-free methods like SPH are under active development[34] and it is foreseeable that these methods will be adopted routinely in the future, much like mesh-based methods such as finite elements. Coarse-grained molecular dynamics simulations also provide a sense of the microstructural organization that continuum models cannot be provided. This can also provide additional insights into nanoscale mechanical forces that act between cell and cytoskeletal components, which cannot be captured in a continuum approximation.

On the other hand, DPD and SPH require all particles to be treated as fluid particles, which may not be the most appropriate description of the cellular environment in some circumstances. The choice of DPD equation parameters, such as the dissipative and stochastic forces, and the number of computational nodes (which are difficult to experimentally constrain) for each type of fluid in the simulation significantly affect the behavior of the simulation (in addition to the computational cost).[26] This makes extrapolation of model predictions beyond the simulation's original purpose more challenging than those from classical methods such as finite element method (FEM); FEM simulation predictions of cell mechanics are only dependent on parameters such as cell stiffness once a sufficiently high density mesh has been defined. SPH is based on the Navier–Stokes equations and its parameters are not as arbitrary as those in the DPD equations. However, as the method also implicitly solves the Navier Stokes' equations, representing viscoelastic and solid properties can be challenging.

The computational cost of tracking the motion and mechanics of thousands of particles (representing molecules or groups of molecules) also preclude coarse-grained molecular dynamics simulations from simulating time scales longer than a few seconds or spatial scales larger than a few micrometers (one could consider each particle as representing a larger spatial scale as a trade-off for computational cost). Mesh-free methods such as DPD and SPH enable simulations at similar spatial scales to mesh-based methods for solving continuum-scale models, such as the FEM. The need to dynamically track the relationship between different computational nodes within a neighborhood however make these methods computationally more intensive than mesh-based, continuum methods.

Simulation Equations for Modeling the Regulation of Cytoskeletal Mechanics by Signaling Pathways

Cells undergo cytoskeletal remodeling on the order of seconds, which affect measurements of mechanical stiffness.[32,35–40] Hence, cellular mechanics must be measured and studied alongside biochemical signaling processes that regulate cytoskeletal organization. The general framework used to describe spatio-temporal biochemical signaling processes are the reaction–diffusion (RD) equations:

$$\frac{\partial c}{\partial t} = D\nabla^2 c + R(c,t), \tag{5}$$

where c is the concentration of a signaling module, D is the diffusivity of the environment in which the signaling molecules interact and $R(c,t)$ represents the list of chemical reactions that represent a signal pathway. Analogous to particle-based mechanical dynamics methods, particle-type and Markov-chain-type methods[41,42] are available for simulating signaling as well. These methods are predominantly used to simulate conditions where there are few signaling molecules in a small domain and the stochastic nature of particle interactions become important. Such methods are rarely used in the cell mechanics context because signaling molecules are typically abundant.

Intracellular fluid flow plays a crucial part in transporting cytoskeletal proteins from the leading edge to the trailing edge (and back) in migrating cells,[21,43] thus reaction advection diffusion (RAD) equations can also be used in such contexts.[14] As fluid pressure also plays a role in the temporal mechanical response of cells,[22] some models incorporate equations for fluid flow[18,44] in the mechanical equilibrium equations. The fluid velocities are coupled to the advective terms in RAD models of signaling, thus incorporating a mechanism of coupling between cellular mechanics and cellular signaling. In a later section of this review we will explore how chemical signals can further modulate cytoskeleton composition, organization and hence cellular mechanics, as well as how mechanics can influence signaling.

Model Parameterization and Validation

Estimation of model parameters is an important yet infrequently discussed challenge in computational modeling of cells. Here we compare methods for parameterization of models across mechanics and signaling.

The continuum-based solid mechanics Eqs. (1) and (2) can only be solved after prescribing the stress–strain relationship that describes the mechanical behavior of the cell. As alluded to in *Simulation Equations for Modelling Cellular Mechanics*, parameters of continuum-based models are typically measurable experimentally. The values of these parameters are usually estimated by iteratively running finite element simulations with different parameter values until a match with an experimental metric (such as a reaction force, displacement or morphology) is achieved. This approach is particularly advantageous when some of the model

parameters cannot be directly related to an experimental measure. This calibration is however only the first step. One should (when possible) validate the model and its parameters by making predictions beyond the initial calibration. For example, Zhou et al.[45] estimated parameters based on creep tests during micropipette aspiration. This parameterized model was subsequently used to predict the effects of micropipette ramp tests, which compared well to predictions. Simulation results from models that do not have this additional check[46] must be used with careful consideration.

As one increases the spatial resolution that the model represents, the parameters become more difficult to estimate and validate. Parameters for DPD, such as dissipative forces or stochastic forces cannot be measured experimentally but can be correlated to physical parameters if carefully addressed for each simulation.[47] Coarse-grained molecular dynamics simulations can suffer from a lack of experimental techniques to measure specific parameters with regards to the cross-linking proteins but can provide insights if the model simulation is validated against bulk or segment rheology experimental data.[31,48]

Fundamental measurements when modeling chemical signaling include concentration, diffusivity, and biochemical reaction rate constants. While diffusivity can be readily measured for regulators, their kinetic properties and even their concentration are significantly more difficult to quantify. Direct measurements of kinetic rate constants are not always feasible and are therefore often inferred from other experiments or estimates based on other indirect observations. Fluorescence images are routinely used to measure qualitative changes in cytoskeletal proteins within cells. Care must however be taken to account for properties of the fluorescent probe and optics if one is interested in quantifying signaling properties. Due to these difficulties, quantitative observations about spatial localization and temporal dynamics of regulators are lacking.

These challenges in parameter estimation make model validation and simulation reproducibility a challenge. While cell morphology and cytoskeletal protein distributions within the cell are relatively easy to acquire, the multitude of parameters in these multi-component models of the cell have varying degrees of uncertainty associated with them. This challenge is compounded by cell to cell variability in mechanical response as well. Parameter sensitivity analysis provides some insights into the effect of this uncertainty. More high throughput measurements of cell to cell variability and more efficient methods for

parameter sensitivity and variability analysis would help synthesize the wide variety of model parameters and results in the literature towards an integrated understanding of cell mechanics.

MEASUREMENTS AND MODELS OF THE BULK MECHANICAL PROPERTIES OF CELLS

Experimental measurements have shown that cells exhibit elastic and viscous behavior as they undergo large deformations *in vivo*.[49,50] Some early studies modeled the cell as a cytoplasmic liquid core encased in a cortical shell under tension.[1] Although cells exhibit fluid-like behavior during micropipette aspiration experiments at time scales greater than 10 seconds, these fluid-based models could not capture the elastic behavior that was observed at the onset of aspiration. There are currently three classes of

continuum-scale constitutive equations (as illustrated in Figure 2) that have been successfully employed to characterize the bulk behavior of red blood cells,[51] chondrocytes,[52] neutrophils,[18,53–55] stem cells,[56] and fibroblasts[57–59] to name a few: (1) Hyperelastic and viscoelastic solid constitutive models; (2) Soft-glassy rheology based constitutive models; (3) Poroelastic constitutive models.

Red blood cells are made up of a liquid hemoglobin center encased in a cortical cytoskeleton and lipid-bilayer membrane shell. By representing this membrane composite as a viscoelastic solid, parameters of high order hyperelastic and viscoelastic strain energy functions such as Yeoh (see Figure 2(a) and Eq. (6)) have been fitted successfully to micropipette and optical tweezer data.[51] As shown in Figure 2(a), the hyperelastic component of the in-plane membrane tension (T_s) is scaled by the shear modulus (μ), the extension ratios in the orthogonal directions of the membrane (λ_1 and λ_2). The viscous nature of the
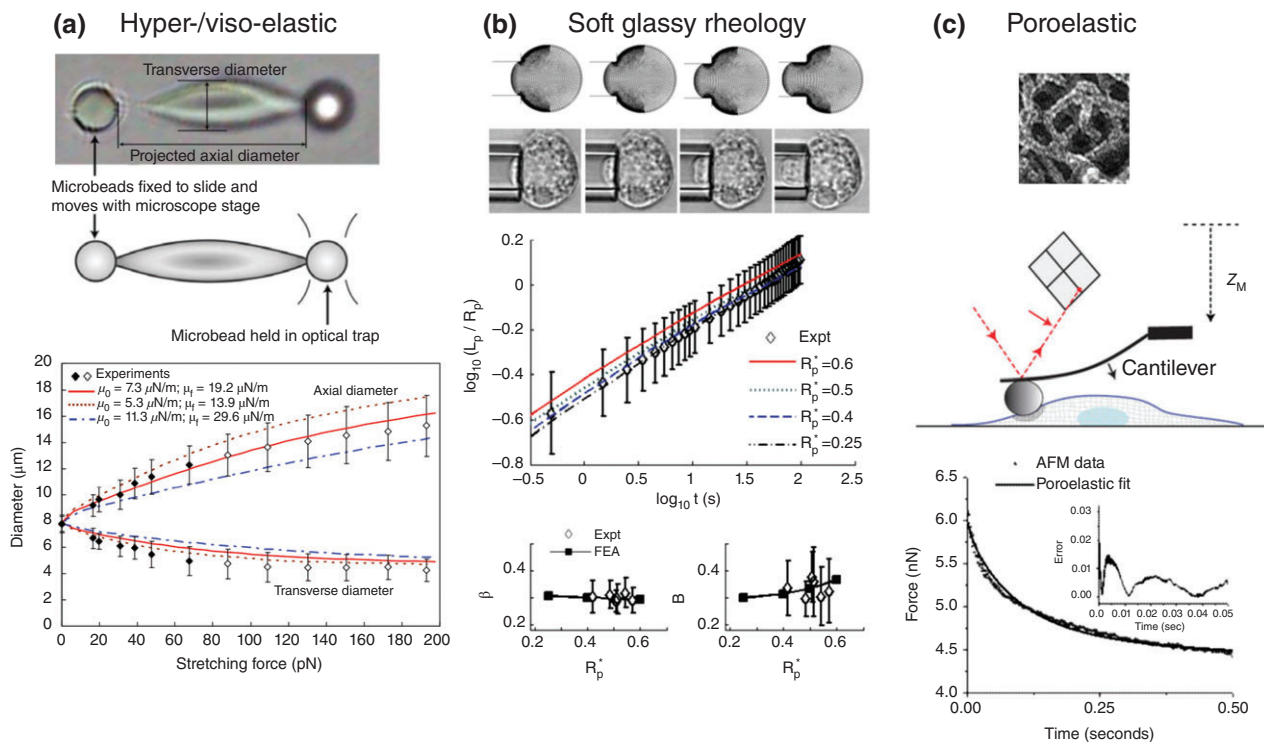

FIGURE 2 | Examples of experimental measurements and mechanical models of bulk mechanical properties of cells. (a) Mills et al.[51] used optical tweezers to perform uniaxial extension and relaxation experiments on red blood cell to parameterize hyperelastic and viscoelastic constitutive equations of its mechanical behavior (Reprinted with permission from Ref 51. Copyright 2004 Tech Science Press); (b) Zhou et al.[45] implemented a finite element model (top image in (b)) with a power-law rheology based constitutive equation to capture the long-time-range soft-glassy like response of cells as measured by creep tests using micropipette aspiration (middle of panel) (Reprinted with permission from Ref 45. Copyright 2012 Springer); (c) Herant and Dembo[18] used the poroelastic continuum mechanics equations to account for the porous nature of the cytoskeletal network (image shown in the top panel of (c), reprinted with permission from Ref 18. Copyright 2010, Elsevier Inc.) and the movement of fluid through these pores. The influence of fluid reorganization during mechanical perturbations and the poroelastic nature of the cell have been recently experimentally measured (and fitted with a poroelasitc model) by Moeenderbary et al.[22] (middle and bottom panels, reprinted with permission from Ref 22. Copyright 2013 Nature Publishing Group).

membrane is accounted for in the second term and is dependent on the strain rate and the viscous co-efficient (η). Linear viscoelastic models such as Maxwell, Kelvin or Voigt models have also been employed previously to capture the viscoelastic nature of the cytoplasm of cells which are richer in cytoskeletal proteins than red blood cells[24,52,60–62]; Gizzi et al.[63] used a transverse-isotropic hyperelastic law to model the mechanical properties of cardiac cells.

$$T_s = \frac{\mu}{2}\left(\lambda_1^2 - \lambda_1^2\right) + 2\eta\frac{\partial ln\lambda_1}{\partial t}. \tag{6}$$

While viscoelastic models can characterize both solid and fluid like behavior of cells, another class of models represent the cell as a soft-glassy material.[45,64–67] Classical spring and dashpot viscoelastic models aim to attribute different time-dependent transitions in cell mechanical behavior to different structural components of the cell, such as the membrane, cortex, and cytoplasm. While these models were found to be sufficient to capture viscoelastic behavior at time scales in the order of seconds, they failed to capture the viscoelastic behavior at extended timescales (10s and 100s of seconds).[45,66] It was shown that a power-law model with a single exponent could adequately describe viscoelastic behavior over much wider timescales.[66] Zhou et al.[45] (Figure 2(b)) developed a power-law model based on a Prony series expansion (see Eq. (7) below) and successfully replicated micropipette aspiration creep test data using a finite element model. They defined the relaxation modulus using a Prony series expansion as follows:

$$G(t) = G_0\left[1 - \sum_{i=1}^{N} g_i\left(1 - e^{-\frac{t}{\lambda_i}}\right)\right], \tag{7}$$

G_0 is the instantaneous shear modulus, and g_i and λ_i ($i = 1,2,\ldots, N$) are material constants characterizing the relaxation spectrum. Experimental time scales of upto five orders of magnitude can be adequately described by a 5-term ($N = 5$) Prony series expansion.

Although the aforementioned constitutive models have successfully reconstructed experimental conditions *in silico*, one of their drawbacks is that they are phenomenological equations, in which material parameters are chosen to ensure that the model simulation results fit the experimental data. To that extent, the parameter values quantify the bulk, emergent properties from several spatial and temporal interactions of the cell's constituents. The cell cytoskeleton is made up of a heterogeneous,

porous network of cytoskeletal proteins and its cross-linking proteins (see Figure 2(c)), immersed within a viscous fluid. A poroelastic framework (see Figure 2(c)) begins to account for how microstructural contributors give rise to emergent behavior of the cell.

As outlined in Eqs. (8)–(10) below, in the poroelastic treatment of the cell, spatially variable volume fractions of solid (θ_s, such as the cytoskeletal network) and liquid phases (θ_n water and solutes) are defined and at each point within the cell, the total volume must be conserved. The stress-equilibrium equations (Eqs. (1), (2) and (3)) are used to solve for the equilibrium shape and stress of the solid phase. Fluid flow equations (typically based on Darcy's law) are used to describe the flow of fluid through the cell volume. In these equations p represents the fluid pressure and ζ represents the drag coefficient between the solid and fluid phases. Movement of this fluid is critical to blebbing-based cell motility and cellular protrusions.[21,68] Continuum-level, two-phase or poroelastic models have been shown to successfully capture the mechanisms of cytoskeletal protein transport and intracellular pressure that these fluid movements provide.[17,18,43,55,69] Recently Moeendarbary et al.[22] experimentally tested the poroelastic nature of cells and showed that cells indeed behave like poroelastic materials at short timescales, and exhibits a power-law response at long time scales.

$$\theta_s + \theta_n = 1, \tag{8}$$

$$\nabla \cdot (\theta_n \boldsymbol{v}_n + \theta_s \boldsymbol{v}_s) = 0, \tag{9}$$

$$\nabla \cdot \sigma_n - \theta_s \nabla p + \zeta(\boldsymbol{v}_n - \boldsymbol{v}_s) = 0. \tag{10}$$

When performing any experimental measurement to interrogate the mechanical behavior of the cell and its cytoskeleton, one must be acutely aware of the conditions under which the experiments are conducted. Temperature and pH are well known to affect many properties of cells but cell stiffness is also modulated by the density of adhesions and the cell's local environment.[70–73] In addition to these factors, the effects of cytoskeletal remodeling on apparent viscoelastic behavior should also be carefully considered. Cytoskeletal remodeling has been routinely observed within 10s of seconds of initiating a micropipette aspiration experiment, for example.[32,35–38] Knight et al.[39] showed that compression loads and hydrostatic pressure can induce cytoskeletal

remodeling in chondrocytes. Song et al.[56] elegantly used fluid flow to exert controlled strains and stresses on the surfaces of stem cells and correlated stress and strain data to measures of gene transcription that mark cell lineage commitment. Therefore, models that incorporate the microstructural arrangement and dynamic reorganization of cytoskeletal proteins, when subject to mechanical loads or chemical treatment, have the potential to give more integrative insights into cellular mechanics and cytoskeletal mechanobiology than continuum-based phenomenological models discussed so far.

MEASUREMENTS AND MODELS OF THE CONTRIBUTION OF CYTOSKELETAL COMPONENTS TO BULK BEHAVIOR

The bulk mechanical behavior of the cell emerges from the organization and stiffness of the cell membrane, its interaction with its surroundings and the proteins that make up its cytoskeleton. This section summarizes the current understanding of how different cytoskeletal proteins contribute to the stiffness of the cell and how their contribution has been explicitly accounted for in computational models of cellular mechanics.

The Mechanics of Individual Filament Types

The cytoskeletal network is made up of three major classes of biopolymers: actin filaments microtubules and intermediate filaments.

Actin filaments have a double helical structure consisting of two strands that spiral around the axis of the polymer (see Figure 3(a)). The coiling occurs every 37 nm (nm) and the two strands span a width of 7 to 9 nm.

Central to understanding muscle biophysics, single actin filaments have been the subject of mechanical studies for several decades. Microneedle manipulation, x-ray and laser techniques[74–76] have been used to measure the axial spring stiffness of actin filaments to be between 11 and 44 pN/μm (piconewtons/μm) for every 1-μm length of actin filament; the reported range of values can be attributed largely to the chemical environment in which the filamentous actin was pulled.[77] The tensile strength of an actin filament—the force at which the filament breaks—is 108 pN, regardless of filament length.[78] Measurement of thermal fluctuations of actin filaments have been used to estimate the bending

stiffness, via flexural rigidity, of single actin filaments in the order of 7.5×10^{-26} Nm².[79,80] Similar methods that observe torsional movement have been employed to estimate torsional stiffness of the order of 8.5×10^{-26} Nm².[81,82]

Microtubules are hollow polymeric structures that are constituted by alpha- and beta- isoforms of a heterodimer called tubulin (see Figure 3(a)). Larger than actin filaments (55 kDa, 25 nm external diameter, 17 nm internal diameter, and 50 nm long repeating unit), microtubules also provide rigidity to the cell, and play a central role in biological phenomena such as mitosis and cargo transport across the subcellular domain.[86]

Schaap et al.[87] provide force-displacement curves from atomic force microscopy (AFM) based cantilever indentation experiments that show that microtubules exhibit a linear elastic response. They showed that microtubules have a higher spring stiffness of ~74 nN/μm, bending stiffness 3 orders of magnitude higher than actin filaments (~22×10^{23} Nm²)[80,88] and deformations become irrecoverable after indentation strains exceed 15%. Hawkins et al.[84] provide an excellent review of the experimental measurements on microtubule mechanics.

Intermediate filaments have been given their name because of their intermediary diameter (~10 nm) between actin filaments and microtubules. Unlike the other two classes of protein filaments, intermediary filaments are made from a more diverse range of proteins and have more varied functions. The wide range of intermediary filaments has been classified into six groups based on the similarity in amino acids.

Unlike microtubules and actin filaments, whose fundamental unit is a globular protein, all intermediate filaments have a distinctly organized, extended α-helical conformation that forms two-stranded coiled coils (Figure 3(a)).[83,89,90] This fundamental difference grants individual intermediate filaments the ability to withstand strains much larger than 100% and a nonlinear stress–strain relationship[91–95]—actin filaments and microtubules exhibit linear stress–strain relationships and much lower yield strains (see Figure 3(b)). Readers are directed to several excellent reviews[4,7,84,89,95,96] for further detailed measurements and models of the mechanical behavior of the three filament types and their networks. Here, we summarize some key measurements of how the different components of the cytoskeleton contribute to the emergent mechanical behavior of the composite cytoskeleton and review models that account for these distinct contributions to cytoskeleton and cell mechanics.
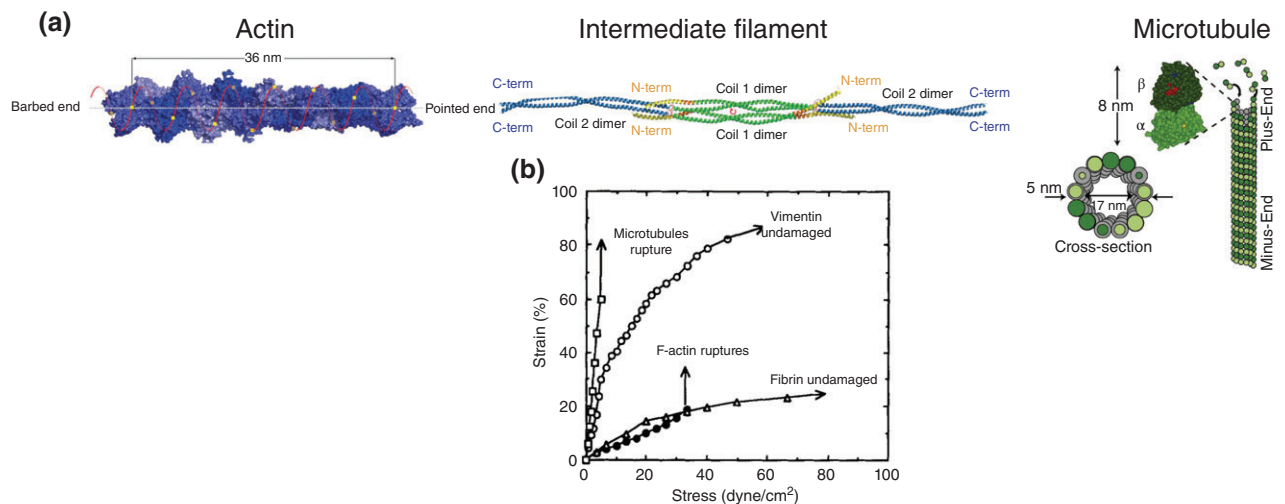
FIGURE 3 | (a) Illustrates the fundamental structure of the three groups of filaments that constitute the cytoskeletal network (Reprinted with permission from Ref 83 and Ref 84. Copyright 2016 Cold Spring Harbor Laboratory Press and Copyright 2010 Elsevier Inc., respectively); (b) Stress-strain relationship for reconstituted cytoskeletal networks purely made from one filament type (Reprinted with permission from Ref 85. Copyright 1991 Rockefeller University Press).

Cytoskeletal Network Mechanics and the Role of Cross-Linkers

Actin Cytoskeleton Mechanics

Actin and associated cross-linking proteins are the most studied components of the cell cytoskeleton due to their role in many biological functions such as skeletal and cardiac muscle force generation,[63] cell migration,[4] cell shape[97] as well as endo and exocytosis. Actin filament network stiffness increases with both filament lengths[98] and density.[99] Biochemical and mechanical signals regulate the lengths and density of the actin filaments within the cytoskeleton and create a wide variety of network morphologies, broadly categorized into branched, parallel and anti-parallel bundles. These morphologies modulate the emergent mechanical behavior of the cytoskeleton composite.[100] Families of actin cross-linking proteins are employed by the cell to affect these modifications.

Fascin, filamin-A, α-actinin, and fimbrin are some of the more commonly utilized cross-linking proteins in studies of cytoskeletal mechanics. Fimbrin and fascin are compact cross-linking proteins that create parallel-aligned actin networks that drive membrane protrusions such as filopodia.[104] On the other hand α-actinin and filamin-A form networks with more widely spaced and orthogonally aligned actin filaments.[102,105] Studies have shown that these cross-linking proteins can cooperatively enhance the mechanical behavior of the cytoskeletal network. Tseng et al.[101] showed that fibroblast cells injected with α-actinin alone exhibited a higher stiffness than a network cross-linked by fascin alone; networks that included both cross-linking proteins exhibited a higher stiffness compared to when the network was only crosslinked by one of them (see Figure 4(a)). Similar observations have been made about the stiffness of networks that contain both filamin-A and α-actinin[102] (see Figure 4(b)). Fimbrin has been shown to make a moderate contribution, compared to α-actinin, to cytoskeleton stiffness associated with the cell membrane as well[32] but its tight bundling capability has been shown to play a more critical role in generating stronger acto-myosin forces at the cortex for cytokinesis and endocytosis.[106,107]

The enhanced stiffness or force generation capacity due to the use of a combination of cross-linkers may suggest that all cross-linkers act cooperatively but different combinations of cytoskeletal proteins also produce different network organizations. Recent studies on reconstituted networks show that cross-linkers may repel other types of cross-linkers and promote cooperative binding of actin to more cross-linkers of the same kind.[105,108] Winkelman et al.[105] showed that fascin and α-actinin repelled each other and formed different actin network topologies—this was observed in the absence of any chemical signaling or local changes to the environment (such as pH). On the other hand, proteins that are similar to fascin, such as fimbrin, bind more cooperatively and in close proximity with fascin. This illustrates the fact that the combination of proteins may not just affect cell stiffness but will also affect the spatial assembly of the cytoskeleton.

Outside of pulling together actin filaments and increasing their stiffness, crosslinking proteins such
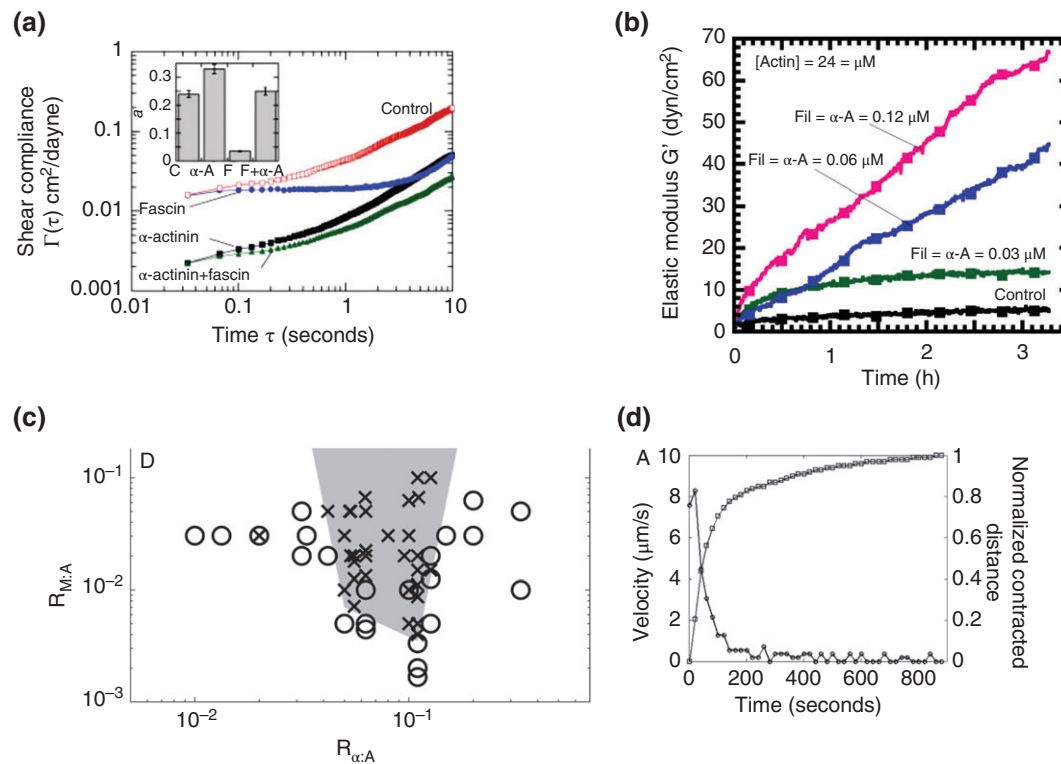
FIGURE 4 | The role of cytoskeletal proteins and their cross-linkers: (a, b) Cytoskeletal networks made from combinations of different cross-linkers exhibit different emergent mechanical behaviour (Reprinted with permission from Ref 101 and 102. Copyright 2005 Elsevier Inc. and Copyright 2009 Public Library of Science, respectively). (c) Myosin-contractility of the cytoskeletal network is ineffective at very low or very high cross-linker concentrations (Reprinted with permission from Ref 103. Copyright 2008 Elsevier Inc.). The shaded region indicates the myosin contractility feasibility. (d) Myosin exerts contractile stresses on the cytoskeletal network that reaches equilibrium after approximately 800 ms (Reprinted with permission from Ref 103. Copyright 2008 Elsevier Inc.).

as fascin, filamin, and α-actinin are passive in nature. Myosin is a more dynamic crosslinker and plays a role not only in modulating cytoskeletal stiffness, but also viscoelasticity, migration, shape changes and apoptotic processes as well.[109] Figure 4(c) and(d) show two key, bulk characteristics of acto-myosin gels[103]: (1) myosin contraction is only effective within a short, middle range of actin cross-linking protein densities (as shown in Figure 4(c)); (2) acto-myosin contractions exhibit exponentially decaying contraction velocities lasting over several 100 s of seconds before reaching a steady state as shown in Figure 4(d). Contraction velocities are concentration dependent, with a one-fold drop in myosin concentration causing an increase in the duration of contraction by an order of magnitude.

Mathematical Modeling of the Actin Cytoskeleton Network

Coarse-grained Brownian dynamics models like Figure 1(c) have captured several features of the actin cytoskeleton behavior mentioned above and have also

provided additional insights on the interactions between different cytoskeletal components that give rise to mechanical properties of actin cytoskeleton.[12,31,110,111] Actin cross-linking proteins that promote orthogonal linkages between actin filaments contribute more to increasing stiffness than cross-links that form parallel bundles.[31] The energy from mechanical loads is absorbed within the network through actin filament bending, cross-link protein bending and stretching of the proteins at much higher strains. More than protein unfolding, actin cross-link protein unbinding regulates the cytoskeletal behavior during strain hardening and stress relaxation.[110]

Myosin also contributes to the emergent mechanical stiffness of the cell but its procession over the actin filaments that it binds to brings about a rich set of characteristics to the mechanical response of cells. Borau et al.[112] showed that when a small cytoskeletal network with myosin motors is formed within a stiff surrounding, myosin motors exert maximal contractile force thus making the cytoskeleton stiffer. At lower levels of boundary stiffness, myosin

motors do not reach maximal force as they are stalled by cross-linking proteins at a sub-maximal stress, making the cytoskeleton softer.

Luo et al.[32] performed a comprehensive, single cell-scale Brownian dynamics simulation coupled with an extensive experimental dataset of cytoskeletal distributions to investigate the mechanisms that govern cytoskeletal reorganization during micropipette aspiration. Localization of cytoskeletal proteins during micropipette aspiration was assessed using live imaging of GFP-fusion proteins. They found that myosin II accumulation occurred at the pipetted tip of the membrane along with some passive cross-linkers such as α-actinin but some cross-linkers such as filamin A accumulated at the neck region instead of the pipetted tip. Their model simulations suggest different cross-linking proteins are more responsive to specific modes of deformation. For example, cross-linking proteins such as α-actinin respond more to dilatational stresses, while filamin-A accumulated in regions of high shear stress. Luo et al.'s model simulation predictions of the localization of different cross-linkers matched with their experimental measurements as well.

Mechanics of Microtubules and Intermediate Filament Networks

The mechanical and structural effects of crosslinking microtubules or intermediate filament networks have been explored less than the actin cytoskeletal network.

Microtubule networks can be crosslinked by a class of proteins called microtubule-associated proteins (MAPs), such as Tau, MAP1, MAP1a, MAP1b, and MAP4.[113] These proteins stabilize and promote the assembly of microtubules and have largely been reported to increase the stiffness of microtubule networks.[84,114] In vitro studies have shown that reconstituted microtubule networks made from purified microtubules alone form homogeneous networks, whereas more heterogeneous networks are formed when crosslinkers are added.[114–116] The networks show a nonlinear stress–strain relationship, with an initial nonlinear stiffening at low strains/forces followed by softening at high forces due to crosslinker unbinding[115,116] (see Figure 5(a)).

Specific crosslinking proteins do not seem to be needed for intermediate filament network formation unlike the actin crosslinking proteins discussed in *Actin Cytoskeleton Mechanics*; divalent cations such as magnesium (Mg^{2+}), calcium (Ca^{2+}), and zinc (Zn^{2+}) can act as crosslinkers of intermediate filament networks[95,117] (see Figure 5(b)). Some reports also suggest that subunits of the intermediate filaments may act as binding sites for other neighboring filaments.[95]

Mathematical Models of Microtubules and Intermediate Filament Networks

Microtubules can be well represented as slender beams or composite fiber reinforcements due to their
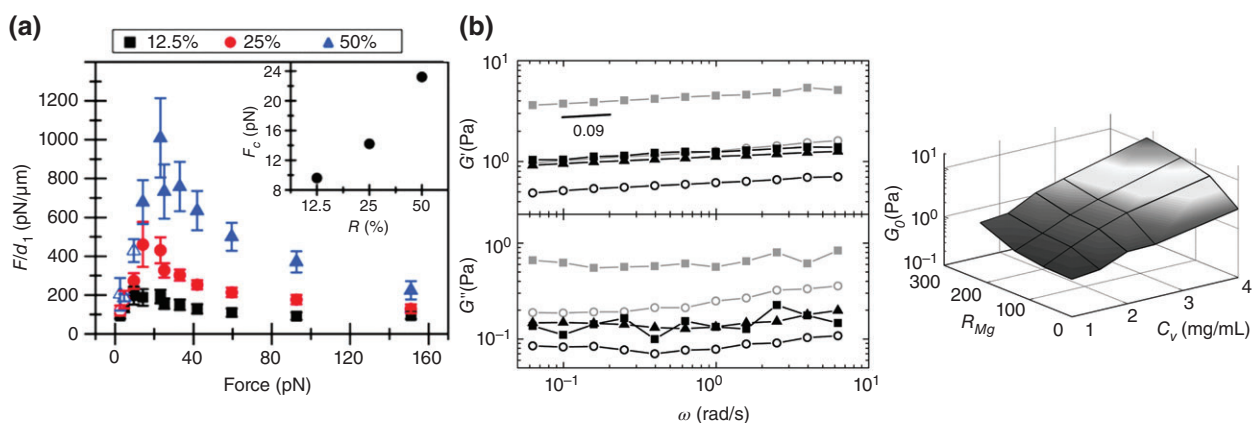

FIGURE 5 | Stiffness of microtubule and intermediate filament networks as functions of cross-linker density. (a) Plot of microtubule network stiffness, calculated by dividing force F by the initial elastic jump distance d1, with three different densities of cross-linkers (12.5%, 25% and 50%). The plot shows network stiffening at low forces and softening at high forces. Inset shows the crossover force Fc from stiffening to softening reginmes increases with crosslinking density (Reprinted with permissions from Ref 115. Copyright 2012 RSC Publishing); (b) Plots of stiffness of vimentin intermediate filament networks cross-linked by different divalent ions. Plot on the left shows linear viscoelastic moduli in the absence of divalent ions (with vimentin concentration c_v = 1 mg/ml, black open circles; c_v = 2.5 mg/ml, gray open circles) and in the presence of Mg^{2+} (with molar ratio, R_{Mg} = 215; c_v = 1 mg/ml black squares; c_v = 2.5 mg/ml, gray squares) and Ca^{2+} (with molar ratio, R_{Ca} = 215; c_v = 1 mg/ml, black triangles). G′ dominates over G″ and exhibits weak power-law scaling with frequency, ω, having an exponent of 0.09. The networks in the presence of divalent ions are two to four times stiffer. Plot on the right summarizes the dependence of the elastic response, G_o, of vimentin networks on R_{Mg} and c_v. (Reprinted with permissions from Ref 118. Copyright 2010 Elsevier Inc.).

very long persistence lengths, bending rigidity and structural dimensions relative to the rest of the cell. There are several continuum-elasticity-based computational models of individual microtubules that have been used to study microtubule mechanical properties,[119] the buckling of microtubules due to deformations in the surrounding elastic matrix[120] and vibration and wave-propagation.[121] Molecular dynamics and coarse-grain techniques have also been used to study microtubule growth and instability.[122] Despite the fact that microtubules are known to play a role in cell morphogenesis and cell migration mechanics,[123] very few mechanical models of microtubule networks have been developed. Allain et al.[124] used particles to define a discretized connected microtubule network and simulated their mechanical dynamics using Newtonian mechanics. Euler-Bernouilli theory was used to derive an elastic force that is applied at each node to account for the rigidity of microtubules. Buxton et al.[125] used a similar approach and although these models were mostly qualitative in their insights, they demonstrated the interplay between microtubule growth kinetics, network morphology and mechanics.

The ability of intermediate filament networks to sustain large deformations and self-assemble into heterogeneous networks makes IF networks ideal for continuum mechanics treatment.[10] Similar to microtubule network models, there are few models of intermediate filament networks. What models are present are mostly qualitative in their insights, using energy potentials to formulate the mechanical dynamics.[126]

New Frontiers for Computational Modeling of Cytoskeletal Mechanics

As outlined above, significant strides have been made to understand the effect of the different constituents of the cytoskeleton on the mechanical behavior of cells and reconstituted networks using a reductionist approach, more so of the actin cytoskeleton than the remaining two groups. Here we outline several additional aspects of mathematical modeling that are open for development and need attention in order to gain an integrative understanding of the emergent mechanical behavior of cells.

Coarse-grained models of the actin cytoskeleton, while providing a framework to examine emergent behavior from the constituent parts, are computationally expensive for extensive use. Extending the Brownian dynamics framework that was used to model the membrane-bound skeleton,[32] to simulate the mechanics of the cytoskeleton of the entire cell volume, is computationally intractable. To this end, homogenization of the emergent properties of cytoskeletal networks in a continuum framework is much sought after. Studies by groups such as Muller et al.,[111] Kwon et al.,[127] and Fallqvist et al.[128] developed microstructure based constitutive equations of the stiffness of actin cytoskeletal networks.

Fallqvist et al.[128] formulated a thermodynamically consistent constitutive law that incorporates anisotropic properties and is based on a strain energy function that can be incorporated into the continuum-based models. Starting with a 1-dimensional (1D) strain energy function, W_{1D} for an individual filament within a network:

$$W_{1D} = W_f + W_s,$$

$$W_f = \frac{\mu_f}{4}\left(\lambda_f - 1\right)^2, W_s = \frac{\mu_s}{2}\int_1^{\lambda_s} f_s\left(\lambda_s'\right)d\lambda_s', \qquad (11)$$

W_f and W_s are strain energies due to filament bending and stretching, respectively. μ_f and μ_s are stiffness parameters associated with filament bending and stretching. λ_f and λ_s are the magnitudes of bending and stretching of individual filaments. f_s is a 1D constitutive force-displacement relationship—to be chosen—to describe the mechanical behavior of individual filaments within the network. The 3D strain energy function contains isochoric (W_{isoch}) and volumetric (W_{vol}) components.

$$W = W_{\text{isoch}} + W_{\text{vol}}, W_{\text{isoch}} = \omega_a W_a + \omega_b W_b, \qquad (12)$$

W_a and W_b are isochoric strain energies due to networks a and b and ω_a and ω_b are 'damage' variables that account for network filament rupture and debonding. One can represent anisotropic properties of a network by defining an orientation distribution function ρ. This function describes the distribution of filament directions in the network. Thus, the isochoric strain energy within a unit spherical domain, Ω, for a given network can be defined as:

$$W_a = \frac{1}{4\pi}\int \rho W_{1D}d\Omega \qquad (13)$$

This model was validated against existing rheological experiments on reconstituted cytoskeletal networks and found to be in good agreement with the data. Some aspects such as the effects of cross-linker unbinding and binding on rheology could not be captured. With our understanding of cytoskeletal

composition, organization and mechanics still incomplete, both particle-based molecular dynamics and continuum-based homogenized models of the cytoskeleton will need to develop in parallel for some time yet before any one model is adopted widely.

While there have been many studies that investigated the stiffness of intermediate filaments and microtubules,[95,96,123] more experimental and computational studies are needed to understand the mechanisms that govern microtubule and intermediate filament network dynamics (as mentioned in *Mechanics of Microtubules and Intermediate Filament Networks* and *Mathematical Models of Microtubules and Intermediate Filament Networks*). Their contributions to the emergent behavior of the cell cytoskeleton have also only gained increased traction recently. Gladlin et al.[10] developed a 3-layered linear elastic shell model of a cell nucleus, a perinuclear intermediate filament network region and a cortical actin network region (see Figure 1(a)) and showed using the model and experiments that drugs that targeted perinuclear vimentin intermediate filaments caused a change to cytoskeletal network density. Mendez et al.[129] showed that vimentin intermediate filaments contribute more to the increased stiffness of cells as their local environment becomes stiffer. Rajthe et al.[130] showed that loss of microtubule integrity caused intermediate filaments to collapse near the nucleus. Guo et al.[131] showed that intermediate filaments could be found in greater abundance within the cytoplasm than within the membrane-bound cortex meshwork. More research into how and when the roles of these two classes of proteins become significant to cell stiffness and biology is a widely untapped opportunity.

Spatial heterogeneity of cell stiffness has also not been incorporated with sufficient accuracy into computational models. Most models have traditionally assumed that most of the relevant mechanics of the cell occurs at the boundary because the cell membrane and membrane associated cortical cytoskeleton is stiffer than the viscous interior. Considering the potential segregation of the highly stretchable intermediate filaments from the stiffer cortical actin, the spatial heterogeneity may in fact be very important when modeling large deformations of the cell. Incorporating the spatial heterogeneity is also important to develop an understanding of how the nucleus senses mechanical forces to send signals for remodeling.[132] New advances in microrheology[133] and non-invasive Brillouin microscopy[134] will provide richer datasets to advance this aspect of cell mechanics in future.

Measurements and Models of Protrusion Forces Due to Actin Polymerization, Depolymerization and Disassembly

Another important contributor to cytoskeletal mechanics and cell stiffness modulation is actin (de) polymerization. It plays a significant role in cell migration along with numerous other processes and the molecules that regulate its dynamics have been studied extensively in the literature.[135–137] We provide a summary of these details within the signaling section of this review. Here, we discuss some key experiments and models that have been used to study the effect of actin polymerization and disassembly on single cell and cytoskeletal mechanics.

Actin polymerization against a membrane produces a force that pushes the membrane forward. Three experimental studies[138–140] provide elegant measurements of the forces generated by actin polymerization. Parekh et al. promoted actin growth within an actin gel against an AFM cantilever tip (see Figure 6(a)) and used the deflection of the beam to measure the force during actin polymerization. Similarly, Marcy et al.[140] promoted growth within an actin gel against an optical trap. Such experiments provide data similar to that shown in Figure 6(b) that relate the force measured at the optical trap or cantilever and the rate of growth of the actin gel.

Models of actin protrusion and migration vary in the spatial scales they represent. Models that explore the role of individual filaments at the leading edge[8,141,142] simulate the attachment and detachment of collections of 1-D actin spring-like filaments against the cell membrane, each of which generates a pulling or pushing force against the leading edge (see Figure 6(c)). Models at this scale are termed mesoscopic. Kim et al.[143] took a macroscopic approach and used a growth-tensor continuum mechanics formulation to simulate cell growth[144] at the leading edge due to actin polymerization. Namely, the mechanical strain is made up of two components: (1) a strain due to mechanical forces that are exerted on the cell and cytoskeleton (these can be due to external forces on the cell, or internal forces due to acto-myosin contractions), which is modeled using a stress–strain constitutive relationship (such as those discussed in previous sections); (2) and a strain due to growth of a region, which in this context arises from actin polymerization (see Figure 6(c)).

Two mechanisms are thought to play major roles in reversing protrusions and local expansions of the actin network: (1) actin network disassembly by ADF/cofilin mediated actin filament severing; (2) and myosin-induced actin network disassembly.[146]
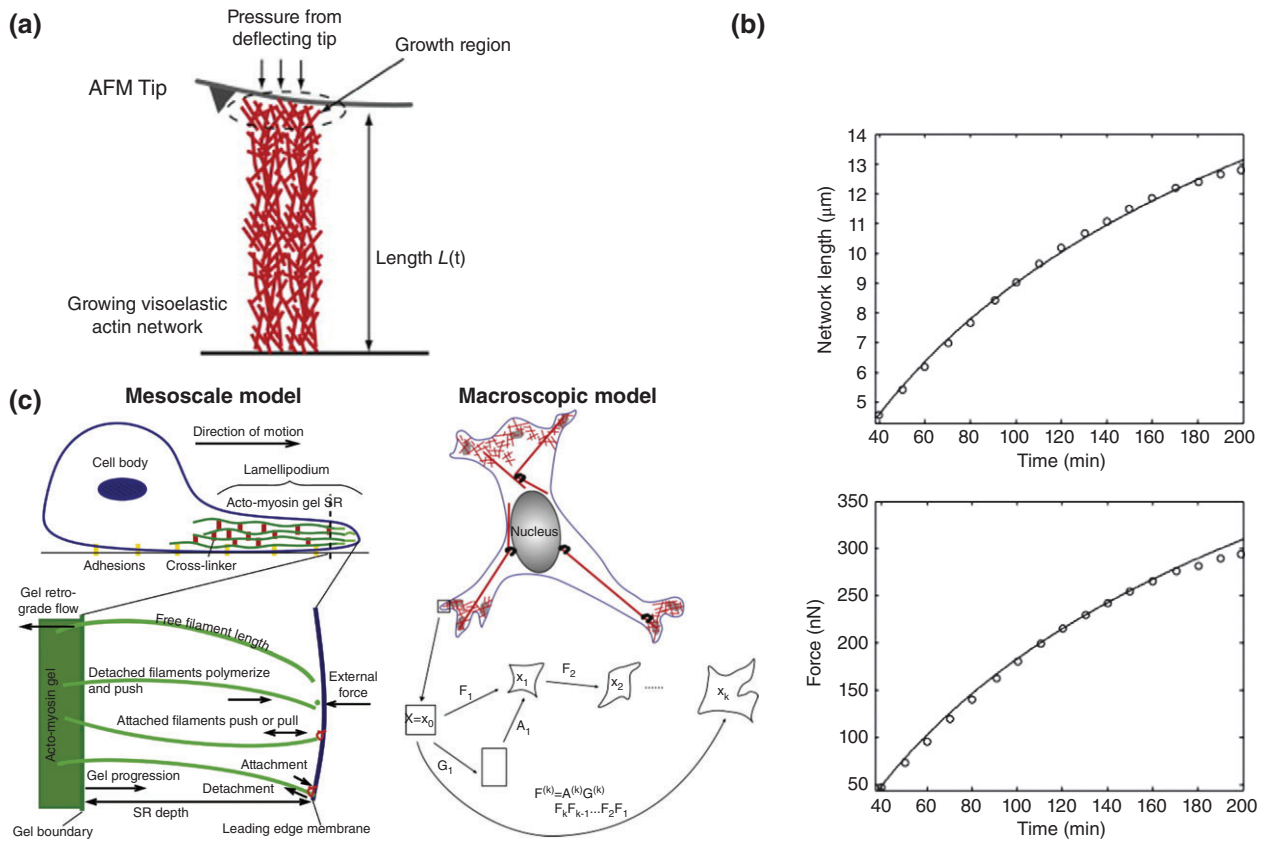
FIGURE 6 | Measuring and modelling actin polymerisation mechanics. (a) A method of measuring the force produced by actin polymerisation where polymerisation is promoted near an AFM tip. The deflection of the tip and imaging the growing actin gel provide sufficient data to parameterise a model of actin gel growth from actin polymerisation. (b) Plots that relate growth rates and protrusion force that can be used to parameterise a model of actin protrusion. Circles represent the experimental data and the curves are predictions from a macroscopic growth-tensor model of the leading edge. (c) Schematic of mesoscopic and macroscopic concepts of the interpretation of the force generation due to actin polymerisation. In the mesoscopic model, actin filaments push and pull the membrane depending on their kinetics. One approach to macroscopic modelling of the forces due to actin polymerisation is to treat the polymerisation as a cell growth strain, G, that is applied on top of any mechanical strains due to external loads. ((a), (b) and macroscopic model of (c) reprinted with permission from Ref 143. Copyright 2009 Elsevier Inc. Mescoscale schematic in (c) reprinted with permission from Ref 145. Copyright 2012 Elsevier Inc.)

ADF/cofilin is a family of proteins that promote actin network disassembly by local binding to actin filaments and consequently reducing the persistence length of actin filaments fivefold and increasing their bending and twisting compliance.[147] This is thought to promote severing of filaments at the boundaries between compliant, ADF/cofilin-decorated regions and stiffer, ADF/cofilin-bare regions. On the other hand, acto-myosin contractions induce stresses onto the actin filament network, which are thought to buckle and fragment the filaments.[146,148]

Fragmentation and severing causes local changes to the actin cytoskeletal network density and its viscoelastic properties, while increasing the availability of actin monomers for reorganization of the cytoskeleton.[146] Jung et al.[149] used a coarse-grained

Brownian dynamics simulation of the actin cytoskeleton to demonstrate stress-relaxation due to actin network buckling and fragmentation. Zhu et al.[142] incorporated network disassembly in their node-and-spring model of the cell cytoskeleton by actively removing nodes and springs at a set disassembly rate; this effectively decreased network density and local network stiffness. These effects could be accounted for in the viscous and elastic parameters of coarser-grained, continuum-based models of the cell cytoskeleton.

The precise relationship between disassembly and cytoskeletal force and mechanics is yet to be fully realized. Rates of disassembly may be affected by two-way feedback mechanisms between the local network morphology, the state of acto-myosin contractions and density of cross-linkers.[146] As with all

other aspects of cytoskeletal behavior that we have discussed in this section, unraveling the interplay between the spatio-temporal forces and the dynamics of different aspects of the cytoskeletal is a major challenge. However, when reviewing the advances that have been made so far, these new advancements are sure to follow.

MEASUREMENTS AND MODELS OF THE CONTRIBUTION OF ADHESIONS AND THE SURROUNDING ENVIRONMENT ON SINGLE CELL AND CYTOSKELETAL MECHANICS

Every computational simulation must prescribe not just what happens in the interior of the cell but also the dynamics of the boundary. Mathematically, this involves prescribing Dirchlet or von Neumann boundary conditions for a computationally tractable simulation result. From this perspective adhesions to the extracellular matrix (ECM) or neighboring cells are where boundary tractions or displacement boundary conditions are specified. Proper prescription of these boundary conditions is not only important for tractable simulations but they are also critical to deciphering the biological mechanisms that are regulated through adhesions. Many studies have shown that mechanical interactions between cells and the ECM or other cells, via adhesions, play a fundamental role in biological processes such as migration, growth and morphogenesis.[43,150–154] Cell–ECM adhesions are formed by a major class of adhesion proteins called integrin receptors and cell–cell adhesions are formed by other classes of adhesion proteins such as cadherins and nectins. While biochemical regulation of adhesion formation and degradation is discussed in *Adhesion Regulation*, here we review measurements and models of the mechanics at the interface of the local environment, adhesions and the cell.

Traction force microscopy is the method of choice for tracking cell–ECM interactions.[50,155–157] The method typically involves tracking the movement of beads or extracellular matrix fibers (or liquid droplets between cell-to-cell adhesions[50]), near the vicinity of the cell when it generates contractile forces against adhesions. Figure 7(a) shows results of tracking beads in a ECM as a cell contracts and migrates through this 3D environment from Legant et al.[157] The bead displacements can be post-processed into strains, which show that cells generate significantly large deformations against the ECM (~30%). These bead displacements are further processed into

traction stress/forces via a constitutive model. This invariably requires assumptions of the mechanical properties of the cell or ECM gel, typically as a linear elastic solid or the solution of the inverse problem whereby the traction strain data are used with a finite element model of the cell to estimate traction forces. Readers are directed to an excellent review by Schwarz et al.[156] on traction force microscopy for further details on the different methods.

Using these approaches, several characteristics about the relationship between ECM density, adhesion size and traction forces have been elucidated. The local orientation of the ECM fibers has been implicated in guiding cell migration by restricting cell protrusions that come in contact with matrix fibers.[158] Traction forces generated by the actomyosin contractions tend to increase in magnitude due to the cascading effect of increased ECM fiber density, causing an increased stiffness in the ECM and increased density of adhesions (and size of adhesion complexes) between cell and ECM. Coarse-grained Brownian dynamics simulations of the actin cytoskeleton also suggest that the increased actomyosin contractions are due to increased processivity of myosin over actin filaments.[112] Passive cross-linkers also play a role at the cell–ECM interface. Both filamin-A and α-actinin have been experimentally observed to trigger maturation of adhesions by linking actin to integrin proteins.[153,159]

Several mesoscopic and macroscopic models of cell-ECM interactions have been used to study the interactions between the cell, adhesions and ECM. Schlutter et al.[160] explored the role of ECM architecture remodeling by explicitly modeling collagen fibers as cylindrical rods that were oriented in ECM-like configurations. The cell was modeled as a point mass whose mechanical cues were regulated by the intracellular acto-myosin contractions and the external orientation and deformation of the fibers (see Figure 7(b)). The force balance between the cell and the ECM was modeled as:

$$F_{\text{drag}} = \sum_f F_{ff} + f_j(t), \qquad (14)$$

where F_{drag} is a macroscopic drag force on the cell that is balanced by the sum of the conservative forces between the cell and every ECM fiber, f, and a noise term $f_j(t)$ to account for other factors. The interaction forces between the cell and ECM fibers were weighted towards favoring cell migration along its direction of polarity. The re-orientation of each fiber was computed using the concept that the fibers were levers that are rotated by the moment generated by

the cellular contraction. Using this formulation, the study showed that ECM fiber re-orientation reduced the persistence of the cell to move in a direction. As the model equations suggest, these observations are limited by the point-wise representation of the cell and the simplified interaction force between cell and ECM.

Zhu et al.[142] represented the cell acto-myosin network and the ECM network as a collection of nodes and springs. This allows for incorporation of additional details such as protrusion forces and adhesion kinetics. Such spatially extended models[163,164] enable more detailed studies of the interactions between the ECM fibers and the cell intracellular machinery.

A continuum approach to modeling cell–ECM interactions is to incorporate the mechanical influence of the ECM into constitutive models of the whole cell. For example Borau et al.[161] incorporated the influence of ECM stiffness on acto-myosin contractility through a spring model (see Figure 7(c)). In this model, the substrate stiffness is represented by a single passive spring stiffness. The cell is modeled as a material with two springs in parallel: (1) K_{pas} representing the passive mechanical properties of the cell that the microtubules, intermediate filaments and membrane contribute to and; (2) K_{act} representing the stiffness of actin filaments which are in series with the acto-myosin complex, AM. The model also incorporated the time-dependent response of myosin motors,[112] thus incorporating a viscoelastic component to the cell's response. While simple and more phenomenological in its detail, the model was successfully able to capture the dynamics of acto-myosin contractile stiffness due to changes in ECM stiffness.
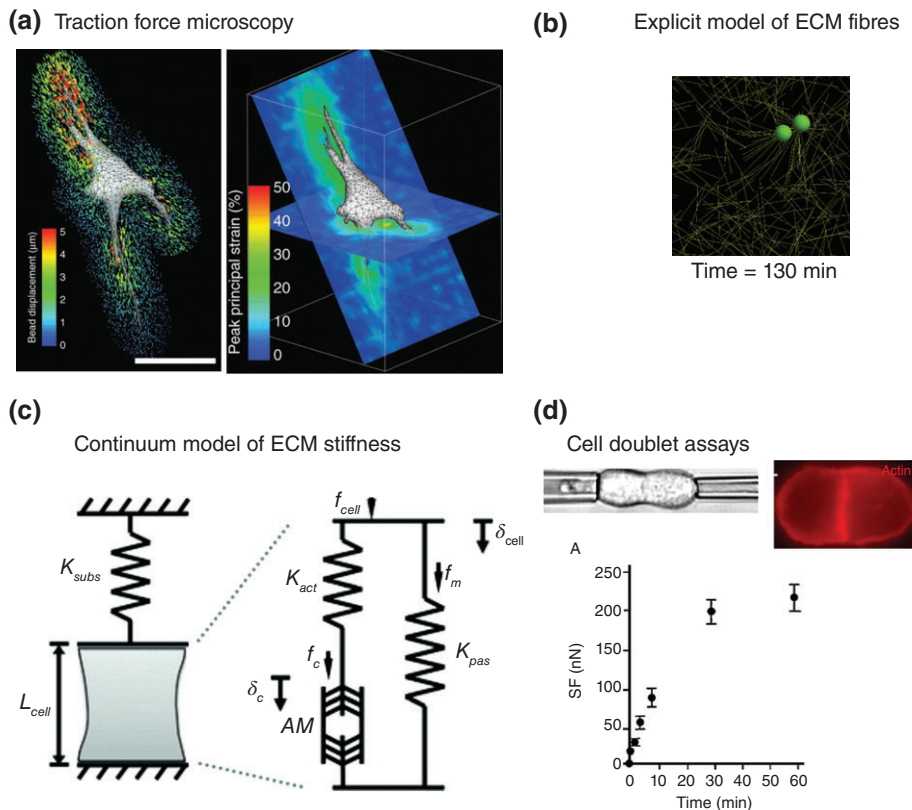

FIGURE 7 | Measuring and modelling cell-ECM tractions. (a) Measurement of bead displacements (left) and calculated peak strains (right) of a cell migrating inside a 3D ECM environment (Reprinted with permission from Ref 157. Copyright 2010 Nature Publishing Group Inc.). Scale bar represents 50 μm. (b) A simulation from a model of cell-ECM interactions in which the ECM fibres are explicitly modelled as cylindrical segments (Reprinted with permission from Ref 160. Copyright 2012 Elsevier Inc.). The two spheres represent cells that are migrating through the ECM, remodelling the local ECM matrix in the process. (c) A conceptual diagram of a continuum model of a cell and its mechanical interaction with the ECM. The model incorporates the role of ECM stiffness in the traction forces felt at the cell adhesions (Reprinted with permission from Ref 161. Copyright 2013 Springer). (d) Cell doublet assays involve pulling two adhered cells apart using micropipette aspiration The fluorescence image shows cortical actin accumulation at the cell-cell junction. The plot shows the temporal change in separation force as the cells are pulled apart (Reprinted with permission from Ref 162. Copyright 2004 Rockefeller University Press).

While we have discussed the mechanical interactions between the cell and ECM fibers above, interstitial fluid flow has also been demonstrated to trigger cytoskeletal remodeling.[56,165,166] Song et al.[56,165] prescribed fluid flow through a tissue scaffold and applied fluid dynamics analysis techniques to compute micro-scale particle image velocimetry (micro-PIV) data. They also tracked the displacement of beads bound to cell membrane glycoproteins to compute a corresponding strain distribution for the prescribed fluid flow and tissue scaffold geometry. A combined computational fluid dynamics model of the fluid flow with measured strains and micro-PIV data was used to estimate the stress distributions over the cell surface and to track the changing cell shape and stiffness. Although only a linear elastic model of the cell was used in these studies, they demonstrate the rich data that can be collected and analyzed using fluid-induced cytoskeletal or cellular remodeling.

The forces in cell to cell interactions are transmitted through another class of adhesion receptors different to integrins, the most well-studied being cadherins. These forces are of significant interest due to their implications in tissue development and tissue homeostasis. An in-depth review of the measurements and models of cell–cell adhesions in tissue constructs are beyond the scope of this review on single-cell mechanobiology and we encourage readers to refer to many excellent reviews of this field for more details.[167–171] Nevertheless, these same forces affect the cytoskeleton and the mechanics of the individual cells that make up the tissue. Therefore, we present a brief summary of some pertinent information that can be used as a starting point for any computational study of the effect of cell–cell adhesions on single-cell mechanobiology.

The actin cytoskeleton is connected to the cell–cell cadherin adhesions *via* a class of proteins called catenins. Acto-myosin contractions play a critical role in immobilizing cadherin proteins during adhesion formation[172] and the actin cytoskeleton transmits forces through these cadherin-based adhesions to neighboring cells,[168] which subsequently activate a wide range of signaling pathways that regulate cell shape, tissue morphogenesis and homeostasis. Micropipette based cell-doublet experiments[162,172](see Figure 7(d)), liquid droplet assays,[49] and laser ablation microscopy[173] are some of the tools that can be used to estimate these forces. As an example, Campas et al.[49] estimated acto-myosin-driven intercellular stresses at the cell–cell interface in the order of 1 nN/µm^2 within embryonic tissues. Bambardekar et al.[174] used optical trapping to pull on cell–cell adhesions and estimated forces in the order of

100 pN. Many computational modeling efforts are also underway to quantify relationships between cell–cell adhesions, tissue homeostasis and morphogenesis,[167,174–176] which can again be classified into particle-based or continuum-based models. For example, Bambardekar et al.[174] proposed and validated a continuum viscoelastic constitutive equation to describe the force dynamics at epithelial cell–cell adhesions, while Coburn et al.[176] used a particle-based method, known as the vertex-based method, to simulate the acto-myosin contractile forces that transmit across the tissue through the cell–cell adhesions. Tightly coupled with experimental data, these models have helped to estimate tensions at the cell–cell adhesion interface[167,174,175] and could therefore be adopted for studies on the role of cell–cell adhesions on single-cell mechanobiology. Thanks to these many advances in measuring and modeling cell–cell adhesions and their acto-myosin generated forces, research in this field is now focused on uncovering how these mechanical forces regulate cellular and tissue remodeling.

THE ROLE OF SIGNAL TRANSDUCTION PATHWAYS IN MODULATING CYTOSKELETAL MECHANICS

It is well established that numerous cellular processes ranging from cell motility[177] to cell wound healing[178] require coordinated action of acto-myosin growth, contraction, and adhesion formation. For example, from a bulk perspective, motility requires three essential events, (1) protrusion of the cell front, (2) retraction of the cell rear, and (3) engagement of adhesions that serve as a molecular clutch to transmit these forces to the substrate. How though are these events coordinated? The historical view is that feed-forward regulatory pathways that convert signaling information into action coordinate where and when these processes take place (Figure 8(a)). More recent observations and modeling however have suggested that complex feedbacks between cytoskeletal remodeling and its own regulation are at play (Figure 8(b)–(e)). Here we review how biochemical and biophysical events regulate cytoskeletal mechanics. This discussion will differ in nature from that in the preceding sections for two reasons. First, quantitative data of spatially localized signaling molecules (e.g., micromolar concentrations of Rac in a specific cell location) is not available. Second, mathematical models encoding signaling dynamics are predominantly described in the language of Reaction Diffusion Equations with
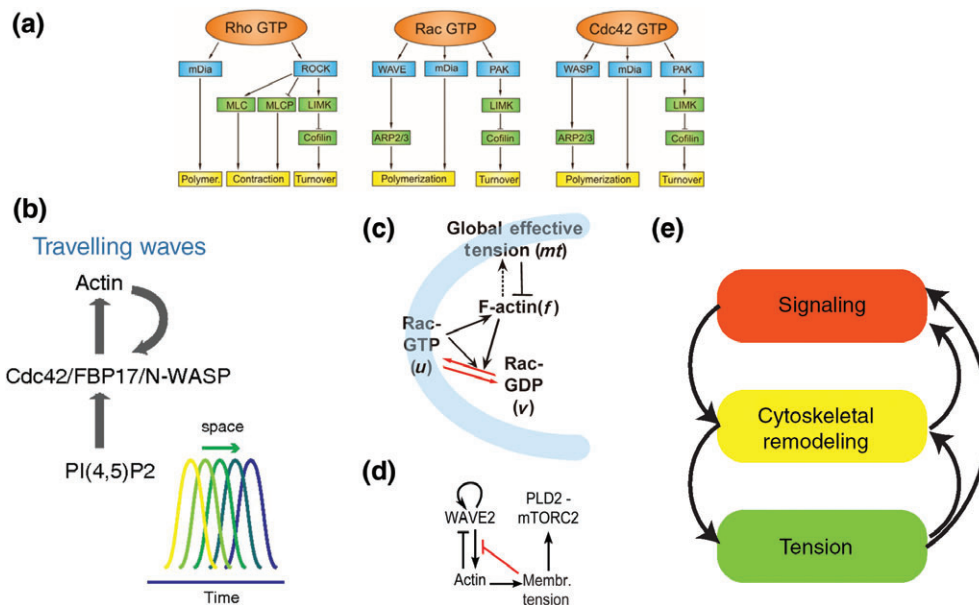
FIGURE 8 | Signalling to cytoskeletal mechanics and back. (a) Well-established biochemical pathways that link signalling molecules to the cytoskeletal machinery (Reprinted with permission from Ref 181. Copyright 2012 Frontiers) (b)-(e) are new ideas of how cytoskeletal mechanics may feedback to signalling as well. The acronyms within each box refer to key signalling molecules involved in modulating cytoskeletal components such as actin (polymerisation, turnover) and myosin (contraction). ((b) reprinted with permission from Ref 182. Copyright 2013 National Academy of Sciences; (c) reprinted with permission from Ref 183. Copyright 2017 Public Library of Science; d reprinted with permission from Ref 184. Copyright 2016 Public Library of Congress).

models differing primarily in their molecular assumptions rather than mathematical formalisms. We chose here to focus on the role of signaling in cell behavior and why it is important to couple it to cell mechanics rather than the mathematical details of the myriad of associated models developed over recent decades. For comprehensive reviews of the mathematical modeling on this topic, see[179,180]

Regulating Actin Growth and Protrusion

When discussing actin growth, it is important to distinguish between different populations of actin. Cortical actin forms part of the structural shell of the cell. Actin bundles, aligned arrays of actin filaments cross-linked by, among other things, myosin motors, are responsible for contractile stresses in the cell and can generate protrusion in filapodia. Lamellipodial actin, located near the protrusive leading edge of a cell, is comprised of a dense, cross-linked meshwork of filaments and is primarily responsible for broad lamellipodial protrusion at the leading edge of cells. While each of these structures contributes to force production in the cell, lamellipodial actin is the most studied and its regulation will be the focus here.

Numerous actin-binding proteins regulate its nucleation and growth. In response to a signal, Arp2/

3, formins, cofilin, and other actin binding proteins are recruited to the cell periphery.[177] Their combined action increases the number of actin barbed ends and their rate of elongation, both of which contribute to force production. Arp2/3 in particular is critical to the generation of the highly branched, dendritic actin network found at the leading edge of motile cells. It is a dimer comprised of two sub-units, Arp2 and Arp3, each of which closely resemble a G-actin monomer.[185] This close resemblance allows Arp2/3 to form a thermodynamically stable bond with an existing actin filament, generating a new growing filament branched from an existing filament at an angle of approximately 35°.[186] It is thus a primarily regulator of the density of force producing filaments.

Cofilin, which binds to F-actin and severs it into two new filaments, also serves to increase the number of growing barbed ends in this network. Spatial modeling has shown that Arp's preference for new actin filaments and cofilin's preference for older filaments leads to a synergy where they jointly produce more barbed ends than would be expected from simple addition of the two mechanisms.[187] While these (and other) binding proteins increase the number of growing filaments, regulation of their growth rate, which depends on the availability of GTP G-actin, also determines the potential for force

production. Profilin, which catalyzes the exchange of GDP → GTP in monomeric G-actin,[188] has a critical role regulating this pool of polymerizable actin, and additional modeling has suggested that advective transport due to retrograde flow of actin can influence this pool as well.[189]

But how are these binding proteins recruited and restricted to the appropriate location? Upstream of these direct actin nucleators, the WASp (Wiskott–Aldrich Syndrome protein) family of proteins, including WASp, N-WASp, SCAR/WAVE among others, regulate the recruitment of Arp2/3 to the cytoskeleton.[190] While the mechanism of action differs among members of this family, each has domains that recruit Arp2/3 and G-actin monomers, respectively. Moving another step up in the regulatory chain, the small Rho GTPases Rac and Cdc42, along with the phospholipids activate this family of proteins.[191,192] Cdc42 activates WASp[193] while Rac in concert with PIP3 (and to a lesser extent PIP2[194]) activates WAVE. Thus, in response to external signaling, localization of the small GTPases and other signaling proteins leads to the activation of WASP/WAVE, which leads to recruitment of Arp2/3 and G-actin.

While Arp2/3 is the star of the show in lamellipodial force production, it is dispensable for chemotaxis. Fibroblast cells depleted of Arp can still undergo chemotaxis, but with lower efficiency.[195,196] Formins appear to pick up the slack in the absence of Arp2/3. These actin-binding proteins have an affinity for both barbed ends of filaments as well as profilin. They bring primed actin monomers into close proximity with barbed ends and increases the rate of filament elongation by a factor of up to 15.[197] Unlike Arp2/3, which becomes incorporated into a filament, formins stay at the barbed end and continually add monomers, leading to linear growth. This promotes the formation of actin cables in yeast,[198] stress fibers in HeLa cells,[199] and filapodia in melanoma and HeLa cells.[200] These filapodia, along with the myosin contractile arcs connecting them[201] are thought to generate force production and protrusion in the absence of Arp2/3.[196]

The complexities of this regulation present a challenge for modeling and understanding how different pathways influence force generation. From a theoretical perspective, force generation is often associated with actin density. However, it is not the density of the actin network that is important, but rather how it grows. This growth depends on a number of interdependent factors including the type of actin (branched versus bundled), how new barbed ends are regulated (branching, severing, capping), and the speed of polymerization. Each of these factors is likely to have different influences on the broader behavior of cells.

Regulation of Myosin and its Interactions with Actin

A second important component in the migration of typical cells is the production of contractile forces behind the lamellipodium to aid retraction. As with F-actin mediated protrusion, there are multiple contractile structures in the cell. Myosin can form filaments interleaved with actin filaments to produce contractile stress fibers.[202] Alternatively, myosin can integrate into a dense, branched actin network resembling an active gel[203] to generate a centripetal inward actin flow[204] that both drives contraction of the rear as well as retrograde flow of actin. In either case, the central player in this process is the molecular motor myosin.

While there are numerous myosin isoforms, non-muscle myosin II (conventional myosin, NMII) is the dominant form responsible for actin crosslinking and cytoskeletal contraction. NMII is found throughout the cell and can perform numerous actions. NMIIA promotes the formation of stress fibers in the lamella while NMIIB promotes the enlargement of those bundles.[205] It has been shown to generate localized pockets of cell retraction at the leading edge of the cell[201] and is known to be important for leading edge protrusion in Arp2/3 deficient cells.[197] More generally however, it's action is primarily located in the rear of the cell[206] where it promotes retraction.

Independent of its location of action, force generation by myosin requires its phosphorylation. Whereas there is a myriad of functionally different ways to regulate actin behavior (branching, severing, capping, etc.), regulation of NMII function appears converge on the phosphorylation of myosin light chain (MLC), which effectively activates NMII.

The two primary myosin binding proteins that phosphorylate MLC are Rho associated protein kinase (ROCK)[207] and myosin light chain kinase (MLCK).[208] ROCK's role in myosin regulation and migration is well established. The role of MLCK, which was first found to activate myosin in muscle cells, is still unclear. It was shown to modulate membrane tension in migratory smooth muscle cells, but was not required for migration.[209] This is in line with observations in fibroblasts[210] that MLCK is responsible for myosin activity at the cell periphery and influences membrane ruffling, while ROCK is responsible for myosin activity in the cell interior. Thus it is

possible that ROCK plays a role in regulating retraction of the cell rear while, in some cases, MLCK regulates cell tension, which itself has a putative role as a mechanical signal regulating migration[211–213]). Whereas Ca+ signaling (more commonly associated with stimulating muscle contraction than cell migration) activates MLCK, the small GTPase Rho regulates ROCK.[214] The mutual antagonism between Rho and protrusive regulators Rac and Cdc42 ensures spatial segregation myosin contraction to the rear of the cell.[178,215–218]

While signaling pathways primarily appear to converge on the activation of NMII, once activated, NMII appears to perform a number of different actions relevant to migration. Its role in leading retraction of the cell rear is clear. The potential of myosin in regulating tension however could be particularly significant as well given the suggested role of tension in regulating bulk cell behavior. Additionally, NMII has the capacity to generate either diffuse, isotropic stresses, if integrated homogeneously into an actin network, or more localized, anisotropic stresses if integrated into stress fibers.

Adhesion Regulation

The actions of protrusive and contractile force generation are not sufficient to generate translocation of the cell. It is well established that the pushing of growing actin filaments against the front of the cell drives retrograde flow from the front to the rear of the cell. Some friction or traction between the cell and its substrate is required to translate those forces into motion. This is where adhesions come into play. During migration, small nascent adhesions (NA) near the front edge (<0.5 μm) of the cell form. As the cell translocates forward these stay and place mature while they flow towards the interior of the cell. During this process, these NA's mature to form focal complexes (FC) which themselves mature into focal adhesions (FA).

The life cycle of a NA to a FA has been well studied. They are complex structures that are constructed in sequential fashion[219] with over 500 proteins, kinases, motors, etc. found to associate with them.[220] Rather than describe this molecular inventory and the undoubtedly long list of regulators involved in their assembly, we give an overview of the signaling pathways involved and discuss the role of FA's as regulators of the cytoskeleton themselves. It is well established that tension helps adhesions mature, but evidence also suggests that it is the structural formation of stress fibers rather than just tension that aids this process.[221] Initial formation of a

NA is independent of NMII,[222] myosin generated tension and stress fiber formation, but maturation requires both. Furthermore, different myosins have different roles in their maturation with NMIIA responsible for the initial maturation and NMIIB responsible for further maturation.[205]

Given the role of stress fibers and myosin generated tension in adhesion maturation, it is unsurprising that the small GTPases have at least an indirect role in their formation.[223,224] Rho's function as a regulator of Myosin contractility makes it a natural candidate to regulate tension induced FA maturation. The role of Rac in FA regulation is less clear however. While it is required for efficient migration, evidence indicates it is not required for FA formation in fibroblast cells.[225] In osteosarcoma cells on the other hand, Rac was recently found to phosphorylate and recruit NMIIA directly to the site of FA's,[226] suggesting that NMIIA is a bona fida FA protein that is integrated in part through phosphorylation by Rac.

In addition to being the target of regulation, FA's have a role in regulating cytoskeletal dynamics as well. They provide a connection between the cell and the outside world, transducing both mechanical stresses as well as molecular signaling, both of which augment cytoskeletal dynamics. In fibroblasts, actin retrograde flow is fast in the lamellipodia and slower in the lamella, where FC and FA are present.[227] When FA's are lost, the speed of retrograde flow becomes uniform due to the loss of traction to slow it. Furthermore, the formation of new NA's correlate with reduced retrograde velocity and advancement of the leading edge. Similarly in fast moving fish keratocytes, there is a gradient of flow velocity that decays away from the front and completely reverses from retrograde to anterograde[206] as a result of the myosin generated centripetal flow in the rear.[204] Thus, FA density and strength, which are modulated by a number of factors including ECM signaling density and mechanical stresses, modulates actin flows and force production within the cell.

They also have a role in molecular signaling to the cytoskeleton.[228] Focal adhesion kinase (FAK) is a critical mechano-sensor that translates integrin and ECM contacts into internal signaling. Among other functions, FAK activates PI3K, which in turn activates the small GTPase Rac,[229] promoting actin growth. Similarly, deletion of FAK was shown to lead to increased Rho activity, cell rounding, and impaired motility.[230] Through a parallel pathway, at sufficiently high levels, fibronectin has been observed to activate Rho with FA's as an intermediary.[231,232] Thus, FA's are a critical element in the signaling

pathway, both receiving signals from and sending signals to the Rho GTPases and the cytoskeleton.

BROADER REGULATION OF CELL BULK BEHAVIOR THROUGH SIGNAL TRANSDUCTION PATHWAYS

While molecular interrogation has yielded extensive insights into the processes responsible for reorganization of the cytoskeleton during migration, one of the major challenges still to be addressed is to understand how these biophysical processes and their control contribute to broader morphology and migratory behavior of cells. In the context of migration, cells can exhibit different sensitivities to stimuli,[233] different levels of persistence,[234] and even different types of migration (amoeboid versus mesenchymal).[235] Modeling has provided numerous insights into how various actin binding proteins interact with the cytoskeleton, and how the actions of those proteins coordinate different types of force production. But how do they contribute to the broader phenomenological behavior of cells?

Beyond controlling where and when acto-myosin remodeling occurs, the Rho GTPases exert significant control over the broader type of motility cells exhibit. Evidence suggests Rho GTPase signaling provides cells with an intrinsic sensitivity to stimuli that can be modulated by either intrinsic or extrinsic factors.[233] GTPase signaling has also been implicated in regulating the types of migration cells undergo. Enrichment of Rho was found to be associated with amoeboid migration, which is characterized by more rounded and contractile cells, while Rac enrichment is associated with mesenchymal migration, characterized by well-defined protrusive lamellipodia.[217,235,236] Similarly, expression levels of Rac and Rho were found to influence the morphology (large, small, polar, apolar) of cells.[237] Cells also exhibit plasticity in these characteristics. Varying the levels of Rac and Rho expression toggle cells between amoeboid and mesenchymal migration[238] and influence cell morphology,[237] which were shown to be linked.[239]

In addition to modulating these features, interactions between GTPases and both upstream (ECM) and downstream (actin) signaling processes modulate bulk cell behavior. The feed-forward model of regulation (depicted in Figure 8(a)) where ECM signaling initiates cascades involving GTPases and other signaling activity, which in turn regulates acto-myosin remodeling (ECM → GTPases or other regulators → actin/myosin) is oversimplified. There are significant feedbacks between these regulatory

layers that influence cell behavior. Dynamic feedbacks from actin back onto GTPase regulation lead to the generation of dynamic waves of actin polymerization that generate force upon colliding with the cell periphery[240–242] (see, e.g., Figure 9(c)). Imaging studies have found that a number of actin binding proteins associate with these waves.[243,244] Furthermore, they exhibit excitable characteristics such as mutual annihilation (two waves converging suppress each other),[245] with interactions between small GTPases and actin being reported to give rise to this excitability[195] (Figure 9(d) for example depicts a relationship between waves of actin activity and FBP17 activity that are out of phase with FBP17 leading the actin wave). Mata[240,241] and Bernitt[245] have further suggested that, in some cases, the unique nature of GTPases as a conserved excitable activator produces a global coupling between disparate regions of a wave. This type of 'conserved excitable system' has distinct wave propagation characteristics, such as size dependent propagation velocity, as observed in.[245]

In addition to actin dependent feedbacks, substrate dependent feedbacks have been observed. While it is known that ECM signaling initiates a number of signaling cascades that regulate acto-myosin reorganization, downstream growth or contraction of lamellipodia (which involve actin and myosin dynamics) influences the level of physical cell contact with the substrate and thus levels of ECM signaling. These feedbacks in turn influence the persistence of migration,[246] which can be manipulated by augmenting the topography of the substrate or the density of fibronectin coating.

Feedbacks involving plasma membrane tension also have a role in regulating cell behavior. Physical manipulations such as stretch, osmotic shock, and aspiration have been applied to cells to assess its effects (Figure 9(a) and (b)). In one case, it was found that aspiration of one side of a cell by a micropipette very quickly suppresses polarity and signaling on the far side of the cell. A joint experiment and modeling study suggested this likely results from tension effects rather than chemical diffusion.[213] The influence of tension on GTPase signaling has been further documented in muscle cells[212] and neutrophil like HL-60 cells.[213]

These feedbacks have been posited to play a role in helping cells navigate complex environments. In the case of wave like actin dynamics, it is proposed that the negative feedback associated with excitability helps cells navigate around barriers by extinguishing growth where there is contact with a barrier. Cell tension based feedbacks provide a potential mechanism for fast, long range
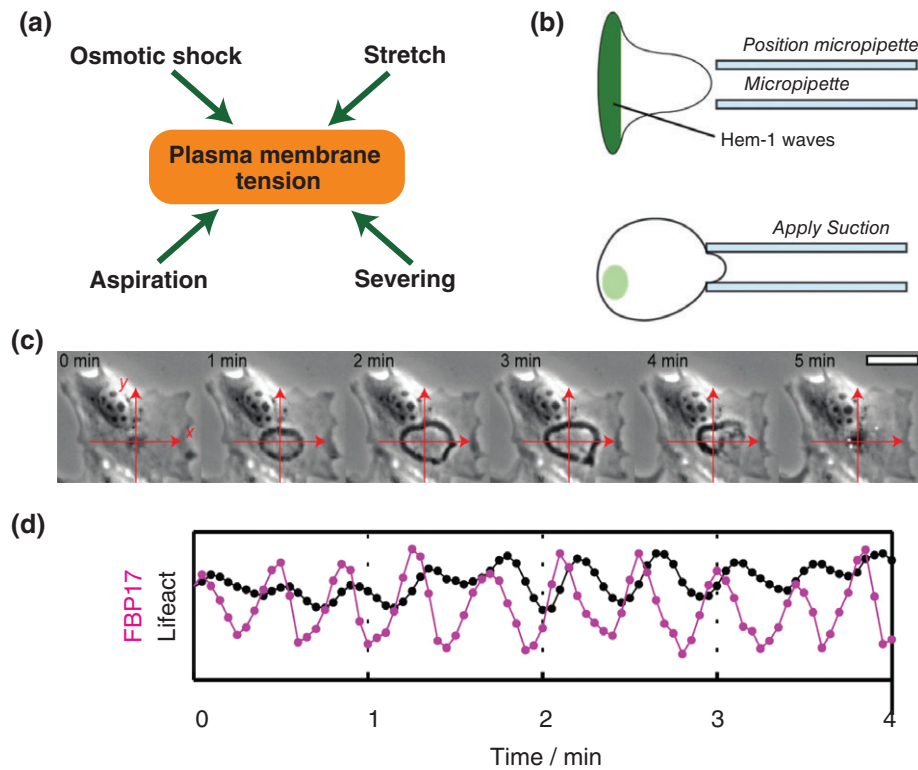
FIGURE 9 | (a) Synopsis of some of the types of manipulations that have been performed to manipulate cell tension and investigate its effects. (b) Illustration of micropipette aspiration used to increase plasma membrane tension (Reprinted with permission from Ref 213. Copyright 2012 Elsevier Inc.). (c) Images of an actin wave (circular dorsal ruffle) expanding and subsequently contracting (scale bar corresponds to 25 μm, reprinted with permission from Ref 245. Copyright 2015 Public Library of Science). (d) Image depicting a phase relationship where actin waves are preceded by and appear to suppress FBP17 (a downstream effector of Cdc42) activity, suggesting a negative influence of actin on its own signaling (Reprinted with permission from Ref 182. Copyright 2013 National Academy of Sciences).

communication that ensures only one protrusion is actively growing at a time.[213] Mathematical modeling has also shown that tension mediated effects promote migratory persistence and make the cell more resistant to random perturbations.[183] Thus, accounting for these mechanochemical feedbacks is necessary to account for real cell dynamics.

The challenge is that there are multiple locations in these signaling cascades that feedbacks could act. Tension could for example act at the level of regulators like GTPases or actin binding proteins. Furthermore, as is the case in actin regulation, there can be multiple qualitatively similar but quantitatively different ways to regulate force production. Finally, ECM mediated feedbacks require an accounting of the structure of ECM (particularly in 3D environments) and how cells interact with and perturb it. Addressing these challenges with a joint modeling and experimentation approach incorporating both mechanics and signaling will be required to link the molecular detail that has been uncovered in recent decades to cellular behavior.

DISCUSSION AND OUTLOOK

A variety of experimental techniques and mathematical models have advanced our understanding of single cell mechanics and cytoskeleton mechanobiology. While initially continuum, phenomenological models of the bulk mechanical properties were the norm, the availability of new, higher-resolution experimental measurements are driving advances in mathematical modeling of cells that incorporate more microstructural details. Based on our extensive review of cell mechanobiology, we believe there are three broad directions in which to focus future efforts.

Microstructurally Informed Models of Cell Stiffness and Mechanics

While models of cell mechanics are prevalent, our understanding of and mathematical models of the contributions of microstructural components of the cell to its stiffness are far from complete. There are few studies that investigate the role of intermediate filaments or

microtubules on bulk cell stiffness. Identifying and quantifying spatial heterogeneity in cell stiffness is critical to evaluating the mechanical stresses that the cell undergoes, which then feed signals to the nucleus.

One brute force approach involves identifying as many cytoskeletal proteins as possible and simulating their contributions to different cell contexts. Perhaps a more fruitful and less time-consuming approach would be to develop a minimal model that includes several proteins that have been implicated in a relatively broad range of biological phenomena and to parameterize and validate the model with a wide range of experimental techniques. Certainly, the relative simplicity of the number of components that contribute to red blood cell mechanics has meant that such an approach has already proven successful.[47] A more in-depth understanding of the role of cytoskeletal components would also help identify and couple biochemical signal pathways to cytoskeletal mechanics remodeling.

Closer Ties between Signaling and Mechanics

As Figures 8 and 9 illustrate, cellular biology is regulated by continuous mechanistic links between biochemical signaling processes and cytoskeletal and cell-wide mechanics. More often than one would like, mechanical and biochemical studies of cells are not performed in tandem. Disciplinary training is a limiting factor to more integrative studies but technological limitations also prevent tandem measurements of mechanical forces and chemical processes simultaneously. Some studies[22,32] show that cross-disciplinary studies provide a more comprehensive picture of cellular mechanobiology than studies that only focus on one aspect.

There are several aspects of cell signaling to mechanics coupling that we have not discussed in this review that are worth mentioning here. A precise understanding of the 'random' myosin motor driven contractions of the cell require attention. Currently, microrheology methods are being used to measure these contractions and to remove them from analysis of cytoskeletal rheology.[133] These processes have been suggested to play a role in sensing the local environment. An investigation into the role of biochemical processes, like calcium dynamics, could

move us closer to determine the source of these random fluctuations. Another open question is how actin polymerization affects cell stiffness in single cell and tissue constructs.

Model Sharing and Standardization

Cellular mechanobiology is a burgeoning field of research with scientists across the STEM disciplines collaborating with biologists and life scientists to discover the fundamental laws that govern cell and tissue function. During the rapid growth of this field, many experimental measurement technologies and computational modeling methods have been brought to bear on a variety of biological contexts, such as the mechanobiology of malaria, cancer cell migration, stem cell differentiation and ostocyte and bone remodeling to name a few. The variety of models and parameters suggest that some efforts to consolidate these advances for future developments would be beneficial. Open-source software packages exist for finite element models[247] and particle based models.[248] Model reproducibility and reuse has the enormous potential to accelerate our research by allowing colleagues to use our models as building blocks in their own research.

Towards Application

We have largely focused this review on the fundamental mechanisms that govern cellular and cytoskeletal mechanics. The motivation that underpins these advances is patient healthcare. Cellular mechanobiology regulates tissue function and therefore insights from our models must ultimately lead to clinical outcomes. While these are still early days, we anticipate that the insights we gain from our computational models will drive further development of new innovative technologies in drug testing and pharmacology. Indeed, as illustrated in Figure 2, experimental measurements need mathematical models to decipher the underlying mechanisms that regulate cell behavior. Models that are tightly coupled with experimental data will make the models more reliable and consequently accelerate innovations that are based on insights that can be gained from such models. When reviewing the modeling efforts to date it is clear that the field is already making advances in this direction.

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