Cellular Responses to Mechanical Stress
Invited Review: Engineering approaches to cytoskeletal mechanics

DIMITRIJE STAMENOVIĆ¹ AND NING WANG²
¹Department of Biomedical Engineering, Boston University, Boston 02215; and ²Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts 02115

Stamenovic, Dimitrije, and Ning Wang. Invited Review: Engineering approaches to cytoskeletal mechanics. J Appl Physiol 89: 2085–2090, 2000.—An outstanding problem in cell biology is how cells sense mechanical forces and how those forces affect cellular functions. Various biophysical and biochemical mechanisms have been invoked to answer this question. A growing body of evidence indicates that the deformable cytoskeleton (CSK), an intracellular network of interconnected filamentous biopolymers, provides a physical basis for transducing mechanical signals into biochemical signals. Therefore, to understand how mechanical forces regulate cellular functions, it is important to know how cells respond to changes in the CSK force balance and to identify the underlying mechanisms that control transmission of mechanical forces throughout the CSK and bring it to equilibrium. Recent developments of new experimental techniques for measuring cell mechanical properties and novel theoretical models of cellular mechanics make it now possible to identify and quantitate the contributions of various CSK structures to the overall balance of mechanical forces in the cell. This review focuses on engineering approaches that have been used in the past two decades in studies of the mechanics of the CSK.

actin microfilaments; microtubules; intermediate filaments; tensegrity; prestress; shear stiffness; strain hardening

It is well established that adherent cells sense their mechanical environment and that mechanical forces affect their function. For example, tensed lung parenchymal tissue, distended by transpulmonary pressure, is the habitat of various types of pulmonary cells. An increase in parenchymal tissue distension due to an increase in transpulmonary pressure results in numerous cellular responses, including an increase in surfactant secretion by epithelial type II cells and in reduction of airway smooth muscle shortening (cf. Refs. 8, 20, 24, 40). A standard explanation is that mechanical signals are only preconditioning for the onset of a series of biochemical events in the cell that are carried out by soluble signaling molecules. However, a growing body of evidence suggests that mechanical forces play a more important role in controlling and regulating cell biochemistry (cf. Ref. 3). It is known that the cytoskeleton (CSK), a three-dimensional intracellular network formed by filamentous biopolymers, is the site of the actomyosin contractile machinery that generates mechanical forces. Furthermore, the CSK also transmits mechanical forces from the cell membrane to the nucleus (23). Importantly, many of the signaling molecules are immobilized in the solid CSK structure, which is highly sensitive to mechanical deformation (cf. Ref. 18). In other words, the deformable CSK lattice provides a physical basis for transducing mechanical signals into biochemical signals. To understand how mechanical forces regulate cellular functions, it is im-

Address for reprint requests and other correspondence: D. Stamenovic, Dept. of Biomedical Engineering, Boston Univ., 44 Cummington St., Boston, MA 02215 (E-mail: dimitrij@engc.bu.edu).

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MECHANICAL MEASUREMENTS

Mechanical forces applied to the cell are transmitted over preferred molecular pathways, specifically across transmembrane receptors that mediate cell-cell and cell-extracellular matrix adhesion and that are physically connected to the CSK. When a mechanical stress is applied directly to cell surface receptors, by magnetically twisted (37) or optically pulled (4) surface-bound microbeads, the measured resistance to the applied stress at steady state is finite and increases with increasing applied stress. This resistance is usually expressed as the ratio of applied stress to the corresponding strain and is referred to as the apparent shear modulus or shear stiffness. The increase in cell stiffness with increasing applied stress or strain is known as the strain-hardening effect. Cell stiffness also increases when cells are stimulated pharmacologically by bronchodilators (16), when the cell contractile apparatus is activated genetically (2), or when cell distension is increased (26, 38). Cell stiffness decreases when cells are treated with bronchodilators (16) or when CSK filament structures are selectively chemically disrupted (36, 37) or genetically knocked out (39). Similar results are obtained when large mechanical loads are applied nonspecifically to the cell surface by either micropipette aspiration (29) or by local compression (25, 27, 31). Values of cell stiffness obtained from those measurements are highly scattered, ranging from order of $10^3$ to $10^5$ Pa, and depend primarily on measuring techniques and models used for data interpretation (33). Taken together, these data suggest that the CSK provides the bulk of cell stiffness and that this stiffness can be modulated by modulating cell distending stress.

Two general approaches have been used in the past to investigate the mechanics of the CSK: one where CSK deformability is assumed to arise primarily from deformability of individual CSK filaments and the other where CSK deformability is assumed to be determined primarily by a preexisting mechanical tension (prestress) and architecture of the CSK lattice. In the following sections we describe these two approaches, their applicability, and their limitations. Because this review focuses on microstructural determinants of CSK mechanics, we do not consider those models of the cell that are not structurally based (e.g., viscoelastic lumped models).

MECHANICAL PROPERTIES OF THE CELL INFERRED FROM RHEOLOGICAL PROPERTIES OF CSK FILAMENTS

Major force-bearing components of the CSK are filamentous biopolymers actin microfilaments (MFs), microtubules (MTs), and intermediate filaments (IFs). Measurements of mechanical properties of isolated MFs and MTs revealed that both have very high Young's moduli ($10^9$ Pa) but that MTs have a much greater bending stiffness than MFs (cf. Ref. 14). Implications of these findings are the following. First, because the Young's modulus of individual CSK filaments is much greater than the stiffness of living cells, then, to accommodate relatively large strains experienced by living cells, MFs and MTs cannot be continuous throughout the cell. Instead, they are organized as an interconnected CSK network, and the nature of this connectedness turns out to be important for cell deformability (cf. Ref. 11). Second, on the basis of their relatively high bending stiffness, MTs may play the role of compression-supporting elements of the CSK.

Additional information about the mechanical role of the CSK polymers comes from in vitro rheological measurements on polymer gels (19, 21, 35). Results from those measurements suggest that, in general, actin MFs provide the cell elastic response at relatively low strains (<20%), whereas IFs provide cell mechanical strength at higher strains (>20%), and they are responsible for the cell strain-hardening behavior.

Actin network model. Satcher and Dewey (28) proposed a microstructural model of the actin CSK in which bending of actin MFs represents the principal mode by which the cell resists applied stress. The model is based on the apparent similarity between the actin CSK microstructure and microstructures of various natural and synthetic materials that resist deformation by bending of their structural components. The general formula for the shear modulus ($G$) of such materials is $G \propto \varphi^2E$, where $\varphi$ is the volume fraction and $E$ is Young's modulus of structural components (cf. Ref. 13). Taking the values of $\varphi = O(10^{-3})$ and $E \approx O(10^9)$ Pa for actin, it follows that $G = O(10^3)$ Pa. This prediction is consistent with rheological measurements on living cell, whereby cells are subjected to short (few seconds) and relatively large local compression stresses (25, 27). Furthermore, the model can predict cell strain hardening in response to local compression (13). However, the predicted value of $G$ is at least an order of magnitude greater than the values obtained from steady-state magnetic twisting and micropipette aspiration measurements on cultured endothelial cells (29, 38). This, in turn, suggests that in those measurements bending of actin MFs is not the principal mode by which the cell resists deformation.

Taken together, the above findings indicate that the mechanical properties of the CSK are not solely determined by the mechanical properties of individual CSK filaments. It is also important to understand how these filaments are organized architecturally.

Actin gels have also been analyzed by using filament dynamics (22) and random network (percolation) (10) models. However, these approaches have not yet produced quantitative predictions of indexes of cell deformability.
MECHANICAL PROPERTIES OF THE CELL INFERRED FROM THE CSK PRESTRESS AND ARCHITECTURE

Measurements on living cells have shown the cell shear stiffness can be modulated by modulating cell distension or cell contractility, i.e., that cell stiffness is determined by cell distending stress. This, in turn, suggests that tensile elements of the CSK carry a preexisting stress (prestress) before application of an external load and that it is this prestress that determines cell deformability.

Recent developments of traction force microscopy (7) made it possible to quantitatively estimate the prestress from measurements of traction at the cell-substrate interface in airway smooth muscle cells treated with different doses of histamine (Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Polte T, and Ingber DE, unpublished observations). Cells were cultured on a deformable gel substrate which served as a strain gauge for measuring traction (τ) at the cell-substrate interface. The prestress (σ) within the cell was calculated at baseline and at steady state after exposure to increasing doses of histamine, by considering a mechanical balance of the traction and the prestress on a free-body diagram of a section of the cell: 

\[
\sigma = \tau S_\sigma/S_\sigma',
\]

where \(S_\sigma\) and \(S_\sigma'\) are the cross-sectional and interfacial areas of the cell section, respectively. It is found that the prestress increases with increasing doses of histamine. This increase directly correlates with an increase in cell shear stiffness measured by magnetic cell twisting; i.e., the shear stiffness is an approximately linear function of the prestress. This relationship is a hallmark of a broad class of tensed structures that require prestress to maintain their structural integrity. The shear stiffness also depends on architecture and elastic properties of microstructure. These two properties describe how the stiffness changes in response to an externally applied load, e.g., strain hardening (5, 34, 39), which is an entirely different phenomenon from the prestress-induced stiffening (Fig. 1).

Tensegrity model. Tensegrity architecture, which has been proposed as a model of the CSK (cf. Ref. 17), falls into the category of tensed structures. This model assumes that the prestress is generated actively by the cell’s contractile apparatus and passively by distension through cytoplasmic swelling pressure and extracellular adhesions. Actin MFs and IFs carry the prestress, and the prestress is balanced internally by compression-supporting structures, such as MTs or thick actin bundles, and externally by focal adhesions to the substrate. Together, the CSK, the cytoplasm, and the substrate form a self-equilibrated, stable mechanical system. According to the tensegrity model, the connectedness of CSK filaments is such that it does not fully constrain their internal freedom of motion. Consequently, the CSK is not an intrinsically stable structure, and thus it requires prestress to maintain its structural stability. An example of a similar structure is a spider web, which requires to be stretched between firm objects to maintain its shape stability. When placed under load, CSK structural components undergo geometric rearrangement until a new equilibrium configuration is attained. The greater the prestress, the smaller the deformation the structure has to undergo before attaining equilibrium. Consequently, as the prestress increases, the stiffness increases in proportion (cf. Refs. 11, 33, 34). Interestingly, lung parenchyma exhibits a similar behavior, i.e., a linear dependence between the parenchymal shear modulus and transpulmonary pressure (cf. Ref. 32). This indicates that a common architecture is reiterated at different levels of organization (11).

A tensegrity model provides a good quantitative correspondence to data for cell stiffness obtained from quasi-static measurements on living cells (33). Most importantly, a nearly linear relationship between the cell stiffness and the prestress predicted a priori from this model is remarkably consistent with an experimentally obtained relationship (Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Polte T, and Ingber DE, unpublished observations). Finally, the model also shows that one part of the cell strain-hardening behavior can be attributed to geometric realignment of cell microstructural elements in the direction of applied load and another part to the intrinsic strain-hardening behavior of IFs (39). This differs from the standard notion that cell strain hardening is primarily due to strain hardening of IFs. Note, however, that strain hardening is not an intrinsic property of tensegrity. Whether a tensegrity structure exhibits hardening, softening or constant stiffness depends on the level of the prestress, direction of applied load, degree of connectedness and mechanical properties of its elements, and external constrains (6, 34, 39).
The presence of internal compression elements, such as MTs, in the tensegrity model of the CSK is controversial. A reason is that there have been no experimental data showing that in living cells MTs carry a substantial compression. If, according to tensegrity, the contractile prestress of the cell is balanced by compression of MTs and by traction at the cell-substrate interface, then disruption of MTs will cause a portion of this prestress balanced by MTs to be transferred to the substrate, increasing thereby the traction. To test this prediction, colchicine (10⁻⁶ M) (a chemical that disrupts MTs) was added to airway smooth muscle cells that were already maximally activated with a saturated dose of histamine (10⁻⁵ M). The traction was measured by traction force microscopy. It was found that the traction increased by ~30% after the addition of colchicine, suggesting that MTs carry a substantial compression (Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Polte T, and Ingber DE, unpublished observations).

Cortical membrane model. An alternative model that describes the effect of the prestress on cellular mechanics is the cortical membrane model. This model was originally proposed to describe mechanics of suspended white blood cells (9) but was later adopted as a model of adherent cells (12, 15, 30). The cortical membrane model depicts the cell as a thin (~0.1-μm) membrane that engulfs liquid cytoplasm (41). The elastic membrane is under sustained tension that is balanced by the pressurized cytoplasm and by traction at the extracellular adhesions. The part of the membrane tension (T) balanced by the traction can be calculated by considering a free-body diagram of a section of the cell, similarly to the calculation of the prestress within the cell. However, for a given traction, membrane tension is much greater than prestress. The reason is that membrane tension is the net force transmitted across a thin layer of elastic membrane per unit cross-sectional area of the membrane (Sm), whereas prestress is the net force transmitted across a cross-sectional area of the cell per unit cross-sectional area, i.e., T = σSm/Sm, where Sm is much greater than S.

To predict the shear stiffness from the cortical membrane model, magnetic cell twisting is simulated as follows. A rigid bead is embedded in a tensed elastic membrane with a thickness (h) that is much smaller than the bead diameter (d). The membrane carries an initial tension. A torque is applied to the sphere in a vertical plane. Rotation of the sphere is impeded by membrane tension. Using standard relationships of mechanics, we calculate the torque as a function of θ from which we obtain that G ∝ (Th/d)sinθ/θ. Taking values for membrane tension inferred from traction measurements and assuming that d equals the diameter of the magnetic twisting bead (4.5 μm) and h = 0.1 μm, we obtain for small angular strains (i.e., sinθ/θ → 1) that G ∝ T. Such obtained values of shear stiffness are quantitatively consistent with experimentally obtained values. However, the slope of shear stiffness vs. membrane tension relationship is much smaller than the slope of shear stiffness vs. prestress relationship inferred from the tensegrity model (33) because membrane tension is much greater than prestress.

The above example shows that measurements of traction and stiffness alone cannot discriminate between the tensed cortical membrane model and the tensegrity model of the CSK. However, some other predictions of the cortical membrane model are not consistent with data obtained from magnetic cell twisting. First, the cortical membrane model predicts that shear stiffness decreases with increasing bead diameter, whereas measurements on living endothelial cells show quite the opposite trend, shear stiffness increases with increasing bead diameter (38). Second, the model predicts that shear stiffness decreases with increasing θ (i.e., strain softening), whereas experimental data show strain hardening (cf. Refs. 37–39). Furthermore, confocal microscopy measurements show that cell actin MF content is not entirely distributed within a thin cortical layer but that the cytoplasmic domain is also rich with filamentous actin in adherent endothelial cells and airway smooth muscle cells (unpublished observations). Taken together, these results suggest that the cortical membrane model cannot explain our observations.

SUMMARY

The body of evidence presented in this review indicates that the CSK, depicted as a three-dimensional interconnected and prestressed network, is a primary determinant of elastic properties of adherent cells. Because of recent developments in measurement techniques and theoretical modeling, it is now possible to identify and quantify the contributions of various CSK structures to the cell elasticity as follows. The role of actin MFs is to carry the prestress, conferring thereby the shape stability of the entire cell. Under applied external stress, taut actin MFs neither bend nor change their length significantly. Instead they rotate and change spacing to attain an equilibrium configuration (33). The greater the initial tensile force carried by those filaments (i.e., the greater the prestress), the smaller the deformation the CSK must undergo before attaining equilibrium. Consequently, cell stiffness increases with increasing prestress. The role of MTs is to carry compression as they balance tension in the MFs. At large tensile strains, IFs may become the primary tension-bearing components of the CSK. If, however, a large compressive load is applied to the cell, the actin MFs may get relieved from tension and bend. In that case, their bending is the key mode by which the CSK resists compression. Another feature of cells is that they may exhibit strain-hardening in response to applied stress. This phenomenon can be attributed to realignment of CSK filaments in the direction of applied stress, bending and buckling of MFs and MTs, and the intrinsic strain-hardening behavior of IFs.
FUTURE DIRECTIONS

Very little is known about the cell’s mechanical and biological behaviors in response to dynamic mechanical perturbations such as cyclic stretching of the extracellular matrix during breathing or pulsatile blood flow. It is reasonable to pose the question whether the prestress may play a similar role in shaping the cell dynamic response as it does in the case of the quasi-static elastic response. To investigate this problem, viscoelastic properties of CSK structures and of the cytoplasm have to be included in future considerations and mechanical measurements that will subject cell to cyclic loading have to be performed.

Future studies should investigate the feasibility of other types of structures, including tensed cable networks (33), polymer chains (1), and hierarchical structures composed of a large number of simple units, as models of the CSK. With increasing model complexity, unique relationships that describe the cell mechanical response to increasing prestress and to applied external stress are expected to emerge. Such obtained relationships should be little dependent on model microstructural geometry and would serve as a link between continuum mechanics approaches and discrete approaches.

An emerging branch of biological research is cellular mechanotransduction, i.e., how mechanical stresses are transduced into biochemical signals. To understand this problem, it is important to know how cells transmit and balance mechanical forces. For example, intracellular mechanical signaling pathways need to be elucidated and compared with known signaling pathway for growth factors. Furthermore, although it has been shown that mechanical force applied specifically to integrin receptors deforms the nucleus via the CSK (23), it remains to be seen whether the force can directly activate proteins and genes inside the nucleus or it needs facilitation from soluble molecules in the cytoplasm.

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