Active soft glassy rheology of adherent cells

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Adherent cells show a wide range of complex mechanical behavior that traditionally has been accounted for by different mechanisms and models, each of which can explain a limited subset of cell behavior. Experimental evidence suggests that nearly all aspects of mechanical cell behavior are closely associated with the cell's contractile machinery of actin and myosin filaments. We propose that the molecular details of actin–myosin interactions can be combined in a unified active soft glassy model that considers the arrangement of stress fibers at the macro-scale, and the soft-glassy non-equilibrium interaction of myosin and actin filaments at the micro-scale.

Introduction

The rheological properties of cells are of utmost importance for some of the most essential and basic cell functions such as migration, proliferation, phagocytosis or contraction. Consequently, derangements of cell rheological functions are often associated with severe disorders such as inflammation, cancer, or cardiovascular diseases.

Adaptations of this statement can be found at the beginning of nearly all reports dealing with cell rheology. It is a compelling beginning, so it seems, but not necessarily so for many biologists to whom the concept of rheology remains alien and unconnected with their daily world of proteins, signaling pathways and gene regulation. The aim of this article is to highlight recent findings that demonstrate how structural and molecular mechanisms contribute to cell rheology, and vice versa, how molecular mechanisms can be understood from cell rheology measurements.

In a strict sense, the rheology of adherent cells describes their stress–strain relationship as measured in a rheometer. Either the cell is mechanically stressed, and the resulting cell deformations are measured, or vice versa.[1] Tremendous progress has been made in the past decade in the development of methods that can resolve forces in the range of pico-Newtons and displacements in the nanometer range. This means that the biochemical and thermodynamic behavior of proteins and protein–protein interactions has become accessible to mechanical measurements with high accuracy and time resolution in the living cell. Moreover, the results obtained with vastly different rheological methods in different laboratories worldwide agree comfortably with each other in that cell rheology obeys few, clearly defined empirical laws, as summarized in a recent review.[2] Despite this fortunate state of affairs, there is an ongoing struggle to gain a mechanistic understanding of what these empirical laws mean. Concepts derived from soft matter physics appear to account for many of the phenomenological effects,[2,3] yet it is unclear how these concepts translate into the language of cell biology.

Biologists rarely use the term "rheology" but prefer "cell mechanics" instead. For the biologist, cell mechanics not only encompasses the stiffness, softness, or fluidity of the cell, but also, and more importantly, its shape, spreading

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area, migration behavior, and contractility. There are good reasons to go beyond the stress–strain relationship if one wants to understand the mechanical behavior of cells. One of them is that cells are not an inert material as, say, colloids and slurries, but rather they are active in the sense that they can generate large internal stresses. The molecular mechanism by which internal stresses are generated is the interaction between the actin filaments and myosin motors. As we discuss below, acto–myosin interactions can account for an extraordinarily wide range of mechanical phenomena seen in cells.

Stress–strain relationship: soft glassy behavior

Cell rheology can be determined using a customized "micro-rheometer" in which the relationship between shear stress and strain is measured. Most commonly, a micron-sized bead is brought into contact with the cell surface, and the bead movements in response to defined forces are recorded.[1] For instance, the bead can be indented into the cell body by an AFM cantilever, or, after the bead has been bound to cell surface receptors, it can be moved about in an optical or magnetic trap. Measurements can then be made either in the time domain by measuring the creep or stress relaxation response, or in the frequency domain by applying small amplitude sinusoidal oscillations of various frequencies to the bead.

One of the most striking results from these measurements is that in the linear range at small amplitudes, the creep response $J(t)$, the stress relaxation response $F(t)$, and the complex modulus $\tilde{G}(\omega)$ all follow a power-law in time, t, or frequency, ω: $J(t) \sim t^{\alpha}$; $F(t) \sim t^{-\alpha}$; $\tilde{G}(\omega) \sim (j\omega)^{\alpha}$.[4,5] This power-law behavior holds over a remarkably broad range of measurement times or frequencies between 0.01 Hz and 1 kHz. Power-law behavior has been observed for a broad range of diverse cell types and for cells treated with almost any imaginable pharmacological intervention.[6,7] Power-law behavior also holds regardless of the measurement method used, the type of bead or probe, its shape, size, or surface functionalization.[7–10]

It has been noted that generic concepts of soft glassy rheology lend themselves straightforwardly to an interpretation of power-law cell behavior. Accordingly, the cell is imagined to consist of many disordered elements held in place by attractive or repulsive bonds, traps, or energy wells formed between neighboring elements.[4] The binding energies are weak enough to allow the elements to occasionally hop out of their trap and change their position. Power-law rheology arises from a wide distribution of energy well depths such that the distribution of element lifetimes is scale-free.[11]

Soft glassy rheology can also account for some other phenomena observed in cells, such as the fluidization after a transient stretch, the subsequent aging as well as yielding at large external stresses.[2,12] But the generic picture of the cell as a soft glassy material, as pleasing as it may seem to physicists, does not answer a pertinent question of particular interest to biologists, namely, what are those elements in the cell, and what is the molecular basis of the interactions between the elements?

The contractile machinery of cells

In the following, we suggest that the two most abundant proteins inside living cells, namely actin and myosin(II), provide a plausible answer to this question. Both, actin and myosin(II) can assemble, or polymerize, into filaments, and then interact with each other by forming acto–myosin bridges. When a bridge is formed and chemical energy in the form of ATP is available, parts of the myosin protein undergo conformational changes, called a power-stroke, that lead to an attractive, or contractile, force generation between the actin and the myosin protein. The myosin protein can then detach from its actin binding site and re-attach at a different location. The attachment, contraction, and detachment of multiple acto–myosin bridges along the filaments leads to their sliding against each other in opposite directions. This so-called sliding filament theory describes the action of skeletal muscle, but it is also applicable to smooth muscle and contractile non-muscle cells.[13,14]

A. F. Huxley, one of the scientists who developed the sliding filament theory, proposed in 1957 a mathematical model of acto–myosin interaction that resembles Sollich's model of soft glassy rheology, with the elements being myosin motors, and the energy wells being the binding energies between myosin and actin.[15] Importantly, however, free elements that fall into a well do so not at the equilibrium position but at some position to the right. Hence, these elements exert a leftward force, which is how Huxley envisioned the forces are generated in muscle. The same simple idea of a Brownian ratchet can be applied to Sollich's model, which would turn an inert soft glass into an active material (Fig. 1).

Contractility and stiffness

Cells are able to actively tune their stiffness by modulating their contractile tone. The stronger the cells contract, the stiffer they become. The relationship between

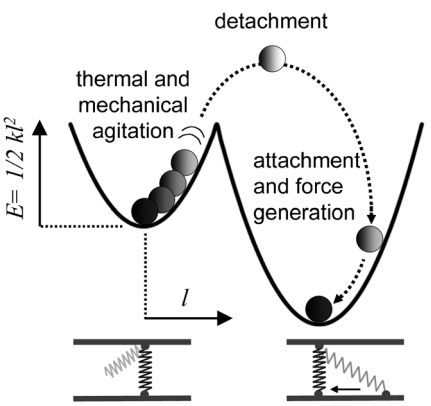

Fig. 1 Potential well picture of the trap dynamics in an active soft glassy rheology model with force generation (adapted from ref. 11). Elements hop between quadratic potential wells of different depth E or yielding force. If attachment takes place at a position l outside the trap minimum, a force kl (with spring constant k) is generated and leads to sliding as the element eventually moves towards the equilibrium position.

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stiffness and contractile tone turns out to be strictly linear.[16] The active soft glassy model would immediately give a plausible explanation for this observation, because the number of elements that have fallen into energy wells set both the stiffness and the force of the system.[15] In cells, however, there appears to be another reason for the proportionality between contractility and stiffness that is not explained by soft glassy behavior. Rather, it has to do with the spatial arrangements of the contractile machinery into stress fibers – bundles of actin and myosin that criss-cross the cell and are connected *via* focal adhesions to the extracellular matrix onto which the cells grow (Fig. 2 left).

These stress fibers are under tension even when no external stress is applied to them, therefore this tension is often referred to as pre-stress.[16] It is trivial to show that when these tensed stress fibers are laterally deformed, they resist with an apparent stiffness that is strictly proportional to the pre-stress (Fig. 2 right), a fact well known by musicians tuning their string instruments, or campers tightening the ropes of their tent. The very same idea is expressed by the tensegrity model of cell rheology,[3] although much unnecessary confusion has been caused by the search for an intracellular component that is under compression to counterbalance the pre-stress. As it turned out, most of the pre-stress in well-spread adherent cells is counterbalanced by the extracellular matrix.[16,17]

Lateral bending of the stress fibers and filaments accounts for another essential mechanical property of living cells and most semiflexible biopolymer networks, namely stress or strain stiffening (Fig. 2 right).[18–21] This stiffening comes from two sources, one being the increase in spring tension with increasing indentation, and hence an increase in stiffness, as explained in the previous paragraph. The other source is the non-linear geometric cosine dependence between lateral indentation and fiber lengthening that ultimately leads to an alignment of the stress fibers in the direction of indentation. Recent AFM measurements of stress fiber stiffness in living cells as a function of strain and pre-stress are in agreement with the prediction that stiffness increases with pre-stress, and that the stiffening response appears more pronounced at lower pre-stress (Fig. 2).[22]

The mechanism of stress-stiffening of the cell as a structure is independent of the mechanisms that give rise to the stiffness of the cytoskeletal network at a microscopic level, which may be enthalpic (bending and stretching of filaments and crosslinkers as in Huxley's model), entropic (stretching out thermal fluctuations), or non-affine (bundle formation) depending on the polymer and crosslinker concentration.[23,24] Interestingly, enthalpic, entropic and non-affine effects can also explain a pre-stress dependence and strain stiffening of semiflexible biopolymers by the same geometric mechanism as illustrated in Fig. 2 but on a much smaller scale, in that both external strain as well as internal pre-stress can pull thermally or otherwise bent individual or bundled filaments straight.[3,18,23,25,26]

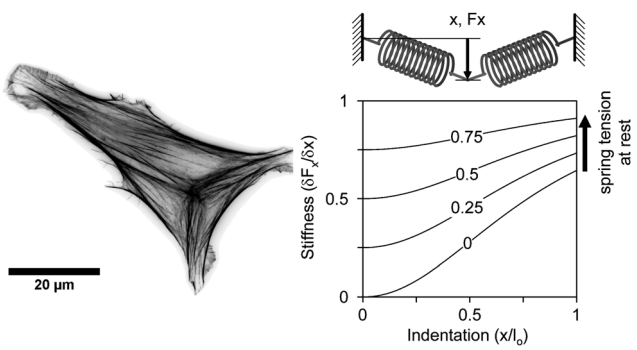

Fig. 2 Left: Contractile acto–myosin stress fibers inside a NIH 3T3 fibroblast. Right: Two connected tensed springs with unit stiffness and unit length are indented in the middle by a distance x under the force F_x. For small indentations, the lateral stiffness is proportional to the spring tension at rest; at higher deformations, geometric stiffening occurs. The stiffening response differs for different resting spring tensions, shown here between 0 and 0.75.

Contractility and dissipation

Power-law rheology implies that the power-law exponent α is a monotonic function of the energy that is dissipated during a mechanical perturbation cycle, G'', relative to the energy that is elastically stored, G': $G''/G' = \tan(\alpha\pi/2)$.[4] In the soft glassy rheology framework, α corresponds to the agitation energy of the elements relative to the average energy trap depth.[11] As elements hop out of their trap, all their elastic energy is dissipated to heat. Hence, dissipation is directly linked to elasticity. In cells, it has been observed that the power-law exponent decreases with increasing cell stiffness and increasing contractile tone.[7,9,27] This observation can be readily explained by the active soft glassy model (Fig. 1). Once the cell's contractile machinery is fully activated and stabilized, the binding affinity between actin and myosin – and with it the energy trap depth – is increased, and the elastic cell properties become more dominant.[28,29]

A decreasing power-law exponent with increasing strain can also be observed in myosin-free reconstituted model systems of the cytoskeleton consisting of actin and crosslinkers.[19,30] Obviously, acto–myosin cycling is not the only source of energy dissipation in cells. Another conspicuous source is viscous dissipation by thermally fluctuating cytoskeletal filaments.[31] As these filaments become stretched, the amplitude of thermal fluctuations and therefore viscous dissipation decreases accordingly.[3,18,25,30] Viscous or non-viscous dissipation by the non-thermal, rheometer-driven motion of cytoskeletal filaments through their surrounding background fluid (or background colloid), as well as cross-linker unfolding or binding dynamics may be another source, although it is less obvious in this situation how an increasing pre-stress would cause a decreasing power-law exponent.[19] But even so, myosin comes into play as it is the cell's dominant mechanism to control the pre-stress of the cytoskeleton.

Conclusion

Several different explanations and models have been constructed to account for the complex rheology of living cells. The soft glassy model accounts for power-law

rheology at low frequencies as well as for strain softening, yielding, and aging behavior. The thermal fluctuation model of semiflexible worm-like chains accounts for high frequency dissipation. Recent work has extended the semiflexible worm-like chain model to high strains such that it can account for the pre-stress- and strain-stiffening. At the level of the whole cell, pre-stress dependence and strain-stiffening is accounted for by simple geometric relationships of pre-stressed elastic networks (tensegrity networks). Pre-stress generation and contractility in cells can be explained by Huxley's sliding filament model.

In this article, we suggest that the ideas expressed in these models need not be mutually exclusive but can be combined in a simple, active soft glassy model that considers the arrangement of stress fibers at the macro-scale, and the soft-glassy non-equilibrium interaction of myosin and actin filaments within the stress fibers at the micro-scale. By adjusting the myosin motor activity, the cell is able to modulate at once its stiffness and its liquidity or solidity, expressed by the rheological power law exponent.

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