Linear and Nonlinear Rheology of Living Cells

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Abstract

Living cells are an active soft material with fascinating mechanical properties. Under mechanical loading, cells exhibit creep and stress relaxation behavior that follows a power-law response rather than a classical exponential response. Such a response puts cells in the context of soft colloidal glasses and other disordered metastable materials that share the same properties. In cells, however, both the power-law exponent and stiffness are related to the contractile prestress in the cytoskeleton. In addition, cells are made of a highly nonlinear material that stiffens and fluidizes under mechanical stress. They show active and adaptive mechanical behavior such as contraction and remodeling that sets them apart from any other nonliving material. Strikingly, all these observations can be linked by simple relationships with the power-law exponent as the only organizing parameter. Current theoretical models capture specific facets of cell mechanical behavior, but a comprehensive understanding is still emerging.

1. THE CELL AS A MATERIAL

1.1. Structure and Dynamics of the Cell

Cytoskeleton: a dynamic network of protein filaments spanning the entire cell body; it maintains the cell's shape and mechanical function

Prestress: permanent mechanical stress, either internally generated by actomyosin contraction or externally applied, in filament networks such as the cytoskeleton

Eukaryotic cells are highly complex structures consisting of a large number of different proteins and other constituents. Mechanically, they are stabilized by the cytoskeleton, a contractile filamentous network that spans the entire cell body. In humans, several thousand different proteins form and regulate the cytoskeleton (1). Together, these proteins assemble into a structure of stunning complexity in a process that is far from thermodynamic equilibrium. To connect two proteins to each other, the cell spends approximately one molecule of high-energy phosphate such as ATP or GTP. It is therefore even more remarkable that this intricate cytoskeletal structure, which costs the cell a large part of its metabolic energy, is constantly destroyed and then reassembled over and over again. This ongoing remodeling makes the cytoskeleton highly versatile and allows the cell to change shape, to move, to contract, to divide, to merge, and to engulf other objects. It is therefore not so much the structural architecture but rather the dynamic behavior of the cytoskeleton that makes the cell fundamentally different from any other nonliving material. But is it then possible or even sensible to treat living cells just like an ordinary inert material and to characterize them in terms of stresses, strains, and deformations? And what can we learn from such a mechanical characterization?

1.2. Cytoskeleton: A Primer

The most abundant proteins in cells of all tissue origin are those that form the cytoskeleton, such as actin and myosin. The cytoskeleton of nucleated (eukaryotic) cells consists of three distinct components: the actin-myosin network, the microtubule network, and the intermediate-filament network (2).

The actin-myosin network is generally located at the cortex and outer edges close to the cell membrane. It is mechanically linked to the adhesive contacts that anchor the cell to the extracellular substrate. These contacts counterbalance the contractile forces built up by the active myosin motors in the network, leading to a constant prestress in the cytoskeleton. In highly contractile cells, the actin and myosin filaments can align and form stress fibers that closely resemble muscle fibrils. These stress fibers are not confined to the cortex but can span the entire cell body. Apart from myosin, there are a large number of other, passive actin cross-linkers with widely varying properties. Such cross-linkers turn the actin-myosin network into a highly versatile structure that can fulfill a variety of functions, such as forming different types of protrusions.

Microtubules are rather stiff filaments that extend from a common origin (centrosome) located near the cell nucleus. They serve as transport pathways for intracellular cargo and play an important role in defining cell polarity and in orienting the chromosomes during mitosis. In most adherent cells, however, they play only a minor mechanical role in stabilizing the cytoskeleton and balancing stress fluctuations (3).

Intermediate filaments consist of a large, tissue-specific group of proteins. Examples include keratin in epithelial cells, vimentin in mesenchymal cells, internexin in neuronal cells, and desmin in muscle cells. They start to significantly contribute to the overall mechanical response of the cell during large cell deformation, when intermediate filaments become fully extended and stretched (4–6).

All these three components of the cytoskeleton are highly connected to each other, to the nucleus, and to the cell membrane (7–10). Their individual contribution to cell mechanical behavior can therefore not easily be separated. This needs to be taken into account when comparing cell mechanical studies with experiments on reconstituted cytoskeletal networks.

2. MECHANICAL BEHAVIOR OF CELLS

2.1. Methods for Measuring Cell Mechanics

Microrheology: measures the microscopic mechanical properties of soft materials by tracking the movement of embedded micrometer-sized particles in response to thermal or nonthermal forces

Rheometers operate by applying a defined mechanical stress to the material and by measuring the resulting deformations or, vice versa, by measuring the mechanical stress necessary to achieve a desired deformation. Because of the exceedingly low stiffness and small size of cells, the mechanical forces and deformations are very low, in the range of pico-Newtons and nanometers, respectively. Precise quantitative mechanical measurements on single, living cells became possible only with the development of modern microrheological techniques in recent decades such as magnetic tweezers, laser tweezers, atomic force microscopy, cell poking, microplates, and cell stretchers. Nonetheless, in 1922 Heilbronn (11), using an early version of magnetic tweezers, reported the first microrheological studies of the cytoplasm. Francis Crick (of DNA fame) & Arthur Hughes (12) improved on this method in the late 1940s. Despite experimental limitations, they observed

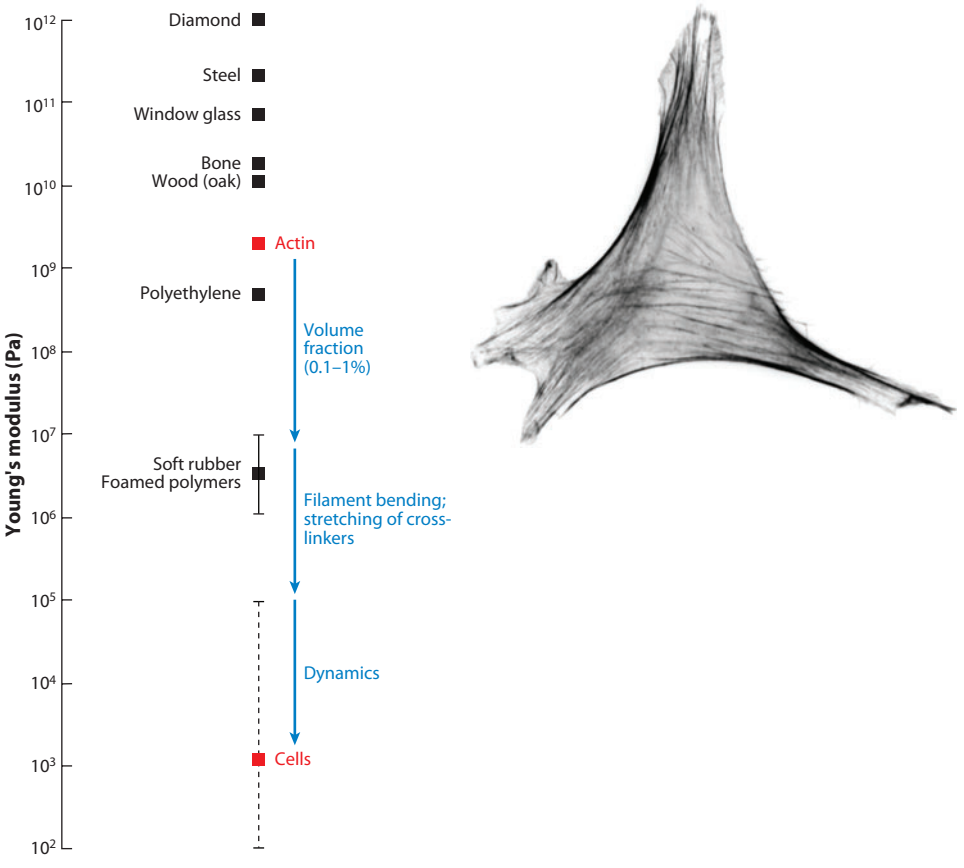


Figure 1

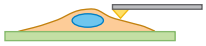
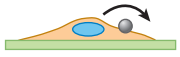
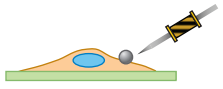
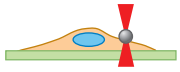
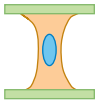
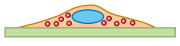
(*Left*) Young's modulus of some common materials. Cells are exceedingly soft, with a Young's modulus many orders of magnitude lower than that of most common materials. Starting with the high stiffness of actin, the main protein constituent of cells, several mechanisms work together to lower the stiffness down to a level comparable to that of soft pastes, slurries, or weak gels. (*Right*) Actin cytoskeleton of an NIH-3T3 fibroblast cell.

the full range of the irritatingly rich and complex viscoelastic behavior of cells, including plasticity and shear thinning, and reported stiffness values of the cell interior in the range of 2 to 50 Pa.

Why are cells so soft? The filamentous network of the cytoskeleton, when viewed in isolation, is actually made of rather strong materials. Intermediate filaments, for example, keratin, which is the main component of wool, have a stiffness (1–5 GPa) and tensile strength (0.2 GPa) that make them a useful material for many technical applications (2). The cytoskeletal protein actin, the most abundant intracellular protein, has a Young's modulus in the range of 1–2 GPa (2). The cell as a material, however, is approximately six orders of magnitude softer (**Figure 1**) for the same reason as why a wool jumper is soft to the touch: The volume fraction of the keratin fibers is low, and the individual fibers undergo bending rather than stretching deformations. In cells, the metastability, or dynamics, of the fiber network reduces the overall stiffness further still.

From the measured value of cell stiffness, however, it is impossible to estimate the relative contributions of volume fraction, filament bending, cross-linker stretching, and dynamic remodeling mechanisms. The concept of cell stiffness as a single number neglects, or rather averages over, any inhomogeneity, granularity, anisotropy, nonlinearity, and time fluctuations. Moreover, the particularities of this averaging depend on the probe, e.g., its shape, size, surface functionalization, contact time with the cell, or position relative to the cell body. The averaging further depends on the applied force or deformation magnitude and on the time or frequency range over which the measurement is performed. Different measurement techniques therefore measure different facets of cell rheology, and each has particular characteristics, advantages, and limitations (**Table 1**).

Table 1 Comparison of common methods to measure cell rheology

Method		Advantages	Limitations	References
	Atomic force microscopy	• High spatial resolution (10 nm, depending on tip geometry) • Quantitative measurements of shear modulus possible • High time resolution of stiffness changes (~1 s) • Large range of forces (up to ~100 nN)	• Low scanning speed • Low throughput	51, 100, 101
	Magnetic twisting cytometry with optical detection of bead movements	• Large range of frequencies (0.01–1,000 Hz) • Parallel measurements of ~100 cells possible • High time resolution of stiffness changes (~1 s)	• Maximum specific torque <140 Pa	44, 55, 64
	Magnetic tweezers	• Large range of forces (up to ~100 nN) • Good measurement throughput (30 cells h^{-1})	• Only for unidirectional forces • No frequency modulation (lock-in) possible	25, 102
	Optical tweezers	• High time resolution of stiffness changes (~1 s) • Good measurement throughput (30 cells h^{-1})	• Maximum forces <500 pN • Heating caused by laser traps	14, 103
	Microplate rheometer	• Large range of forces (up to ~1 °N) • Control of cellular prestress	• Low throughput • No subcellular resolution	35, 63, 65
	Particle tracking microrheology	• Quantitative measurements of shear modulus possible • High frequency (up to 100 kHz)	• Only for soft materials ($G << 100$ Pa)	48, 104

Moreover, whether by ligation and/or mechanotransduction, the interaction of a probe (such as an AFM tip, ligand-coated bead, or microplate) with the cell unavoidably induces local remodeling events that alter the structure being probed (13–20). This remodeling is a principal feature of all approaches in which an external probe is used. Other limitations of bead-based methods, except for the cases in which a bead is glued onto an AFM tip, are that the position at which the bead settles onto the cell surface cannot be controlled (although it can be measured) (21) and that the geometry of the bead-cell interaction is also not controlled (although, again, it can be measured) (22).

As with all the available methods for measuring cell mechanics, with the exception of two-point particle tracking microrheology (23, 24), a length scale must be invoked to convert raw displacement data into cellular strain and subsequently into a proper elastic modulus. The intracellular strain distribution can be analytically derived (24, 25), computed using a finite element model of cell deformation (26), or estimated from simple dimensional arguments (27), all under the assumption that the cell is a homogeneous, isotropic, and mostly linear elastic material. Even under these simplifying assumptions, the strain and stress field within the cell close to a bead or AFM tip is highly complicated (26, 28) but is predicted to decay rapidly with larger distances from the probe. In practice, however, one finds in violation with continuum mechanics predictions that locally applied stresses can be focused and transmitted at great lengths through the entire cell body via stress fibers (28).

From all these considerations, one might conclude that quantitative cell mechanical measurements are a pointless endeavor; indeed, reported values of elastic moduli measured by different techniques vary by more than one order of magnitude, and this scatter in the available data only increases when different cell types are compared (29, 30). The current situation, however, looks much more positive. Several recent experimental investigations have revealed a set of cell mechanical properties that can be reproduced consistently and compared between various probing techniques and even across different cell types (29, 31, 32). Such universal aspects of cell mechanics are the basis for understanding the complex cell rheology from a physical point of view (6, 29, 32) and are discussed below.

2.2. Linear Viscoelasticity: Springs and Dashpots

Depending on the experimental approach and the timescales probed, cells show both solid-like elastic and fluid-like viscous properties. This behavior is termed viscoelastic. In engineering, viscoelastic materials are traditionally described by mechanical equivalent circuits of connected Hookean elastic springs and Newtonian viscous dashpots. Any arbitrary linear viscoelastic behavior can be modeled with networks of springs and dashpots arranged in series or in parallel.

The aim of the spring-dashpot approach is that the different elements of the mechanical equivalent circuit can be ascribed to different structural elements. In the case of cells, different elastic and viscous elements were thought to reflect the membrane, actin cortex, deep cytoskeleton, and so on (25). Due to the limited resolution of early experimental results on viscoelastic creep and stress relaxation in cells, simple spring-dashpot models with only one time constant were sufficient to fit the data (33–35). With the development of more sophisticated experimental techniques, the accessible ranges of time and frequency and the resolution of the obtained data were increased, which made it necessary to introduce more complex models of cell mechanics (25, 36).

The problem with this approach is that larger numbers of model parameters make it ambiguous or outright impossible to equate the spring-dashpot elements with real cell components. Moreover, as we see below, basic underlying assumptions such as linearity or the decoupling of elastic and viscous properties are not correct. Additional components such as nonlinear plastic elements were introduced to explain more complex behavior such as plasticity (36), but the increasing number of

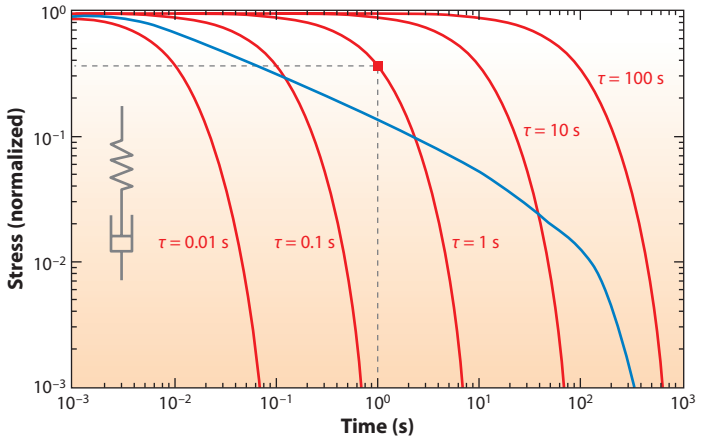


Figure 2

Stress relaxation. A Maxwell body (the combination of an elastic spring with elasticity k in series with a viscous dissipative dashpot element with viscosity ν, as shown at *left*) shows an exponential relaxation of mechanical stress after a step increase in mechanical strain (*red curves*). The ratio ν/k defines a characteristic timescale τ after which a dominant fraction of the initial stress has decayed. A power-law decay of stresses can be reasonably well fitted with a combination of Maxwell bodies (*blue curve*) when, for each decade in time, an additional spring-dashpot element is added in parallel to the other elements. The fitted values of τ, the spring elasticities, and dashpot viscosities, however, are determined solely by the timescale of the experiment and therefore have no meaning and no relationship with the state of the cell (37).

model fit parameters necessarily decreases the confidence of the actual fit. As explained in the next section, traditional viscoelastic models of the cell, except for very special cases, are not mechanistic and have no physical meaning.

2.3. Power-Law Rheology

As the time and frequency resolution of microrheology became better, researchers set out to record a broader viscoelastic spectrum and to find precise time constants describing cell mechanics. Surprisingly, the viscoelastic spectrum of living cells lacks any distinct timescales that can be identified with discrete structural elements or processes. The number of spring-dashpot elements needed to fit the data, and their specific values, depends only on the scale of the measurement time or frequency and for that reason cannot have any physical meaning (**Figure 2**) (37). Instead, the viscoelastic behavior of cells can be described by a power law with a single exponent over many orders of magnitude of time or frequency.

Power-law stress relaxation of biological samples was described in the first part of the nineteenth century (38–40). With the development of linear viscoelasticity, however, the description of stress relaxation by power laws (41) was discarded in favor of mechanistically more intuitive spring-dashpot equivalent circuits with exponential relaxation behavior (42). More than 100 years later, Hildebrandt (43) finally discovered the simplicity and accuracy of power laws for describing tissue biomechanics.

Power-law behavior can best be explained by a creep experiment in which the material is mechanically loaded at time $t = 0$ with a constant force F, and the material deformation d versus time t is recorded. The ratio $d(t)/F$ defines the creep function $J(t)$:

$$J(t) = d(t)/F = j_0 \cdot (t/\tau_0)^{\beta}. \quad 1.$$

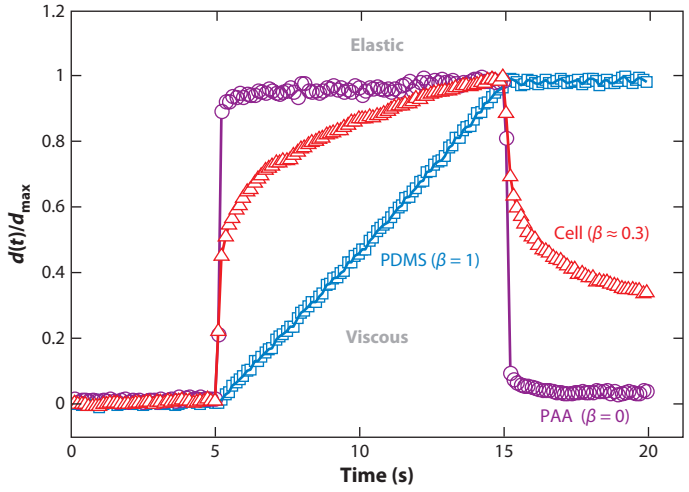


Figure 3

Power-law creep response. Experimental creep response of an elastic material [polyacrylamide-bis-acrylamide (PAA) hydrogel; *blue curve*], a viscous material [polydimethylsiloxane (PDMS) silicone oil; *red curve*], and a cell (F9 embryonic carcinoma; *purple curve*) measured with a magnetic bead rheometer. In each case, the creep response follows a power law, with an exponent of zero for the elastic hydrogel, unity for the viscous silicone oil, and 0.3 for F9 cells.

In this equation, the prefactor j_0 characterizes the softness or compliance of the material, which is the inverse of stiffness. Time is normalized by a timescale τ_0, which can be arbitrarily set to 1 s or any other convenient value. Changing τ_0 does not change the value of the power-law exponent β. Such behavior is termed timescale invariant.

Often, the material is probed not with a constant force but instead with a sinusoidally varying force, in which case the ratio of the Fourier-transformed displacement and force defines the complex modulus of the material $G(\omega) = d(\omega)/F(\omega)$ as a function of the radian frequency ω. Equation 1 then transforms to a power law with the same exponent β (43),

$$G(\omega) = 1/j_0 \cdot (i\omega\tau_0)^{\beta}\Gamma(1-\beta),$$

with Γ denoting the gamma function and i denoting the imaginary number $i^2 = -1$.

The power-law exponent describes the viscoelastic behavior of a material in a very economical way. If β approaches zero, then Equation 1 simplifies to $d/F = j_0$, which is Hooke's law of elastic deformations. All the deformation energy is elastically stored in the material, and after the external force is removed, the material springs back to its original shape. If β approaches unity, Equation 1 simplifies to $d/F = j_0 \cdot t$, which is Newton's law of viscous deformations. The material is unable to elastically store the deformation energy, which is dissipated as heat. After the external force is removed, the material remains in its deformed state. At intermediate values of β, both elastic and dissipative mechanisms coexist (**Figure 3**). Higher β values point to a more dissipative behavior and lower values to a more elastic behavior. In cells, measured β values are typically between 0.1 and 0.5.

2.4. Implications for Our Understanding of Cell Mechanics

Power-law rheology and the applicability of Equation 1 have been confirmed in a large number of different cell types as measured with many different techniques and over an exceedingly large

range of timescales or frequencies (**Figure 4**) (31, 44). Scale-free power-law rheology appears to be a universal property of adherent cells and holds even when the cells are treated with an exhaustive range of cytoskeletally active drugs (45). Considering this universality, Equation 1 is a remarkably simple empirical relationship. It captures the essence of the data with only a single parameter: the power-law exponent β. (We show below that the parameters j_0 and τ_0 are not free-fit parameters but are constants for a given system such as a cell line.)

This behavior may at first glance appear somewhat disappointing because if no characteristic timescale is evident, it is not possible to identify a dominating relaxation process. If all imaginable pharmacological interventions or differences between different cell types can be characterized by changes in the power-law exponent β alone, then obviously β cannot explain a molecular mechanism. But what does it explain?

A possible answer comes—surprisingly—from a theory of soft glassy materials (SGMs) (46). SGMs are a diverse group of substances that includes foams, pastes, colloids, emulsions, and slurries. These substances are very soft (in the range of pascals to kilopascals) and are scale free according to Equation 1, with power-law exponents on the order of 0.1. Because these materials are so diverse, it has been reasoned that the common rheological features must be not so much a reflection of specific molecular mechanisms as a reflection of generic systems properties at the level

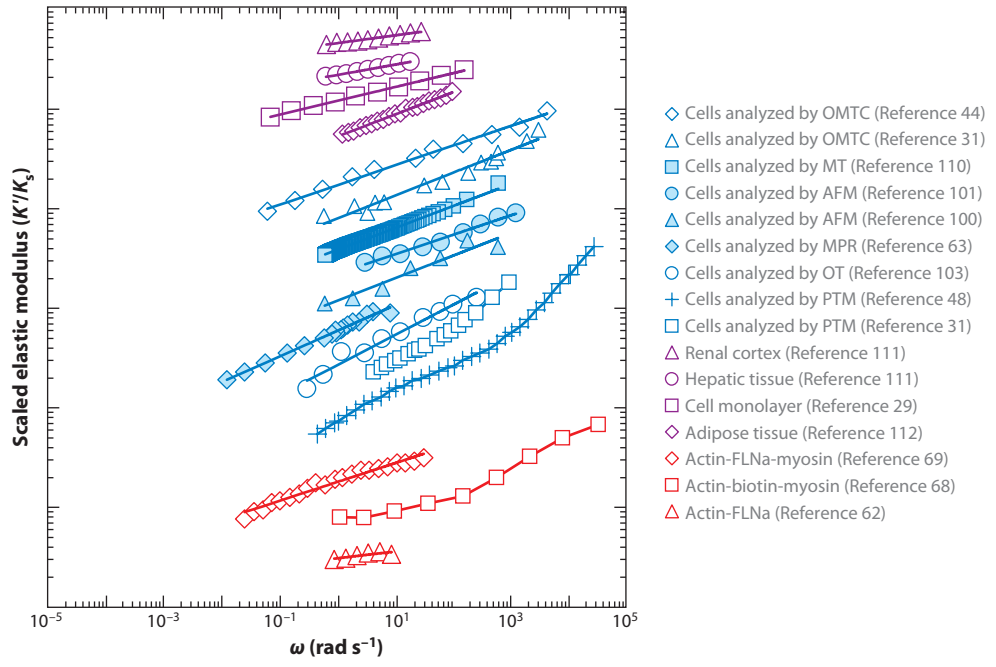


Figure 4

Universality of power-law responses in biological materials. The frequency response of cells (*blue*) follows a weak power law over several decades of frequency, regardless of cell type or experimental technique. Cytoskeletal networks under active or passive stress, cell ensembles, and soft tissues also display a power-law frequency. The absolute values of the differential elastic modulus K' measured at a fixed prestress vary according to specimen type and experimental technique and are difficult to compare between different studies. For clarity, the curves have been shifted in the y direction to emphasize the similarity of the frequency dependency. In some cases, the reported time-dependent responses were Laplace transformed into the frequency domain. Abbreviations: AFM, atomic force microscopy; FLNa, filamin A; MPR, microplate rheometer; MT, magnetic tweezers; OMTC, optical magnetic twisting cytometry; OT, optical tweezers; PTM, particle tracking microrheology.

of structural organization (46). All SGMs are composed of elements that are discrete, numerous, and aggregated with one another via weak interactions. In addition, these materials exist far away from thermodynamic equilibrium and are arrayed in a microstructural geometry that is inherently disordered and metastable. The data presented above establish that living cells satisfy all these criteria and features, and it has been proposed that they be added to the list of SGMs (44).

Soft glassy rheology (SGR): a concept to explain glassy behavior and power-law rheology in soft disordered materials such as foams and colloids

Soft glassy rheology (SGR) theory considers that each individual element of the matrix exists within an energy landscape containing many wells, or traps, of differing depth E. In the case of living cells, those traps might plausibly be thought of as being formed by binding energies between neighboring cytoskeletal proteins through hydrophilic interactions, charge effects, or simple steric constraints. Each energy well is regarded as being so deep that the elements are unlikely to escape the well by thermal fluctuations alone. Instead, elements are imagined to be agitated by some other energy source (46). In cells, ATP-dependent mechanisms are thought to be the cause of such nonthermal agitation (47). The magnitude of this agitation is represented by an effective temperature, or noise level, x.

For a soft glass to deform elastically, its elements must remain in energy wells; to flow, the elements must hop out of these wells. Once an element has escaped its energy well, all its elastically stored deformation energy is dissipated as heat. Dissipation is therefore intrinsically linked to an elastic stress, not to a viscous stress, and the locus of both friction and elasticity lies within the same elements and their binding energies and cannot be attributed to distinct components (32).

With increasing effective temperature x, the elements more frequently manage to hop out of their well only to fall into another. The hop can be thought of as the fundamental molecular remodeling event and the origin of fluid-like behavior. An increase in x causes the system to melt, and a decrease in x causes the system to freeze. If x tends toward unity, the elements become trapped in deeper and deeper wells; all hopping events abate, and the system approaches a glass transition.

In the special case of an exponential distribution of energy well depths, the creep response of such a system follows Equation 1. The parameters in this equation are defined as follows. The power-law exponent β can be identified as the noise temperature x minus unity ($x - 1$); the prefactor j_0 is the lowest possible compliance (i.e., the highest stiffness) of the system, which occurs at the glass transition temperature ($x = 1$); and the timescale τ_0 is the minimum residence time of an element in a well before it can hop out. The parameters j_0 and τ_0 are thus system-specific structural constants, whereas the power-law exponent β (or the noise temperature $x - 1$) reflects the system's dynamics.

If j_0 and τ_0 are constants, the consequences for cell behavior are striking. The cell cannot change its elastic and dissipative properties independently but can change such properties only along a special trajectory and only through changes in the exponent β. To increase its stiffness, the cell must become more solid like (i.e., have a smaller exponent β), and vice versa, to decrease its stiffness, it must become more fluid like (i.e., have a larger exponent β). Measurement of the creep response of cells with different stiffnesses experimentally demonstrates this behavior. The creep curves may differ dramatically, but when extrapolated, they intersect at the point j_0 at time τ_0 (or at frequency $2\pi/\tau_0$) (**Figure 5**). This striking behavior has been seen in a wide range of different cell lines treated with a plethora of drugs and has been measured using a range of different methods (45, 48–51). This behavior is still utterly mystifying, but it implies that the power-law exponent β plays a central organizing role leading to the collapse of all data onto universal master curves (**Figure 6**) (32, 49).

The extrapolation of the creep response $J(t)$ to very short timescales, or equivalently the extrapolation of the complex modulus $G(\omega)$ to very high frequencies, should not be confused with a prediction by SGR theory. Equation 1 is valid only in the limit of long timescales or low frequencies. At higher frequencies, a viscous dissipation of the thermally driven bending

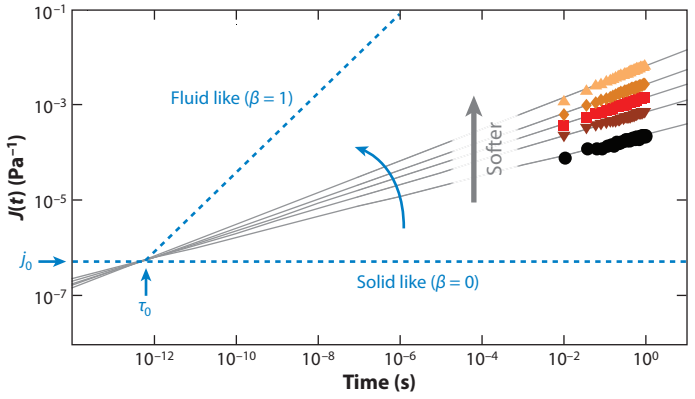


Figure 5

Intersection of creep responses. Data between individual cells varied, but when the cells were arranged in groups with different stiffnesses, a relationship emerged. Cells in the stiffest group displayed the lowest power-law exponent β, whereas cells in the softest group displayed the highest power-law exponent β. Remarkably, the data defined a family of curves that, when extrapolated, appeared to intersect at a single value (j_0) at a very small timescale τ_0.

fluctuations of the cytoskeletal filaments is thought to dominate the system's frictional response. Theoretical considerations of the relaxation spectrum (or creep behavior) in this high-frequency regime also predict a power law, with exponents of 3/4 for freely fluctuating filaments (52) and exponents of 1/2 for tensed filaments (53). Microrheological measurements on cross-linked actin filament networks and living cells are in agreement with these predictions (54, 55).

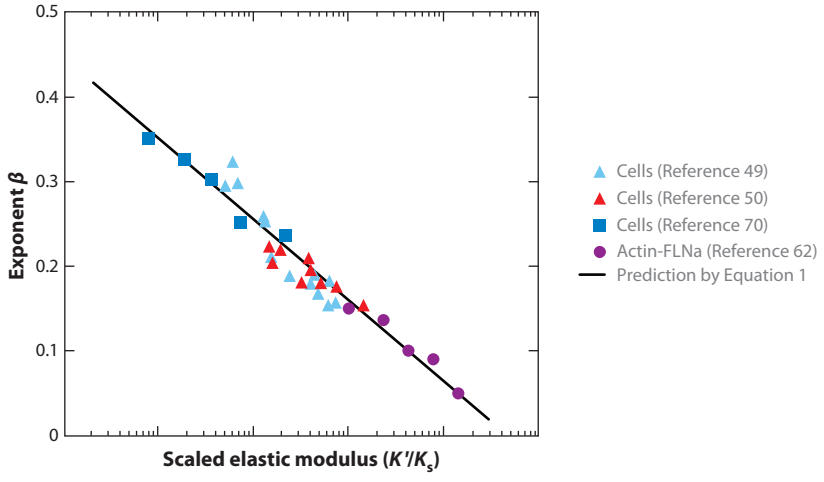


Figure 6

Scaling the data. The elastic modulus and the power-law exponent in cells are not independent but are related to each other by Equation 1. When data from different cell types as well as flexibly cross-linked actin networks are scaled by the prefactor j_0 and the timescale τ_0, such data collapse into a single master relationship. FLNa denotes filamin A. Note the logarithmic scaling of the x axis. The differential elastic modulus K' is the inverse of the creep compliance $J(t)$ measured at time $t = 1$ s or the elastic modulus $G'(\omega)$ measured at a radian frequency $\omega = 1$ s^{-1} for a fixed prestress.

2.5. The Role of Cytoskeletal Prestress

A second law of cell mechanics states that the stiffness of adherent cells increases with contractile tension (56). This can be demonstrated in a simple experiment by pressing the biceps muscle while lifting a heavy weight: The stronger the muscle cells contract, the stiffer they become. Such behavior can also be seen in contractile cells other than skeletal muscle. Moreover, the relationship between the differential elastic modulus K' (measured at a fixed time or frequency) and contractile tone is linear (with a geometry-dependent prefactor a) except for a small offset K_0' (**Figure 7**) (21, 56–58):

$$K' = K_0' + a\sigma_p. \qquad 2.$$

The contractile tone is here expressed as the internal stress σ_p in the cell body (56, 57). This internal stress exists even when no external stress is applied to the cell, and it is therefore referred to as prestress. K' is the response to small forces or deformations at a fixed value of prestress.

The theory of SGR does not explain force generation, prestress, or contractile stiffening. Unlike colloids and slurries, cells are not an inert material. Rather, cells are active and can generate large contractile stresses through the interaction between actin filaments and myosin motors. This interaction occurs at the molecular level through the formation of actomyosin bridges. When a bridge is formed and chemical energy in the form of ATP is available, parts of the myosin protein undergo conformational changes, termed a power stroke, that lead to a contractile force generation between the actin filament and the myosin filament. The myosin protein can then detach from its actin-binding site and reattach at a different location. The cyclic attachment, contraction, and detachment of multiple actomyosin bridges lead to the sliding of myosin and actin filaments against each other in opposite directions.

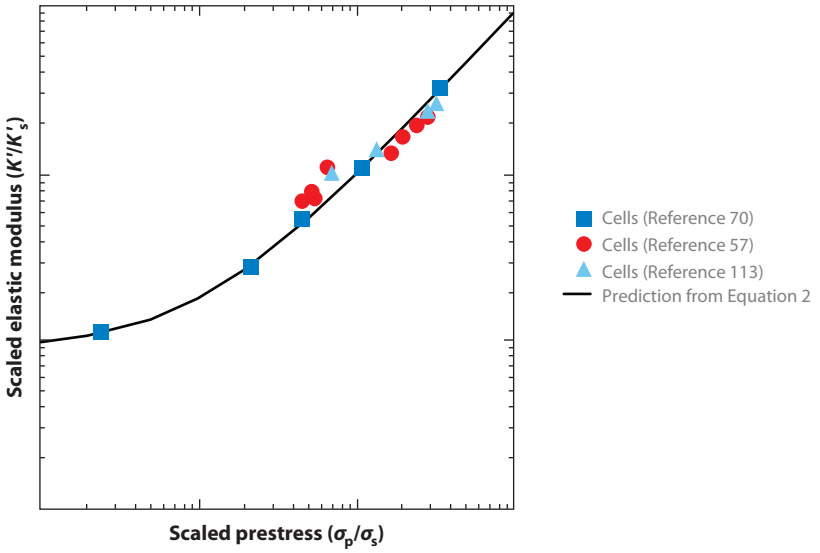


Figure 7

Contractile stiffening. The elastic modulus of cells is proportional to the active cytoskeletal prestress except for a small residual stiffness at zero prestress. Different data points correspond to different cell types. Data from different experiments were scaled (shifted in the x-y direction) to account for differences in experimental conditions or cell types.

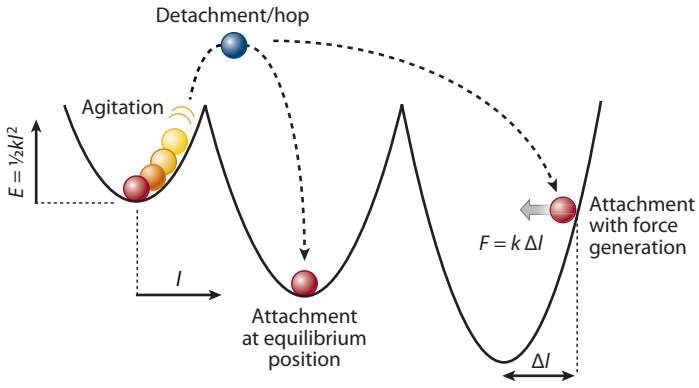


Figure 8

Potential well picture of the trap dynamics in a soft glassy rheology model with active force generation. Elements hop between quadratic potential wells of different depths or yielding forces. If attachment takes place at a position outside the trap minimum, a force is generated and leads to sliding as the element eventually moves toward the equilibrium position (60).

The sliding-filament theory by A.F. Huxley (59) captures this behavior. In its mathematical structure, Huxley's theory closely resembles Sollich's SGR model (46, 59). The SGR elements can be identified with myosin motors, and the energy wells can be identified with the binding energies between myosin and actin. The important difference is that in Huxley's model, the free elements that fall into a well do not fall in at the equilibrium position. Hence, these elements exert a force. The same idea of a so-called Brownian ratchet can be applied to Sollich's model and turns an inert soft glass into an active force-generating material (**Figure 8**) (60). Huxley's model as well as the active soft glassy model give a plausible explanation for a strictly linear relationship between force and stiffness because the number of elements that fall into energy wells sets both the stiffness and the force of the system.

An alternative explanation for contractile stiffening considers the spatial arrangements of the contractile actin and myosin network. When prestressed network fibers are laterally deformed, they resist with an apparent stiffness that is strictly proportional to the prestress (60), a fact well-known by musicians tuning their string instruments or by campers tightening the ropes of their tent. The tensegrity model of cell rheology expresses the same idea (3, 56, 57).

2.6. Connection Between Power-Law Response, Prestress, and Stiffness

We introduce above two universal laws of cell mechanics, one stating that stiffness is a function of the power-law exponent and the other stating that stiffness is a function of contractile prestress. For both laws to hold at the same time, the following relationship between prestress, stiffness, and the power-law exponent must also hold (61):

$$\beta = \frac{\ln\left[j_0(K_0' + a\sigma_p)\right]}{\ln \tau_0}. \qquad 3.$$

Indeed, the creep exponent obtained from the data in **Figure 5**, when plotted against the prestress, closely follows Equation 3 without any further adjustment of parameters (**Figure 9**).

Equation 3 can also be written with the exponent β as the independent parameter. In the framework of SGR theory, the exponent β reflects the noise temperature that determines the cytoskeletal prestress according to Equation 3, which in turn links the universal master

Tensegrity (tensional integrity): a concept adapted from architecture; stability in a prestressed structure arises from a balance between tension and compression

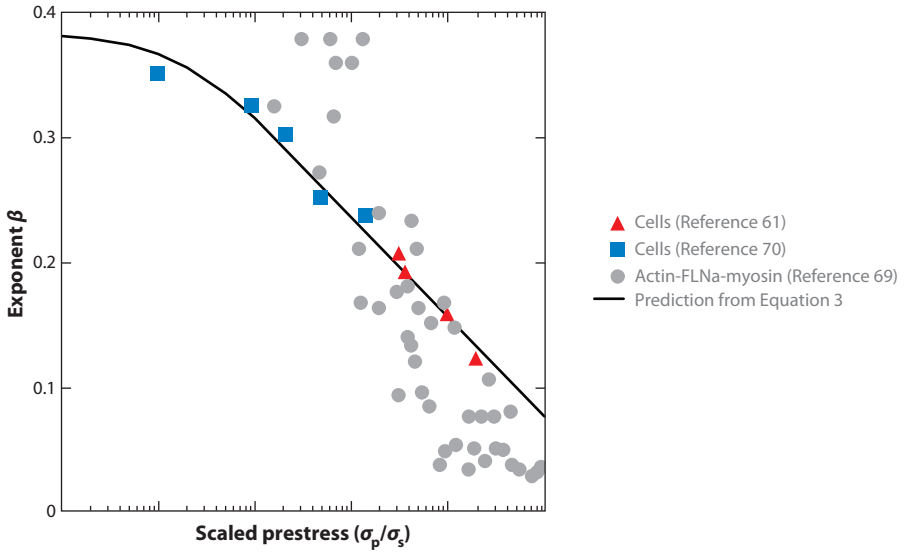


Figure 9

Power-law response and prestress. The power-law exponent decreases with increasing prestress according to Equation 3 (*black line*). Prestress data from different cell types and experimental conditions were scaled (shifted along the *x* axis) to account for differences in contractility. Also shown are data from actin–filamin A (FLNa) networks with active (myosin) cross-linkers.

relationships expressed in Equations 1 and 2. For cells and actin gels cross-linked with myosin motors, ATP-consuming processes set the contractile prestress, and they may also be at the root of the agitation energy and noise temperature *x* (47).

Surprisingly, Equation 3 also holds for inert cross-linked actin gels in the absence of active myosin motors. An externally applied stress can replace the contractile prestress in these systems. Under increasing external stress, cross-linked actin polymer solutions show a decrease in the power-law exponent and exhibit a more solid-like behavior (62). This implies that the total mechanical stress in the filament network is important, not so much whether the stress is internally generated by active motors or externally applied. As we see in the next section, this equivalence of internal and external stress has important consequences for the nonlinear behavior of cells under large external stresses.

3. NONLINEAR MECHANICAL PROPERTIES

3.1. Passive Stress Stiffening

As long as the applied external stress or strain is small, the viscoelastic response of the cell is linear, that is, independent of the magnitude of the external stress or strain (50, 63). This considerably simplifies the analysis of cell mechanical measurements and viscoelastic modeling. The studies discussed above were carried out mostly in the linear regime. In some cases, deviations from linear behavior were noted, but such deviations were not important enough to sacrifice linear response theory (25). Only a few studies explicitly reported a dependency of stiffness on external stress (34, 64), but the stiffening observed in these experiments was later attributed to geometrical effects of bead-based microrheology (26).

Stress stiffening: increase in a material's stiffness in response to increasing mechanical stress (externally applied or internally generated)

The first comprehensive study of the nonlinear viscoelastic properties of cells was carried out by Fernandez et al. (65), exploring for the first time the mechanical response of cells over a large, physiologically relevant range of cytoskeletal stresses. This was done by superimposing small stress fluctuations (for which linear response theory holds) over a larger stress offset using a microplate rheometer. Differential stiffness depended linearly on the externally applied stress, except for a small offset at small stresses. Identical behavior was reported for in vitro cytoskeletal networks consisting of actin and the flexible cross-linker filamin A (66). The conclusion from these data is that cells and cytoskeletal polymers are a stress-stiffening material in which the network stress controls the differential stiffness (66, 67).

Instead of application of an external stress, reconstituted cytoskeletal networks can be turned into a contractile material by adding active myosin motor proteins as cross-linkers and by controlling the amount of contractile prestress by adjusting the ATP and cross-linker concentration (68). By use of such a system, researchers showed that an actively generated stress and an externally applied prestress are equivalent and have the same effect on the differential stiffness (69). Recent data on a wide range of living cells support this finding and demonstrate that different amounts of stress stiffening can be fit by a master relationship; the total cytoskeletal stress (the sum of prestress and externally applied stress) is the only parameter that sets the differential stiffness (70). This master relationship is a generalization of Equation 2:

$$K'(\sigma) = \frac{\delta\sigma}{\delta\gamma} = K_0' + a\sigma, \qquad 4.$$

where K' is the differential stiffness and σ is the sum of contractile prestress σ_p and externally applied stress σ_e.

Data from the studies discussed above can be scaled into a single master relationship (**Figure 10**), establishing that Equation 4 describes a universal property of living cells and cytoskeletal networks. Moreover, Equation 4 readily explains why cells with high contractile prestress exhibit a much smaller amount of stress stiffening compared with soft cells under the same external force (70): The external force increases the total stress of the cytoskeleton in cells that are already highly prestressed by only a small fraction.

Integration of Equation 4 yields an exponential stress-strain relationship. Exponential stiffening at large strain values is a highly unusual property for most common materials, but as noted more than 150 years ago (71), it is a universal, characteristic property of many biological materials, in particular connective tissue (72, 73). Use of a microplate rheometer has also revealed exponential stiffening to occur in fixed cells (which prevents them from yielding at large strains) (65). The universality of stress or strain stiffening points to a structural mechanism that occurs at multiple different length scales rather than to a specific molecular origin.

When stress stiffening in cells and in vitro networks is compared, one should take into account that cells, in contrast to inert nonliving systems, exhibit active mechanosensing responses (74). Externally applied force is sensed and transduced into biochemical signals that result in remodeling and reinforcement of the cytoskeleton, leading to a drastic change of mechanical properties. The matter is further complicated by the fact that some cross-linker proteins such as filamin A seem to play an important role for the passive properties in cytoskeletal networks but are additionally involved in active mechanosensing and force regulation in living cells (27). Here we consider only the passive mechanical response of cells to an externally applied load. The contribution of active mechanochemical signaling responses can be filtered out by oscillatory loading and lock-in techniques or minimized by keeping the measurement time sufficiently short. Data on the low-frequency or long-time passive behavior of cells are therefore exceedingly difficult to analyze (75).

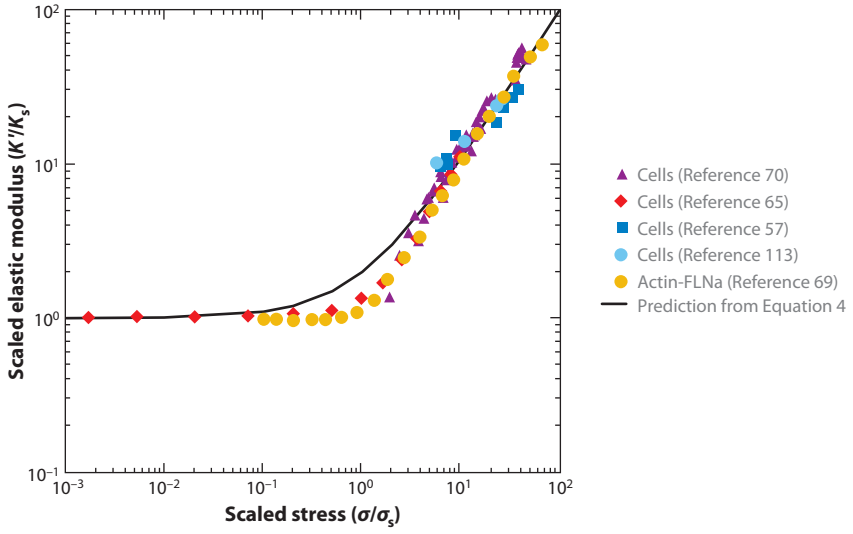


Figure 10

Universality and scaling of stress stiffening. The differential elastic shear modulus of different cell types and flexibly cross-linked actin networks versus internal cytoskeletal stress can be scaled into a single master curve. Scaling of stress and differential stiffness leaves the slope of the relationship unchanged. FLNa denotes filamin A.

3.2. Fluidization and Rejuvenation by Stretch

We see above that the relationship between stiffness and prestress (Equation 2) can be extended from the linear regime to the nonlinear regime at high loading stresses σ_e if we substitute the prestress σ_p by the total cytoskeletal network stress, $\sigma_p + \sigma_e$. The same approach, however, fails if applied to Equation 3, which would predict that cells become more solid like (i.e., the power-law exponent decreases) as they stiffen with increasing stress. Instead, the opposite behavior is observed: Cells fluidize in response to large stresses, as indicated by an increasing power-law exponent (70).

This behavior is not entirely unexpected. If cells, as suggested above, belong to the class of SGMs, then mechanical stretch contributes to energy injection, intermatrix agitation, and increased noise temperature, which in turn lead to more frequent hopping of elements out of their binding energy wells and hence to fluidization. Fluidization of adherent cells after a transient stretch has been experimentally confirmed (47, 76, 77); here we add that fluidization occurs during the application of stretch and is masked only by the concurrent stiffening behavior (70). Importantly, the amount of fluidization depends directly on the cytoskeletal prestress (or equivalently the power-law exponent through Equation 3) immediately before stretch. The stiffer, more contractile, solid-like cells fluidize during stretch more than the softer, less contractile, and fluid-like cells do (47, 70).

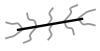
Stretch-induced fluidization is accompanied by a relaxation of contractility, a phenomenon that is well-known in smooth muscle physiology (78) and mechanistically explained by Huxley's sliding-filament model of muscle contraction (59, 78). Interestingly, the fluidization of most cells under stress-controlled loading conditions does not exceed a limit that corresponds to a power-law exponent of ~0.5. This value is also theoretically predicted for tensed semiflexible polymers (53). Power-law exponents larger than 0.5 during creep experiments are observed only immediately prior to catastrophic cell rupture or detachment (70).

4. THEORETICAL DESCRIPTION

4.1. Theoretical Models of Cell Mechanics

The constitutive relationships between elastic modulus, power-law exponent, and prestress described by Equations 1–4 are purely phenomenological. The small number of empirical parameters greatly simplifies the description of cell mechanical behavior but does not provide a mechanistic understanding. Researchers have developed a number of theoretical models that are able to explain and to predict certain facets of cell mechanics (**Table 2**), but currently no single model captures the full phenomenology of cell mechanical behavior.

Table 2 Comparison of the predictions of different theoretical models of cell mechanics[a]

Top down						
Model		**Concept**	**Stress stiffening?**	**Power-law rheology?**	**Stress-dependent exponent?**	**References**
	Linear viscoelasticity	Equivalent circuit of elastic springs and viscous dashpots	No	Requires a large number of elements	No	25, 33, 35, 36, 103
	Tensegrity	Prestressed structure made of stiff rods and tensed cables	Stiffness proportional to prestress + external stress	No	No	105–108
	Soft glassy rheology	Elastic elements hopping between energy traps, effective noise temperature	No (softening at high stress)	Yes	Fluidization with stress	44, 47, 109
Bottom up						
Model		**Concept**	**Stress stiffening?**	**Power-law rheology?**	**Stress-dependent exponent?**	**References**
	Worm-like chain	Entropic filament elasticity, viscous background, prestress	Yes	Crossover from elastic plateau to $G' \sim \omega^{0.75}$	Prestress broadens crossover regime $G' \sim \omega^{0.5}$	52, 54, 81, 83
	Nonaffine network deformation	Geometrical effects, bending-to-stretching transition of filament networks	Yes	No	No	87–90
	Cross-link unfolding	Network of stiff polymers with serially unfolding cross-links	Yes	Broad distribution of unfolding forces	No	85, 86
	Glassy worm-like chain	Filament relaxation slowed down due to sticky interactions	Yes	Power-law regime	Prestress decreases apparent exponent	94, 95
	Prestressed semiflexible chain	Propagation of stress, thermal fluctuations	Yes	Yes	Prestress decreases exponent	93
	Stiff filaments with flexible linkers	Randomly oriented cross-links and effective medium	Yes	No	No	91
	Sliding filament	Stiff filaments linked by active crossbridges	Yes (for prestress) No (for external stress)	Broadly distributed relaxation spectrum	Fluidization with stress	59, 96

[a]Red text denotes that the model does not predict the behavior in question. Blue text denotes that the model does predict the behavior in question.

4.2. Cytoskeletal Filaments and Networks

Although the springs and dashpots of linear viscoelasticity (Section 2.2) as well as the elements of SGR (Section 2.4) and the cables and rods in tensegrity structures (Section 2.5) can be identified with distinct structural components of the cytoskeleton, these models rest on generic principles that are valid independently of the details of the microscopic structure. All three approaches can therefore be considered phenomenological, or top-down. The reductionist method of physics, in contrast to the phenomenological approach, explains the properties of a system from the properties of its constituents. In the case of cell mechanics, this bottom-up strategy starts with quantitative in vitro studies of the constituents of the cytoskeleton. Such in vitro reconstituted systems can be prepared in a reproducible way with well-defined structural properties such as filament lengths or cross-linker densities (79). Once these systems are understood theoretically, it is expected that the functional modules can then be linked together into higher-order structures with emerging biological complexity (80).

Worm-like chain (WLC): a minimal physical model that describes the entropic elasticity of individual semiflexible polymers such as cytoskeletal filaments

The cytoskeleton of eukaryotic cells is a cross-linked network of semiflexible filaments (with a persistence length on the order of the distance between cross-links). A minimal model for the behavior of individual semiflexible filaments is the worm-like chain (WLC) model. The elasticity of a semiflexible polymer is entropic, with the individual filaments more likely to be curved than to be straight. Upon stretching, the number of available conformations and therefore the entropy are reduced, which generates an opposing force (81). The dynamic frequency-dependent properties can be obtained by considering friction of the thermally fluctuating filaments in a viscous background fluid (52). This model accurately describes the elastic as well as dynamic properties of reconstituted actin and other semiflexible polymer networks (82, 83).

The elastic and dissipative properties, however, are determined not only by the filaments and the viscous background but also by the properties of the cross-linkers. For instance, unbinding of transient cross-links introduces additional dissipation and alters the frequency response (84). Simulations of filament networks with serially unfolding cross-links showed that under prestress, many links are at the threshold of domain unfolding, which can give rise to collective unfolding, a broad distribution of lifetimes, and power-law rheology (85, 86).

When filaments and cross-linkers are combined into networks, additional geometric effects come into play. At large strains, the deformation is nonaffine (87, 88), meaning that individual filament strain and network strain differ. As more and more fibers are pulled straight, filament stretching begins to dominate over filament bending (89, 90). Broedersz et al. (91) use a self-consistent effective medium approach to examine the role of flexible cross-linkers in stiff filament networks. Here, the onset of stiffening occurs when the cross-links are stretched toward their full extension. These investigators show that the stiffening response crucially depends on cross-linker and filament length, in good agreement with experiments (92).

These models provide several mechanisms to account for the stress-stiffness response of cytoskeletal networks, but they are less successful in modeling the weak power-law dependency of elastic moduli on frequency. Two recently developed theoretical descriptions deal with this issue. Rosenblatt et al. (93) relate the propagation of force along a prestressed nonlinear semiflexible chain to the creep response of cells. The model is a combination of tensegrity and the WLC description, and it correctly predicts a decrease in the power-law exponent with increasing prestress. The other approach, termed the glassy worm-like chain (GWLC) model, combines the WLC model with concepts from SGR (94). It describes the retardation of filament relaxation due to sticky interfilament interactions, leading to an exponential stretching of the $\omega^{3/4}$ relaxation spectrum of individual filaments. The idea behind this concept is generic and applicable to cells and other heterogeneous biological materials. The model can explain power-law rheology and stress

stiffening as well as fluidization and plasticity (94, 95). As with all microscopic filament–based descriptions, however, a quantitative prediction of the shear modulus for cells is difficult because structural parameters of the cytoskeleton such as mesh size or filament length are highly variable.

None of the above models can account for the active contractility of the cytoskeleton. On a microscopic level, the mechanism of contractile force generation is the interaction of actin and myosin filaments, which is well captured by Huxley's sliding-filament model of skeletal muscle (59, 96). Highly contractile cells such as fibroblasts and myoblasts exhibit a remarkably muscle-like mechanical phenotype, and Huxley's model captures the dynamics of force generation in these cells against an elastic load (97, 98). Moreover, the model can explain the linear dependency of stiffness on prestress but in its simple form does not predict a power-law frequency dependency. The Huxley model resembles the SGR model in that it contains multiple binding interaction energies with the addition of a force-generating mechanism based on a Brownian ratchet. From this perspective, a combination of the SGR model and muscle contraction models appears to be a promising approach for describing cell mechanics (60).

4.3. Top Down Versus Bottom Up: Lessons from Muscle

The history of muscle modeling provides an interesting example of how phenomenological and mechanistic models can complement each other. When A.V. Hill formulated his active-state model of muscle contraction (99), which is a viscoelastic equivalent circuit with springs and dashpots, he knew next to nothing about the microscopic structure of the muscle and the mechanisms of force generation. Huxley's sliding-filament model, on the contrary, follows a reductionist approach to quantitatively explain overall muscle tension from the molecular interactions between actin and myosin. Both models can generate valid macroscopic predictions, and neither of them is correct or incorrect; they differ only in their complexity. The Hill model is still used today to fit data whenever the molecular details are not considered relevant or are unknown.

The quantitative match between bottom-up and top-down predictions in the case of muscle contraction was possible because muscle has a well-defined and regular structure. In the case of cells, however, scaling up molecular-level models to the whole-cell level is difficult due to the structural complexity of the cytoskeleton. Although the mechanical properties of various molecular constituents such as cytoskeletal filaments and cross-linkers are well characterized, deriving macroscopic mechanical predictions from this knowledge in any but the most simple model systems is not possible. Moreover, theoretical models based on a full microscopic description are not likely to yield much more additional insight. The structural complexity of the cell requires a different approach, discarding the reductionist search for a microscopic mechanistic model to which all macroscopic observations can be traced back. A successful comprehensive model of cell mechanics will likely be both generic and mechanistic. It will incorporate mechanisms such as filament fluctuations, protein unfolding, bond dynamics, actin-myosin cycling, and geometric network mechanisms on different hierarchical levels to relate microscopic interactions in the cytoskeleton to the macroscopic mechanical behavior of cells.

SUMMARY POINTS

1. Measurement of mechanical properties of living cells has become a standard tool in cell biology. A wide range of microrheology techniques are available, each with specific advantages and limitations.

2. The linear and nonlinear viscoelastic properties of cells conform to a limited set of universal scaling laws. Creep and stress relaxation behavior follows a power law over several orders of magnitude of time or frequency. The power-law exponent characterizes the state of the cell between a solid and a fluid and is related to the contractile prestress of the cytoskeleton as well as to stiffening and fluidization behavior in response to large stresses or strains.
3. This universal behavior can be reproduced consistently with different microrheology techniques and across different cell types treated with different pharmacological interventions. It can also be reproduced in simplified model networks of cytoskeletal filaments.
4. The biological relevance of such universal scaling behavior is wide ranging. By a single mechanism that is present in all eukaryotic cell types, namely, by modulating the activity of actomyosin contraction and hence the cytoskeletal prestress, cells can stiffen or fluidize and thus adapt to different mechanical loading conditions. Moreover, by modulating their contractile prestress, cells also change the mechanical state of their environment, which is again sensed by neighboring cells. This mechanical feedback loop may be essential for the control and robustness of many developmental and growth processes.
5. Investigators have proposed numerous different theoretical models that capture different aspects of cell mechanical behavior at different functional and structural levels, ranging from considerations of the cytoskeletal network architecture down to single-protein binding kinetics. A comprehensive understanding of the physical mechanisms that govern these universal scaling laws, however, is still emerging.

DISCLOSURE STATEMENT

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94. Combined the mechanistic WLC description with the phenomenological SGR concept.

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Errata

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