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Rheological Behavior of Living Cells is Timescale Dependent

Dimitrije Stamenović,* Noah Rosenblatt,* Martín Montoya-Zavala,[†] Benjamin D. Matthews,[†] Shaohua Hu,[‡] Béla Suki,* Ning Wang[¶] and Donald E. Ingber[†]

*Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA; [†]Departments of Pathology and Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA; [‡]Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA; [¶]Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

ABSTRACT The dynamic mechanical behavior of living cells has been proposed to result from timescale-invariant processes governed by the soft glass rheology (SGR) theory derived from soft matter physics. But this theory is based on experimental measurements over timescales that are shorter than those most relevant for cell growth and function. Here we report results measured over a wider range of timescales which demonstrate that rheological behaviors of living cells are not timescale invariant. These findings demonstrate that although SGR appears to accurately predict certain cell mechanical behaviors, it is not a unified model of cell rheology under biologically relevant conditions and thus, alternative mechanisms need to be considered.

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Address reprint requests and inquiries to Dimitrije Stamenović, Tel.: (617) 353-5902; Fax: (617) 353-6766; E-mail: dimitrij@bu.edu

The ability of mammalian cells to deform in response to mechanical forces is essential for behaviors that are critical for life, and thus many seek to identify general principles that govern cell rheology. It has been proposed recently that cell deformability results from timescale-invariant processes governed by soft glass rheology (SGR) theory derived from soft matter physics (1-5). The pivotal idea of SGR is that soft glasses are non-equilibrium systems that flow slowly when stressed such that their material moduli scale with the time and frequency of loading according to a single weak power-law (6). This behavior indicates that the rheology of these materials is governed by timescale-invariant processes, regardless of the physical basis for this behavior. SGR has recently gained great interest in biology because similar single power-law behavior has been observed in various cultured cell types with different techniques when analyzed over limited timescales using creep (10^{-2} - 10^2 s) (2,3,7) and oscillatory (10^{-2} - 10^3 Hz) (1,4,8) measurements. Based on these observations, SGR has been purported to be a fundamental property of living cells (5). The finding that a theory from soft matter physics can describe certain cell behaviors is extremely intriguing. But the timescales explored in those studies are not relevant for most physiological cellular processes (e.g., mitosis, motility, differentiation, apoptosis, secretion) that take place over minutes to hours within cells that continuously remodel their cytoskeleton. Thus, the biological relevance of SGR remains unclear.

To explore whether SGR is applicable at these physiological timescales, we measured the oscillatory response of the same cultured human airway smooth muscle (HASM) cells that were used in the original studies that described the SGR phenomenon (1-3) and with the same oscillatory twisting magnetometry technique (1), except that we used a wider range of frequencies (10^{-3} - 10^3 Hz). The magnitude of the cell dynamic modulus ($|G^*|$) was determined using two protocols: one where $|G^*|$ was measured over a high frequency (f) range (10^{-1} - 10^3 Hz), and the other where the effects of lower frequencies (10^{-3} - 10^0 Hz) were studied. Detailed description of experiments is given in Supplementary Material.

Interestingly, rather than observing a single power-law response, we found that the $|G^*|$ vs. f relationships exhibited two distinct power-law regimes – one (10^0 - 10^3 Hz) less frequency-dependent, with exponent $\alpha_1 = 0.2 \pm 0.004$, and the other (10^{-3} - 10^{-1} Hz) more so, with exponent $\alpha_2 = 0.33 \pm 0.034$, separated by a third plateau regime (10^{-1} - 10^0 Hz) (Fig. 1); the values of α_1 and α_2 were significantly different ($p < 0.001$). Importantly, analysis of the data from 10^0 to 10^3 Hz is fully consistent with previous reports claiming that HASM cells exhibit a single power-law (1); however, by including the data from 10^{-3} to 10^0 Hz, it is obvious that these same cells exhibit multiple regimes that cannot be described by a single power-law. Furthermore, when we modulated the cytoskeletal prestress – which is an important determinant of cell rheological behaviors (1-4,9) – by uniform (equibiaxial) stretching the substrate beneath

adherent HASM cells (10), we still observed the two power-law regimes (Fig. 1), indicating that this behavior is robust.

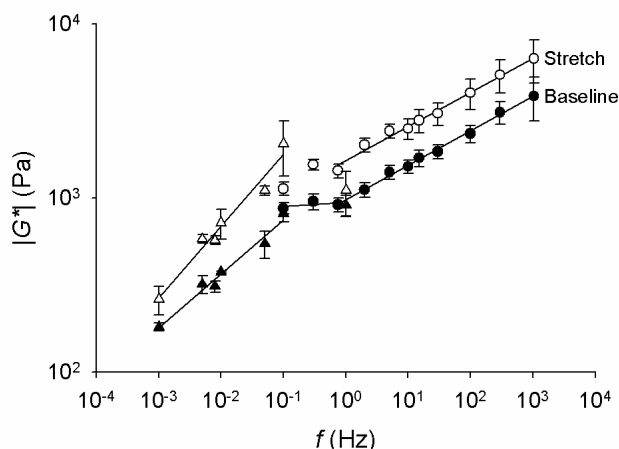


FIGURE 1 The magnitude of the dynamic modulus ($|G^*|$) vs. frequency (f) relationship measured in cultured HASM cells at the baseline (solid symbols) and following cell stretching by a $\sim 12\%$ uniform substrate strain (open symbols) using oscillatory magnetic cytometry. Cells displayed two power-law regimes at 10^0 - 10^3 Hz and at 10^{-3} - 10^{-1} Hz, separated by a plateau (10^{-1} - 10^0 Hz). Each regime was fitted by a function $\sim f^r$ (solid lines). Data are means \pm SE of median values of $|G^*|$ obtained from 180-500 magnetic beads distributed in 3-5 different wells for each condition (circles and triangles indicate data from two protocols).

To rule out the possibility that these results are an artifact of using two different experimental protocols, and to independently confirm the generality of this multi-power-law mechanical behavior, we measured the creep response (0.01-120 s) of a different cell type – bovine capillary endothelial (BCE) cells – using a different technique (electromagnetic pulling cytometry) that applies sustained tension rather than oscillatory shear (11). Again, we confirmed the existence of two power-law regimes with an intervening plateau (see Supplementary Material), and this is consistent with previous reports (7,11,12).

An *a priori* prediction of the SGR model is that if living cells are glassy systems, then they should deform slowly following a single power-law regime. Our oscillatory and creep data, taken together with past sporadic findings (7,11,12), clearly show that this is not the case. Thus, the underlying processes that govern the rheology of living cells are not timescale-invariant; instead cells exhibit a transition between power-law regimes that occurs over a well defined timescale. Moreover, the transition time appears to be cell type-specific: in HASM cells, it occurs between 0.1 and 1 Hz (i.e., 1-10 s) (Fig. 1), whereas it appears around 10 s and 100 s in BCE cells (11) (see Supplementary Materials)

and myocytes (7), respectively. Hence, SGR is not as universal a model as purported (5) when it comes to describing the rheological behavior over the full range of timescales that are biologically relevant for living cells.

While SGR has effectively described several dynamic mechanical behaviors of cells (1-5), it has not provided insight into underlying molecular mechanisms, hence its value for cell biology remains limited. In contrast, analysis of cell rheology over a wider range of timescales, as described in the present study, could lead to new and more biologically meaningful explanations. For example, theoretical studies of the viscoelasticity of myosin-activated entangled actin networks show that they exhibit multiple power-law behavior during stress relaxation (13) that is consistent with the behaviors observed here. This results from increased longitudinal fluctuations of individual actin filaments due to the mechanical action of molecular myosin motors. However, the longitudinal motion of actin filaments is constrained by multiple crosslinks in living cytoskeletal networks. Moreover, this theory also predicts that increased longitudinal motion of actin filaments will lead to fluidization of the network (13), whereas living cells (1-4) and crosslinked actin gels (14) stiffen when activated by myosin motors. Thus, it seems unlikely that the multiple power-law behavior we observed is due solely to the behavior of activated entangled actin networks in living cells.

Alternatively, the emergence of the second power-law regime at low frequencies could be due to cytoskeletal remodeling. However, when we treated HASM cells with jasplakinolide (0.1 μ M, 3 min), a drug that induces polymerization of actin filaments, we observed no changes in the low-frequency response (see Supplementary Material). Another possibility is that the high-frequency response is determined primarily by the rheological properties of individual actin filaments (15), whereas the low-frequency response results from these properties combined with the behavior of the higher order (cell-wide) cytoskeletal network; but currently there is no experimental evidence to support this claim. Finally, the high-frequency response might be based on the mechanics of the highly flexible submembranous ankyrin-spectrin-actin cytoskeleton, whereas the low-frequency response could be due to the internal microfilament-intermediate filament-microtubule lattice.

A novel observation is the existence of a well defined timescale regime – the plateau (Fig. 1) – where no power-law behavior can be seen. The mechanism responsible for the plateau also remains unclear. This type of plateau behavior can occur when one power-law regime saturates for some reason, such as due to finite system size. Thus, for example, it could be due to a limited length distribution of actin filaments in the

cytoskeleton when analyzed at high frequencies. In contrast, a completely different mechanism may come into play at lower frequencies (e.g., limited length distributions of intermediate filaments and/or microtubules), and this could generate another power-law regime with a distinct exponent. It is not clear what biological advantage this plateau region might offer from an evolutionary perspective, but considering that energy dissipation is reduced when $|G^*|$ is frequency independent, it may significantly increase the cell's functional efficiency. In this context, it is noteworthy that breathing and heartbeat frequencies fall within the range of the plateau region in most species.

We also considered the possibility that nonlinearities of the cytoskeleton may contribute to the emergence of the second power law since the nonlinear behavior of HASM cells became apparent below 10 Hz, indicated by the change in the shape of bead displacement vs. specific torque loops from elliptic (linear) to sigmoid (non-linear) (see Supplementary Material). This emerging nonlinear behavior may reflect material nonlinearities of intermediate filaments (16) and actin stress fibers (17), that might become prominent with increasing bead displacements amplitude at low frequencies, or some other mechanisms, and together they could contribute to a new power-law regime. To explore this further, we calculated an index of nonlinearities, the harmonic distortion index (K_d), which characterizes the extent to which the input-output loop deviates from the linear elliptic shape. In a linear system, $K_d = 0$, and in the presence of nonlinearities $K_d > 0$ (18). These studies revealed that K_d steadily increased with decreasing f , but this dependence did not become suddenly greater at low frequencies, indicating that the nonlinearities did not correlate with the transition to a new power-law that was observed in the $|G^*|$ vs. f relationship (Fig. 1) (see Supplementary Material).

In summary, regardless of the underlying mechanisms that govern rheological behaviors of cells, our data reveal that these mechanisms are not timescale-invariant, as previously reported (1,2,4). Instead, multiple power-law behavior, often with an intervening plateau, appears to be a property of various types of adherent living cells over the full range of time scales that include physiologically relevant frequencies. These findings demonstrate that SGR is not a unified model of cell rheology and suggest that different micromechanical mechanisms also need to be considered. Whether the SGR model can be integrated into a more general model of cell mechanical behavior remains to be seen. However, an effective model needs to incorporate quantitative descriptions of cell behavior amenable to computational and structural analysis in the context of physiological relevant molecular structures in living cells.

SUPPLEMENTARY INFORMATION

An online supplement material of this article can be found by visiting BJ online at <http://www.biophysj.org>.

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