

invited editorial

Biology lessons from oscillatory cell mechanics

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HISTORIANS AT THE TURN OF this century, reflecting on the tremendous advances in science and technology, have argued that the 20th century will be forever remembered as the century of physics, whereas the 21st century will belong to the biologists. As the human genome project has neared completion, it is hard not to speculate how detailed knowledge of our genetic heritage will forever change our world. At the same time, it is becoming ever clearer that knowing our blueprint generates many questions about the function of genes and their gene products. In the pursuit of these questions, we are frequently reminded that molecular interactions occur in an ordered environment and that order, as represented in cell and tissue architecture, is a pivotal determinant of all function. This is true on many scales, ranging from the whole organism to individual organelles. Sophisticated new imaging techniques have allowed biologists to describe cellular and subcellular architecture in ever increasing detail, while challenging them to incorporate the added information within the framework of functioning mechanisms. However, capturing architecture on all biologically relevant scales, including that of individual molecules, requires more information than a detailed image. Defining the mechanical properties of biological structures might be one way to fill this gap.

The mechanical properties of cell membranes, cytoskeletal proteins, and whole living cells have all been measured. The various techniques used for this purpose include atomic force microscopy, optical traps, glass micromanipulators, disc rheometers, and magnetic twisting cytometry. In this issue of the *Journal of Applied Physiology*, Maksym and colleagues (7) describe their initial observations on the oscillatory behavior of adherent human airway smooth muscle (HASM) cells measured with magnetic cytometry. The idea to use magnetic beads in cell research dates back to the 1950s (1) and was revisited in 1984 by Valberg (9), who wanted to study particle handling by phagocytes. In 1993, Wang, Butler, and Ingber (10) introduced step cytometry, which, for the first time, made it possible to probe the mechanical properties of the cytoskeleton directly through specific transmembrane adhesion receptors. To this end, ligand-coated ferromagnetic beads are allowed to associate with cells; the beads are magnetized and then manipulated (“twist-

ed”) with magnetic fields of known strength and orientation. The apparent average bead rotation is measured with an in-line magnetometer and serves as an estimate of average local strain. To the extent to which cells resist a local shape change, their mechanical properties can be computed from applied torque (proportional to magnetic field strength) and apparent bead rotation. Step cytometry provided new and important insights into the mechanical coupling between cytoskeleton and transmembrane receptors and lent support to the idea that the cytoskeleton of living cells behaved like a tensegrity network (6).

The report by Maksym and colleagues on the oscillatory mechanics of HASM cells in this issue of the *Journal* is of interest for several reasons. Compared with conventional step cytometry, changes in system configuration (i.e., the orientation of the twisting field) have substantially improved the signal-to-noise ratio. As pointed out earlier by Fabry and colleagues (2), heterogeneous bead binding biases step cytometry toward low apparent stiffness values. This is because unbound or weakly bound beads, which experience large rotations, contribute disproportionately to the change in the remnant magnetic field from which average stiffness is calculated. Although this bias cannot be totally eliminated in any configuration, magnetic oscillatory cytometry (MOC) seems to be less prone to it. Maksym and colleagues also reaffirm that the mechanics of HASM cells are sensitive to changes in prestress and interconnectedness of the actin cytoskeleton and emphasize that this would not be the case if step cytometry and MOC simply probed cell membrane properties. However, the most interesting and thought-provoking finding is the strong correlation between elastic and frictional stresses under virtually all experimental conditions. What are biologists to take away from this?

To answer this question, one must begin by critically examining which structures are being probed with MOC. Although truly quantitative data on the topic are sorely missing, in general, apparent cell stiffness seems to vary with the amount of focal adhesion proteins and the number of microfilaments that are being recruited to the bead binding sites [i.e., to the focal adhesion complex (FAC)]. To the extent to which “FAC size” determines the ease with which a bead can be

pivoted on top of a cell, matrix-dependent adhesion receptor clustering and activation must be very important determinants of step cytometry and MOC-derived cell mechanical properties. The fact that apparent cell stiffness also tracks more distant events, such as myosin activation and cross-bridge interactions, means only that the bead-associated FACs are coupled to the cytoskeleton but does not tell us how strong this coupling really is. Because smooth muscle agonists might induce FAC remodeling in addition to activating cross bridges (8), the apparent stiffening of HASM cells during agonist stimulation cannot be attributed to a single mechanism.

Notwithstanding this caveat, why is it that fundamentally different interventions, such as cytochalasin D-induced microfilament breakdown, histamine exposure, and adenylyl cyclase activation, seem to produce highly correlated changes in apparent cell stiffness and resistance (as reflected in storage and loss moduli)? The authors remind us that the mechanical behavior of many biological materials can be described with an empiric law, “structural damping” (5). Accordingly, frictional stress (i.e., resistive or out of phase behavior) is a fixed fraction of elastic stress (i.e., in-phase behavior). Fredberg and Stamenovic (4) likened this fraction, the so-called hysteresivity (η), to a tax that must be spent any time elastic energy is stored. Because frictional energy loss and elastic energy storage are coupled, their source probably resides within the same structure, the cytoskeleton. However, the structural damping law is descriptive and the molecular basis of η remains obscure. To a translational biologist, that might seem disappointing. After all, in contractile tissue strips, subtle changes in the correlation between storage and loss moduli (i.e., changes in η) have been attributed to changes in myosin ATPase activity and cross-bridge cycling rates (3). However, it would be inappropriate to a priori attribute a change in MOC-derived mechanical properties of HASM cells to the same molecular mechanisms. Not only were the changes in η that accompanied cytoskeletal manipulations of HASM cells quite small but the ubiquity of structural damping behavior argues against a single, molecule-specific mechanism.

If storage and loss moduli cannot teach us about specific molecular interactions and signal transduction pathways, why should translational biologists profess interest in cytomechanics? One simple answer is that the phenomenon, resistance to deformation, is frequently correlated with form and function (6). To define such correlations in specific cell systems might not be viewed as mechanistic, but the information is nevertheless relevant considering that it can provide new

ideas about disease management strategies. Is a contracted and stiff endothelial cell more likely to express adhesion receptors and bind leukocytes in response to a shear stress than one that is relaxed and deformable? Do changes in shape and stiffness of cells that participate in alveolar wound repair determine their proinflammatory and proliferative deformation responses? These are but two of many questions that should concern clinicians and investigators interested in the biophysics of acute lung injury. The excellent correlation between changes in storage and loss moduli, at least in the frequency range described by Maksym and colleagues, suggests that a simple index of apparent stiffness is probably sufficient to establish such structure-function correlations.

There is, of course, a more fundamental perspective to structural damping and cytomechanics. What does a phenomenon that has been observed in foams, gels, tissue strips, and now living cells teach us about molecular order and the control of structure? It is impossible to predict whether the answer to this question will result in a payoff for translational research. However, even those who frown on the pursuit of science for the sake of science must admit that this question is both intriguing and fundamental.

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