

controversies in physiology

Opposing views on tensegrity as a structural framework for understanding cell mechanics

DONALD E. INGBER

*Departments of Pathology and Surgery, Children's Hospital
and Harvard Medical School, Boston, Massachusetts 02115*

STEVEN R. HEIDEMANN, PHILLIP LAMOUREUX, AND ROBERT E. BUXBAUM

Department of Physiology, Michigan State University, East Lansing, Michigan 48824-1101

Donald E. Ingber: Important new theories in science often ignite heated debates. If they do not, they are probably of little significance. Thus a strong argument in support of the importance of the tensegrity model of cell and tissue architecture first proposed almost 20 years ago (23, 24) is the large number of public and private criticisms that have been mounted against this theory. Demonstration of the ability of the tensegrity model to explain complex mechanical behaviors in viruses, nuclei, cells, tissues, and organs in animals as well as in insects and plants (reviewed in Refs. 4, 5, 7, 10, 17, 20–24, 26, 30, 32, 42) has led to a drastic reduction in the number of these confrontations. Nevertheless, some intransigent critics remain. However, their remaining objections are limited in scope and largely result, I believe, from an overly concrete definition of what tensegrity is and how it can be applied. My purpose here, at the request of the Editor and Associate Editors of this journal, is to present the argument in support of the tensegrity model and to respond to some of these remaining concerns.

The tensegrity model states that cells, tissues, and other biological structures at smaller and larger size scales in the hierarchy of life gain their shape stability and their ability to exhibit integrated mechanical behavior through use of the structural principles of tensegrity architecture (5, 20, 22–24). The term, “tensegrity” (contraction of “tensional integrity”) was first created by the architect R. Buckminster Fuller, who first explored use of this form of structural stabilization as early as 1927 in his plan for the Wichita Dymaxion house, which minimized weight by separating compression members from tension members (31). To create this cylindrical building, Fuller proposed to set a central mast in the earth as a vertical compression strut and to suspend from it multiple circular floors (horizontal wheels) using tension cables. Tensile guy wires that linked the mast to surrounding anchors in the ground provided the balancing tension necessary to stabilize the entire structure. “Fuller called this special discontinuous-compression, continuous-tension

system, the Tensegrity” (31) to emphasize how it differs from conventional architectural systems (e.g., brick-on-brick type of construction), which depend on continuous compression for their shape stability. Fuller’s more formal definition in his treatise, *Synergetics*, is “Tensegrity describes a structural-relationship principle in which structural shape is guaranteed by the finitely closed, comprehensively continuous, tensional behaviors of the system and not by the discontinuous and exclusively local compressional member behaviors” (16). Note that there is no mention of rigid struts, elastic strings, tensile filaments, internal vs. external members, or specific molecular constituents in this definition. In fact, Fuller describes a balloon with non-compressible gas molecules pushing out against a tensed rubber membrane as analogous to one of his geodesic domes when viewed at the microstructural level (i.e., the balloon is a porous, tensed molecular network on the microscale) and explains that both structures are classic examples of shape stability through tensegrity. Fuller also described hierarchical tensegrity structures in which individual struts or tensile elements are themselves tensegrity structures on a smaller scale; key to this concept is that smaller tensegrity units require external anchors to other tensegrity units to maintain higher order stability. In fact, he argued that nature utilizes this universal system of tensile structuring at all size scales and that it provides a way to mechanically integrate part and whole (16), a view I recently explored in greater depth (22).

In 1948, Fuller’s student, Kenneth Snelson, constructed the first “stick-and-string” tensegrity sculpture, which thrilled Fuller because it visibly communicated the essence of this novel form of shape stability to those who could not “see” it in more complex structures (e.g., geodesic domes with rigid struts; see Fig. 5 in Ref. 5). Snelson’s sculptures contain isolated compression members that are suspended in midair by interconnections with a continuous tensile network. Some of these structures require anchorage to the ground to remain

stable (e.g., large cantilevered structures); however, most are entirely self-stabilizing. Similar stick-and-string tensegrity models have been used to visualize tensegrity in cells and other biological structures for those who cannot easily visualize them (Figs. 1 and 2). The appearance of geodesic patterns in biological structures, including viruses, clathrin-coated vesicles, and actin geodesomes in the cytoskeleton of mammalian cells, provides additional visual evidence of nature's use of this form of architecture (20, 22).

My own view of tensegrity has been refined over the years as a result of extensive reading, personal correspondence with Fuller, conversations with Fuller's close associates (including Snelson), collaboration with expert mechanical engineers, and many hours of thinking about how to best respond (experimentally) to some very intelligent critics. In simplest terms, tensegrity structures maintain shape stability within a tensed

network of structural members by incorporating other support elements that resist compression. The stiffness of the stick-and-string tensegrity structures, and hence their ability to resist shape distortion, depends on the level of preexisting tension or "prestress" in the structure before application of an external load. The distinguishing microstructural feature accounting for this behavior is that, when placed under load, the discrete structural elements move, changing orientation and spacing relative to one another, until a new equilibrium configuration is attained. For this reason, a local stress can result in global structural rearrangements and "action at a distance."

To visualize tensegrity at work, think of the human body: it stabilizes its shape by interconnecting multiple compression-resistant bones with a continuous series of tensile muscles, tendons, and ligaments, and its stiffness can vary depending on the tone (prestress) in its muscles. If I want to fully extend my hand upward to touch the ceiling, I have to tense muscles down to my toes, thus producing global structural rearrangements throughout my body and, eventually, upward extension of my fingers. However, the body is also multimodular and hierarchical: if I accidentally sever my Achilles tendon, I lose form control in my ankle module, but I still maintain structural stability in the rest of my body. Furthermore, every time I breath in, causing the muscles of my neck and chest to pull out on my lattice of ribs, my lung expands, alveoli open, taught bands of elastin in the extracellular matrix (ECM) relax, buckled bundles of cross-linked (stiffened) collagen filaments straighten, basement membranes tighten, and the adherent cells and cytoskeletal filaments feel the pull; however, nothing breaks and the deformation is reversible. Tensegrity provides a structural basis to explain all these phenomena.

In the cellular tensegrity model, the stabilizing prestress is generated actively by the cell's contractile apparatus and passively by distension through extracellular adhesions, by osmotic forces acting on the cell's surface membrane, and, on a smaller scale, by forces exerted by molecular filaments extending through chemical polymerization. The model assumes that the prestress is carried by tensile elements in the cytoskeleton, primarily actin microfilaments and intermediate filaments, and that the cell is both a hierarchical and multimodular structure (5, 20–23) (Figs. 1 and 2). This prestress is balanced by interconnected structural elements that resist being compressed at different size scales, including the cell's external adhesions to the relatively inflexible ECM and internal cytoskeletal filaments, specifically microtubules that stretch across large regions of the cytoplasm and cross-linked bundles of cytoskeletal filaments that stabilize specialized microdomains of the cell surface (e.g., actin microfilaments in filopodia; microtubules in cilia). In this model, the internal cytoskeleton is surrounded by an elastic submembranous cytoskeleton (e.g., actin-ankyrin-spectrin network) and its associated lipid bilayer, which may or may not mechanically couple to the internal, tensed microfilament-microtubule-intermedi-

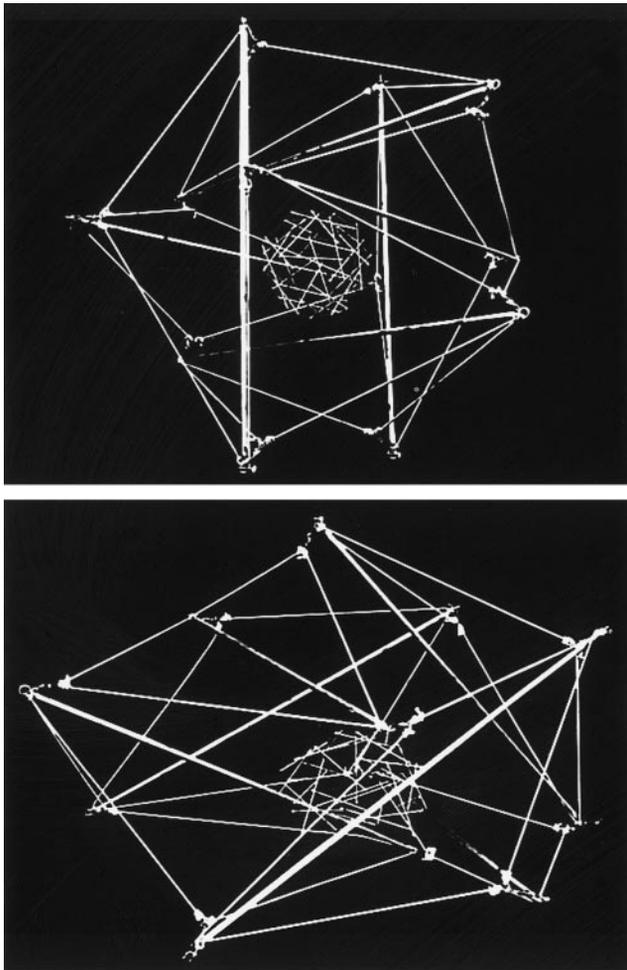


Fig. 1. A hierarchical tensegrity model of a nucleated cell composed of sticks and elastic string when unanchored and round (*top*) vs. attached and spread on a rigid adhesive substrate (*bottom*). The independent nuclear tensegrity sphere is mechanically connected to the surface of the larger tensegrity unit by black elastic filaments that are not visible against the black background. This model predicts that rapid pulling on surface receptors that mechanically couple to linking filaments in the cytoplasm may promote immediate changes in nuclear structure, as confirmed experimentally (Ref. 30 and Wang et al., unpublished observations; also see Fig. 3 below).

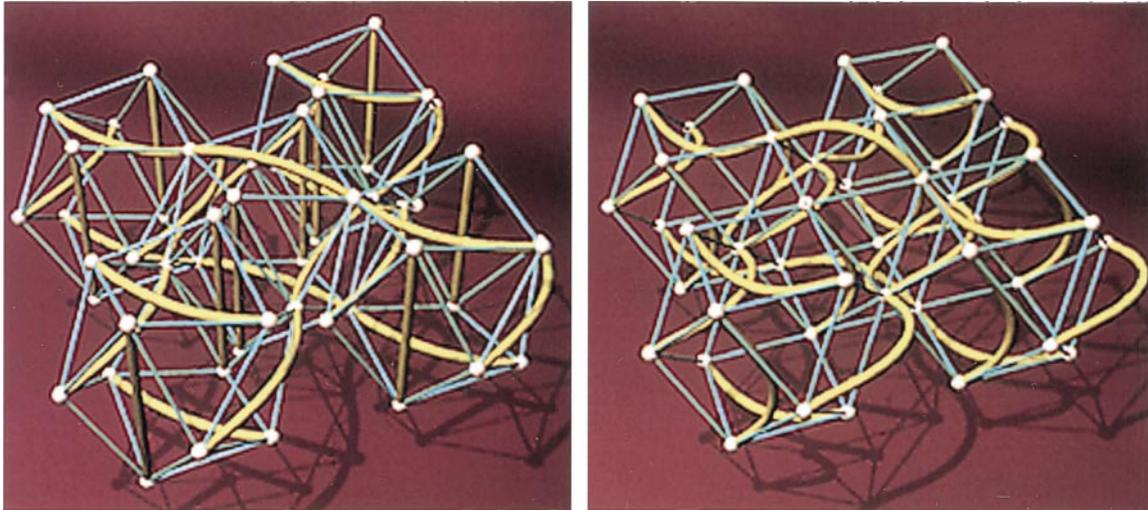


Fig. 2. A multimodular tensegrity model of a portion of the internal cytoskeleton containing long microtubules (yellow) that interconnect and stabilize multiple smaller polygonal networks comprised of contractile microfilaments (blue). Microfilament contraction induces compressive buckling in the semiflexible microfilament struts (*right vs. left*). This model is consistent with the finding that drugs that stimulate cell contraction increase microtubule curvature, whereas compounds that suppress this response promote straightening (Ref. 45 and Wang et al., unpublished observations).

ate filament lattice depending on the type of adhesion complex that forms. The entire cytoskeleton is permeated by the viscous cytosol. Most importantly, this micromechanical model leads to specific predictions relating to the mechanical role of distinct cellular and molecular elements in cell shape control.

In contrast, a conventional model of cell structure (12), which is espoused by my esteemed counterparts in this article (18), depicts the cell as an elastic cortex that surrounds a viscous cytoplasm with an elastic nucleus in its center. In engineering terms, this is a “continuum” model, and, by definition, it assumes that the load-bearing elements are infinitesimally small relative to the size of the cell. It is essentially the balloon model considered by Fuller, but in this case all microstructure is ignored. Because they ignore microstructural features, continuum models cannot provide specific predictions that relate to the functional contribution of distinct cytoskeletal filaments to cell mechanics. Furthermore, although these models can provide empirical fits to measured mechanical properties in cells under specific experimental conditions, they cannot predict how these properties alter under new challenges to the cell.

Future advancement of our understanding of the relation between cell mechanics, molecular structure, and biological function requires a more unified cell model. This model must build on our existing knowledge of cell microstructure and take into account experimental observations that reveal that the cytoskeleton is organized as a porous molecular network composed of discrete structural elements that physically interconnect with external support networks in the ECM and in neighboring cells (14). I would argue that tensegrity provides this model. In fact, we and others (including my counterparts in this article) have

shown that both buildable tensegrity structures (17, 20, 23, 26, 42) and a theoretical tensegrity model developed from first principles (9, 38, 39, 46) are robust in terms of their ability to predict complex cell behaviors in various experimental systems and across many different size scales. Then why the continued criticisms? Let’s explore this in greater detail.

One of the most important features of the tensegrity model, as opposed to the viscous cytosol model, is that it predicts that applied mechanical forces will not be transmitted into the cell equally at all points on the cell surface. In the tensegrity model, the submembranous cytoskeleton (cortical actin-ankyrin-spectrin lattice) is viewed as an independent tensegrity structure, which is itself stabilized by the presence of a prestress within its discrete porous (and geodesic) molecular network, as recently demonstrated in the purest form of this structure, the red blood cell membrane (11). Depending on the molecular composition of the attachment substrate (e.g., ECM, surface of another cell) to which a cell anchors, this highly elastic cortex may or may not mechanically couple to the internal microfilament-microtubule-intermediate filament lattice, which, in turn, distributes loads throughout the cell and to the nucleus. A simple example of how the tensegrity model has contributed to the advancement of science is that it has led to the proposal that adhesion receptors, such as integrins, which form a transmembrane molecular bridge between the ECM and the internal cytoskeleton, provide a preferred path for transmembrane mechanical signal transfer and, hence, play a central role in cellular mechanotransduction. On the basis of subsequent experimental confirmation (8, 30, 35, 42), this role for integrins is now well established (7, 21).

The point here is that, if cells use tensegrity, then long-distance force transfer should be observed in liv-

ing cells. However, this action at a distance will only be observed if the correct series of molecular couplings are formed between the surface receptor and the internal cytoskeletal lattice; externally applied stresses would dissipate at the cell surface under other conditions. In contrast, the elastic cortex-viscous cytosol model (12, 18) would predict that living cells will never exhibit directed action at a distance inside the cell. Importantly, when we applied mechanical stresses directly to transmembrane integrin receptors using surface-bound micropipettes that were precoated with the ECM molecule fibronectin, we observed immediate repositioning of cytoskeletal filaments and elongation of nuclei along the applied tension field lines as well as molecular realignment within nucleoli deep in the center of the nucleus within living cells (30) (Fig. 3). In contrast, no changes in intracellular structure were observed when tension was applied to other transmembrane receptors on the cell surface that only couple to the submembranous actin cytoskeleton. More recently, similar studies were carried out using pipettes to pull on ECM-coated microbeads bound to cell surface integrin receptors on cells that were transfected with en-

hanced yellow fluorescent protein (EYFP)-cytochrome *c* to make mitochondria fluorescent throughout the entire cell. Real-time fluorescence microscopic analyses of these living cells revealed coordinated movement of mitochondria during the entire course of the pull and realignment of these natural fiducial markers; this was observed as far as 20 μm into the depth of the cell (N. Wang, K. Naruse, D. Stamenovic, J. J. Fredberg, S. M. Mijailovich, G. Maksym, T. Polte, and D. E. Ingber, unpublished observation). Again, pulling on other transmembrane receptors that do not couple to the internal cytoskeletal lattice (but do couple to the cortical actin cytoskeleton) did not result in long-distance force transfer as predicted by the tensegrity model. Because mitochondria directly associate with microtubules, these results indicate that forces transmitted to microfilaments via integrins can result in displacement of microtubules at distant sites and that these different filament networks are mechanically connected inside living cells.

The main reason for Dr. Heidemann's change of heart regarding tensegrity (he was one of the first proponents of this model) is described in his recent publication (18) in which the action at a distance he expected to see was not observed when he pulled on cell surface receptors using ECM-coated micropipettes. However, the ECM protein laminin, which was used in that study, binds to classes of integrin receptors different from fibronectin and focal adhesion formation was not demonstrated in that study. In fact, his results are not new or surprising: we and others have experimentally observed similar local responses and high cell membrane deformability when cells were probed with beads coated with antibodies to certain integrin subtypes (44) and even with fibronectin when analyzed during the first few seconds after binding (i.e., before focal adhesion formation) (35) or when dragged over short distances in the plane of the membrane (i.e., when the submembranous cytoskeleton is the primary load-bearing element) (1). Thus, consistent with the tensegrity model, the cell may appear to behave like an elastic cortex surrounding a viscous cytosol, if the submembranous cytoskeletal network is probed independently of the internal cytoskeleton (microfilament-microtubule-intermediate filament lattice). In contrast, action at a distance can be observed when other receptors that provide deeper linkages (e.g., integrin $\alpha_5\beta_1$) are ligated, although the specific molecular species involved will vary depending on cell type.

The cellular tensegrity model also differs from other models of cell mechanics in that it predicts that cytoskeletal prestress is a critical determinant of cell shape stability. This has been demonstrated directly in studies in which cytoskeletal prestress was altered by modulating actomyosin-based contractility using drugs (19), transfecting cells with constitutively active myosin light chain (MLC) kinase (3), varying transmembrane osmotic forces (3) or quickly distending the flexible ECM substrate on which the cell is adherent (34), resulting in immediate changes in the cellular shear modulus (a quantitative measure of stiffness or shape

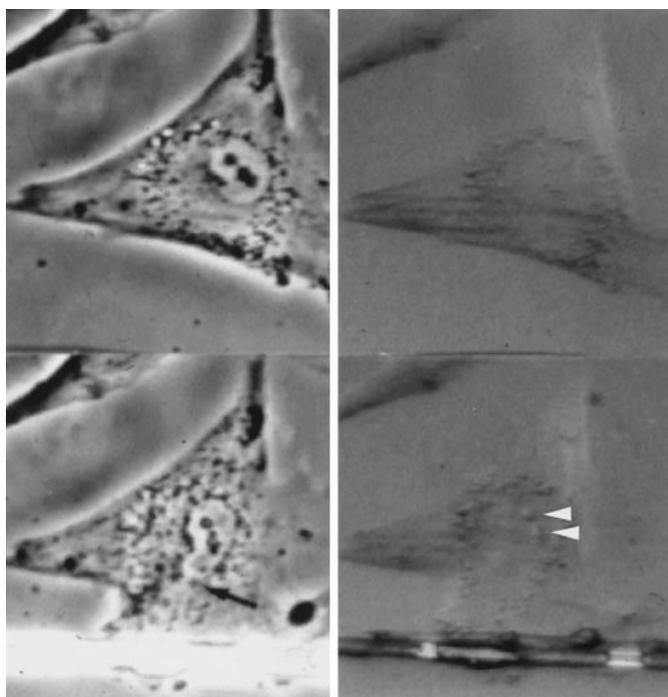


Fig. 3. Phase-contrast (*left*) and polarization optic (*right*) views of an adherent endothelial cell immediately before (*top*) and after (*bottom*) a fibronectin-coated micropipette (visible in *bottom*) was bound to integrin receptors on its surface and pulled laterally (downward in this view) using a micromanipulator. Arrow in *bottom left* indicates downward extension of the nuclear border along the applied tension field lines. Arrowheads in *bottom right* point to white birefringent spots, which indicate induction of molecular realignment within nucleoli in the center of the nucleus by applying mechanical stress to integrins micrometers away on the cell surface. These results directly demonstrate that action at a distance can occur in living cells if external forces are applied via the correct set of transmembrane molecular linkages (e.g., integrins that form intact focal adhesion complexes), as predicted by the nucleated tensegrity model shown in Fig. 1 (see Ref. 30 for more details).

stability). One may argue (and some have) that it may be prestress in the cortical cytoskeleton (the elastic cortex in the continuum models, which view the cell as an inflated balloon or rubber ball) that is responsible for these effects. However, when cell mechanics was measured by twisting on two differently sized magnetic beads bound to the same type of cell surface integrin receptor using cell magnetometry, cell stiffness scaled directly with bead size for a given applied stress (cells appeared to be less stiff using the smaller beads) (43); this result is the opposite of what would be predicted by a prestressed membrane cortex model. Furthermore, when cell mechanics was measured through cell surface integrins that connect to the internal cytoskeletal lattice, cell stiffness was found to be increased in spread vs. round cells (43) and in cells expressing constitutively active MLC kinase (3), whereas no significant difference in stiffness was measured when the same cells were probed through transmembrane receptors that only connect to the cortical cytoskeleton in those studies. Thus differences in shape stability due to altered prestress in these cells cannot be explained solely by changes in the cell cortex.

The reality is that transmission of tension across molecular connections within the cytoskeletal network influences shape stability throughout the entire cell. For example, the shape and stiffness of the cell, internal cytoskeleton, and nucleus can be altered by using drugs (30, 42) or genetic techniques (e.g., vimentin knock-out mice; Ref. 13) to disrupt the intermediate filament lattice, which is known to extend throughout the depth of the cytoplasm. Coordinated retraction and rounding of the entire cell, cytoskeleton, and nucleus also were observed in membrane-permeabilized cells when ATP was added under conditions that supported microfilament contraction but not when a synthetic peptide that specifically blocks actomyosin filament sliding was present (37). Quantitation of changes in cell stiffness in these permeabilized cells confirmed that tension within the internal cytoskeleton directly determined cell and nuclear shape stability, independently of transmembrane osmotic forces (43), clearly demonstrating the inappropriateness of the “water balloon” or “inflated rubber ball”-type models of the cell. Finally, Dr. Heidemann’s own elegant studies on neurites show that the elastic cortex-viscous cytosol model alone is not sufficient to explain how nerve cells produce highly extended processes such as neurites (17, 26). These cells also must be able to shift mechanical forces between tensile microfilaments in the cortex, central microtubule compression struts, and external ECM tethers to extend these specialized projections. In short, continuous transmission of tension through the depth of the cytoskeleton and between the cytoskeleton and ECM tethers is critical for cell shape stability.

Probably the most common concern raised over the years has been, Where are the compression elements? The answer depends on the size scale and hierarchical level that one examines. If we ask how the whole cell controls its shape in living tissues (the ultimate question), then we have to take into account the contribu-

tion of the cell’s adhesions to ECM and to other cells as well as internal support elements. The reality is that most cells cannot stabilize their shape in the absence of these adhesions: cells with highly specialized forms retract and round when detached from their anchoring substrate *in vivo* as well as *in vitro*. The reason that an adhesive substrate must be stiff (relative to the cell) to promote cell spreading is that isolated regions of the substrate located between the two integrin-containing focal adhesions that form at the opposite ends of each contractile stress fiber must resist local compression produced by the contraction and shortening of each fiber. The finding that cells can spread over multiple focal adhesion-sized ECM dots that are separated by nonadhesive regions many micrometers in length (6) clearly demonstrates this point.

However, if the ECM were the only compression element, then all cells would be flat and smooth as a fried egg. The reality is that cells also use many different types of internal compression struts to further refine their shape, both in microdomains and at the whole cell level. Internal microtubule struts are used to stabilize local regions of the cytoplasm (25, 41), to stiffen the mitotic spindle (32), and, when oriented vertically, to maintain a cylindrical cell form (2). Bundles of cross-linked (and, hence, further stiffened) microtubules help to create specialized membrane extensions, such as cilia, and long cell processes, as in neurites (26). Stiffened bundles of cross-linked actin filaments similarly stabilize the shape of exploratory projections (filopodia) that extend from the cell surface at the leading edge of migratory cells (36). These locally rigidified structural elements are interconnected by a continuous cytoskeletal lattice that is otherwise under tension; severing the cell in any location results in spontaneous cell retraction (34). Again, we see local compression balanced by continuous tension, the defining features of Fuller’s tensegrity systems.

What is the evidence that these structures actually bear compression in living cells? Cilia and filopodia, which are rigid enough to resist distortion when probed by micropipettes (36), clearly must act locally to resist the inwardly directed compression caused by the tensed cortical membrane to maintain shape stability, regardless of the theoretical model one favors. Microtubules have also been directly shown to resist compression in the mitotic spindles of living cells: when an ultraviolet microbeam was used to sever one microtubule, the remaining microtubules buckled as expected if the same total compressive load was now distributed among a decreased number of semi-flexible compression struts (32). This is an example of tensegrity at a lower hierarchical level. Importantly, studies with green fluorescent protein (GFP)-labeled microtubules also revealed local buckling in the cytoplasm when polymerizing microtubules impinge end-on onto surrounding cellular structures and thus become compressed (Ref. 27 and Wang et al., unpublished observations) (Fig. 4). My counterparts in this editorial have argued that this form of microtubule buckling involves very small compressive loads; hence, it could result

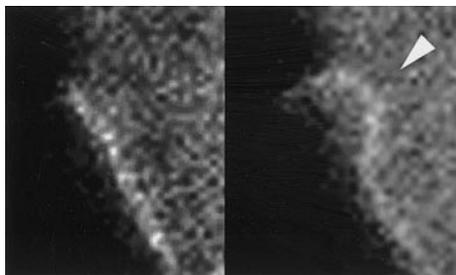


Fig. 4. Two sequential time-lapse immunofluorescence views of the same endothelial cell expressing GFP-tubulin showing a straight microtubule that extends through a large region of the cytoplasm (*left*), which then buckles locally due to compression (indicated by arrowhead) when it elongates through polymerization and impinges end-on on the stiffened cell cortex (*right*).

from fluid flow in the surrounding cytosol (18). However, analysis of time-lapse video recordings of cells expressing GFP-microtubules reveals no evidence of flow; rather, individual buckled microtubules can be seen to immediately straighten when they slip by an obstacle and then only buckle again when they hit end-on on a second obstacle (Ref. 27 and Wang et al., unpublished observations). Furthermore, when cells containing EYFP-mitochondria or GFP-microtubules were repeatedly extended and compressed, with the extension sometimes held for more than 2 min before release, no evidence of intracellular cytoskeletal flow could be observed (Wang et al., unpublished observations). In addition, the curvature of GFP-microtubules (a visual read-out of compressive buckling) decreases when drugs are used to inhibit tension generation in the surrounding actin cytoskeleton, whereas buckling increases when constrictors are added (Ref. 45 and Wang et al., unpublished observations). Disruption of microtubules also significantly reduces the shear modulus (stiffness) of the cell and induces retraction of long processes in various cell types (26, 41, 42), thus confirming the structural importance of their compression-bearing role.

If microtubules are compression elements that maintain cell shape stability by supporting a substantial part of the tensile prestress, then their disruption should cause the prestress (or a significant portion of it) to be transferred to the ECM, thereby increasing the traction at the cell-ECM interface. In contrast, if microtubules were tension elements, then their disruption would inhibit transfer of traction to the ECM. In fact, many cell types increase tractional forces on their ECM substrate when treated with microtubule depolymerizing agents (10, 20, 29), whereas disruption of tensile microfilaments dissipates stress (29). However, part of the effect of microtubule disruption has been attributed by some to increases in MLC phosphorylation in response to release of free tubulin monomers after microtubule depolymerization rather than to a tensegrity-based force balance (28). Importantly, similar transfer of prestress from microtubules to the ECM was recently demonstrated in cells that were pretreated with chemical constrictors to optimally stimulate MLC phosphorylation before microtubule disruption

(Wang et al., unpublished observations) and we have found that MLC phosphorylation does not increase when tubulin monomers are released in cells in which cytoskeletal tension is decreased using relaxant drugs before microtubule disruption (Polte and Ingber, unpublished observations). In other words, the increase in MLC phosphorylation observed after microtubule disruption (28) does not result from release of tubulin monomers; rather, it appears to be a compensatory mechanism that is activated in response to transfer of mechanical stress from microtubules to the ECM and the remaining cytoskeleton in these cells. This is yet another example of a complex behavior that can be explained by tensegrity and not by the other cell models.

Some of those who accept that microtubules bear compression locally within an otherwise tensed cytoskeleton, a clear example of cellular tensegrity, then argue whether this contributes significantly to cell mechanics. To explore this idea in greater detail, studies were recently carried out in pulmonary airway smooth muscle cells cultured on flexible polyacrylamide gel substrates containing small fluorescent microbeads as fiducial markers, which permit quantitation of cell tractional forces and prestress within individual cells (by quantitating bead displacement relative to the traction-free state of the gel after the cells are released using trypsin). Colchicine was used to disrupt microtubules in adherent cells that were activated with a saturating dose of the chemical constrictor histamine, again to ensure optimal MLC phosphorylation. These studies revealed that microtubules counterbalanced approximately one-third of the total cellular prestress within an individual histamine-stimulated cell (Wang et al., unpublished observations). Thus these data confirm that the ability of microtubules to bear compression locally contributes significantly to cellular prestress and that prestress, in turn, is critical for maintenance of cell shape stability. However, because of complementary (tensegrity-based) force interactions between microtubules, contractile microfilaments, and ECM, microtubules may bear less compression in cells when high levels of stress are borne by a rigid ECM substrate, just as tent poles may bear less compressive load if the tent is partially secured by tethers to an overlying tree branch. Thus, although the demonstration that microtubules do carry compression in living cells is a strong support for tensegrity, a negative result in a particular cell would not necessarily rule out this model.

Importantly, many biologists fail to recognize the important difference between engineering models that can describe ("curve-fit") a complex cell behavior vs. one, such as tensegrity, that can explain and predict multiple behaviors at many different size scales from mechanistic principles. For example, one can argue that a tensed (prestressed) rubber ball, a liquid droplet, or a spring and dashpot can mimic mechanical behaviors (e.g., strain-hardening behavior) observed in living cells and tissues, as can tensegrity. This is true. In fact, living cell aggregates can be modeled with

quantitative accuracy as liquids (15). However, we know that these biological structures are not constructed in this manner, and, indeed, the viscous cytosol-elastic cortex model (12,18) does not mesh with the microarchitectural complexity that is observed within the cytoplasm of living cells (14). Essentially, these are all ad hoc models, and, as such, they do not provide a means to explain these behaviors in mechanistic or molecular terms and do not lead to specific predictions that are independent of the experimental system. In contrast, Stamenovic and colleagues have formulated a theoretical description of the tensegrity model of the cytoskeleton starting from first principles of mechanics (9, 38, 39). This micromechanical model provides multiple a priori predictions of which the strain-hardening behavior of living cells is only one. For example, another key quantitative prediction arising from the tensegrity model is that the static shear modulus of the cell should change approximately linearly with the prestress, that is, with the internal tensile stress that preexists in the cytoskeleton before stress application (this is distinct from strain-hardening behavior). This model also suggests that cell mechanical impedance can be decomposed into the product of a prestress-dependent component and a frequency-dependent component. Specifically, tensegrity predicts that, at a given frequency, both the storage and loss moduli should increase with increasing prestress, whereas the hysteresivity coefficient (the fraction of the frictional energy loss relative to the elastic energy storage) should be independent of prestress. Recent studies (Wang et al., unpublished observations) demonstrate that these a priori predictions are supported by experimental measurements of static and dynamic mechanical behaviors in living cells and thus clearly demonstrate the validity and relative value of the tensegrity model. In short, the tensegrity model provides mechanistic, theoretical, and quantitative bases to begin to define the molecular basis of cell mechanics as well as mechanotransduction; the rubber ball model leaves us with, well, a rubber ball.

In summary, I hope that I have convinced you that, although the elastic membrane-viscous cytosol model embraced by my counterparts in this discussion may be able to describe certain behaviors of cells, it cannot explain others. This continuum model also does not provide insight into the molecular basis of cell mechanics or the hierarchical basis of cell organization. In contrast, tensegrity represents a unified model. Tensegrity can explain and predict from mechanistic principles how complex cellular behaviors observed at different size scales and under different experimental conditions emerge from collective interactions among specific molecular components. The cellular tensegrity theory also takes into account the molecular intricacy of living cells and can incorporate increasing levels of complexity, including multimodularity and the existence of structural hierarchies (5, 20, 22). These features may help to explain how molecular structures in specialized regions of the cell are independently stabilized on progressively smaller size scales, although also

displaying integrated mechanical behavior as part of the larger cell and tissue (4, 5, 7, 17, 20, 22, 30, 33, 34, 42). Because the tensegrity model is a mechanical paradigm, it does not per se explain chemical behavior in living cells. However, as many investigators (including Dr. Heidemann) have shown, tensegrity provides a framework to distribute and focus mechanical forces on specific molecular components; hence, it may help to explain how mechanical forces regulate cellular biochemistry and influence gene expression (7, 17, 21, 33). The other cell models that still dominate the literature cannot.

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Steven R. Heidemann, Phillip Lamoureaux, and Robert E. Buxbaum: We have been thinking about tensegrity architecture for cells since a scientific meeting, 15 years ago, at which Dr. Ingber pointed out to us that our evidence on the mechanical roles of actin and microtubules in neurons fit a tensegrity structure. We had just conducted a mechanical reinvestigation (19) of the classic anti-cytoskeletal drug experiments of Yamada et al. (32) on neurons. He and others had shown that depolymerizing microtubules caused axons to retract suddenly, suggesting to us that the axon may be under tension, which was normally balanced by compression of the microtubules. Direct force measurements on axons before and during treatment with anti-microtubule and anti-actin drugs seemed to confirm this mechanical hypothesis. Tension in axons increased when microtubules were depolymerized, and tension decreased when axons were treated with actin-disrupting drugs. Furthermore, increased tension in axons caused microtubule depolymerization (9). Combined with the well-known spatial arrangement of these filaments in axons, the simplest interpretation was that the outer actin network of axons is under a sustained tension that is normally supported in part by the inner bundle of microtubules. This complementary force balance between separate tensile and compressive elements is a basic feature of tensegrity. On this basis, we also proposed an idea related to, but separate from, tensegrity per se that shifts in this force balance regulate microtubule assembly during axonal growth (1, 2).

The problems began when we assessed the tensegrity model more critically and compared it to older models of cell architecture. In our view, the tensegrity model of cells has at least two necessary features, both fundamental according to Fuller’s own account of tensegrity (12). One, implied by the name derived from “tensional integrity,” is that continuous tension in the actin cortex fully integrates overall shape and structure. Thus global integration of the cellular structure is key; local mechanical inputs should produce distributed cytoskeletal responses (“action at a distance”) because cytoskeletal elements are interconnected throughout the cell (4). Indeed, pull on one side of a classic stick-and-wire tensegrity sculpture and the structure as a whole shifts slightly toward the side

with relative motion even on the other side of the structure. In support of this aspect of cells as tensegrity structures, Maniotis et al. (21) showed that a needle attached to one side of a cell and pulled caused the relatively distant nucleus to change shape. The second necessary feature that cells should manifest if tensegrity is to be a useful model is that a significant portion of the compression balancing the surface tension must be borne by discontinuous internal elements, e.g., cytoplasmic microtubules, not by attachment to the dish or general compression of a fluid interior, as in a balloon. This requirement for internal compressive support is a key difference between tensegrity structures and other tensile structures. That is, Fuller (12) makes a clear distinction between tensegrity and other tensile structures based on anchoring to an external compressive support:

“I also saw that man had long known of tensional structures and had experienced and developed those tensional capabilities but apparently only as a secondary accessory of primary compressional structuring. For instance, he inserted a solid mast into a hole in ‘solid’ earth and rammed it in as a solid continuity of the unitary solid earth. However, to keep it from blowing over and breaking off when hurricane raged, he added a set of tension stays triangulated from the top of the mast-head to the ground, thus taking hold of the extreme end of the potential mast-lever at the point of highest advantage against motion. But these tensions were secondary structuring actions.”

Insofar as cultured cell shape is clearly dependent on attachment to and compression of the “unitary solid” dish (cells round up when “trypsinized” from the dish), it would appear that, at best, cells can only approximate true tensegrity structures as envisioned by Fuller. Nevertheless, we would find tensegrity useful if axial compression along the microtubules would be seen to hold up part of the tensile forces known to exist in the actin cytoskeleton and this structuring was interconnected throughout much of the cell.

Sadly, our recent experiments with cell structure failed to support either of these key properties of tensegrity. We tested cell tensegrity (14) by pushing, pulling, prodding, and cutting the cytoskeleton of fibroblasts whose actin and microtubule arrays were visualized in real time using cytoskeletal proteins labeled with GFP (20). If actin and microtubules are highly integrated by a tensegrity interaction, or indeed attached to one another in any way, we should have seen distributed, generalized changes in cell shape and/or in the filament array when force was applied to various regions of the cell. Rather than integrated, spatially broad responses to forces, we repeatedly observed highly local responses. The outer actin network did behave elastically, but the internal microtubule cytoskeleton behaved primarily like a fluid. Most disappointing, the outer elastic network of actin behaved independently of the underlying cytoplasm with its microtubules and other organelles. The most telling series of experiments were those in which glass needles at the surface were strongly and effectively engaged

with the underlying actin cortex. In these experiments, glass needles were treated with an adhesion protein, laminin, to engage integrin receptors. As predicted by current models of cell adhesion, we observed a rapid recruitment of GFP-actin on the cytoplasmic side of the needle tip, which had a robust mechanical attachment to the cell (Fig. 8 in Ref. 14). Relatively weak tension exerted by the needle caused the newly recruited spot of actin to move with the needle along the surface without disturbing the underlying actin or microtubules. Larger forces exerted by actin-attached needles caused the cell to change shape, but only a local extension of cytoplasm formed. Rather than the cell and its substructure moving toward the pulled side, as predicted by tensegrity, the cell shape changed so that most cytoplasm moved away from the needle! Most damaging to our view of tensegrity was that quite large forces exerted by or on these short cellular extensions produced little change in the shape, position, or arrangement of microtubules directly adjacent the extension. In addition, it was clear that the attachment had an effective functional connection to the actin cortex in that the cell was able to exert large contractile forces on the needle (Fig. 10 in Ref. 14). Whether the attached needle exerted forces on the cell or the cell exerted forces on needle, we repeatedly observed independence of actin and microtubule behaviors among themselves and failed to observe any effect of actin deformation on microtubule arrangements. Indeed, we were particularly surprised by the lack of evidence for any significant cytoskeletal interconnections in our recent experiments. Our deformations of the cell, with and without needle linkage to the cortical cytoskeletal, produced movements only among those microtubules or actin filaments directly contacted by the needle. Even cytoskeletal fibers quite near to the site of intervention were unaffected. Thus our observations not only contradicted the global integration of the cytoskeleton required for the tensegrity model of the cell but generally changed our view of the extent of interconnection among cytoskeletal elements.

Dr. Ingber and colleagues (17, 18, 26) have defined tensegrity as continuous tension and local compression. However, we find this definition too broad. On this basis, tensegrity would include pup tents (i.e. Fuller’s compressional mast stabilized by a tensile cloth in place of discrete guy wires), suspension bridges, and rubber membranes stretched out on a board with multiple pins. These are all structures that long predate Fuller’s conception of tensegrity. We would define tensegrity structures as those with tension-induced structural integrity resulting from a continuous structure of tension-bearing elements and discontinuous compressive elements integrally connected to but dispersed within the structure by the tension elements. In other words, in our view, cellular tensegrity requires architectural features that are quite similar in mechanical and shape properties to those of the classic string-and-strut tensegrity sculptures of Snelson. These sculptures, it should be noted, have been used repeatedly as the models, illustrations, and the

sources of predictions for cellular tensegrity (7, 17, 28, 31).

In addition to the multiplicity of tensile structures, old and new, that fit a broad definition of tensegrity, we propose our more narrow definition of cellular tensegrity because two properties of the tensegrity model emphasized by Dr. Ingber and colleagues are shared by cell models quite distinct from anything we could fairly call "tensegrity." These properties are prestress of the outer actin network and linear stiffening of the cell in response to deformation or increased stress (4, 7, 17, 18, 26, 28, 31). In addition, these properties have both been well described for years, if not decades, without recourse to tensegrity models. Thus sustained tension of the cell surface, i.e., prestress in the actin cell cortex, has been analyzed as far back as the 1930s (6), and there have been well-controlled measurements of rest tension at the cell surface and associated modeling throughout the decades (10, 11, 15, 22, 23, 25, 33). Perhaps the simplest mechanical models for cells have been an inflated rubber ball (15) and a liquid drop (33). Although both of these structures would have unmistakable prestress on the surface (due to elasticity and surface tension, respectively), it is clear that neither qualify as tensegrity structures.

As shown in Fig. 5, our analysis of these classic data on liquid drops (Fig. 5A) and rubber balls (Fig. 5B) indicates that they share with tensegrity structures the property of being linearly strain/stress hardening, i.e., of behaving like an increasingly stiff spring with greater force loads or extensions (15, 33). This is an interesting property, but it cannot be regarded as diagnostic for tensegrity. Indeed, cellular stiffening is fascinating to us because it appears at every scale of force, length, and time. That is, stress/strain hardening has been measured with subcellular deformations and forces in the pico- to nanonewton range using atomic force microscopy and laser optical trapping (5, 16, 27); in whole cells with deformations in micrometers and forces in the 0.1- to 1- μ N range by plates, needles, and suction (8, 10, 14, 29, 30); and in cell layers and tissues with forces in the dyne to gram range with deformations in the millimeters (3, 13, 24). However, this stiffening effect can be explained by a wide variety of models in addition to tensegrity, including simple viscoelasticity (27), a liquid drop surrounded by an elastic cortex (10, 30), and active responses (5, 14).

Thus we are skeptical of the value of data on cellular prestress and/or stiffening for support of the tensegrity model of cells. In our view, tensegrity requires clear evidence for cell-wide integration of the cytoskeletal structure as seen by motion integration. We further require evidence of discontinuous, dispersed compressive support for the universally observed tension in the animal cell cortex. This compressive support could be supplied by microtubules or other discrete cytoplasmic elements in arrangements similar to the classic strut-and-string tensegrity structures. For these reasons, we continue to hold out some hope that neurons will be shown to resemble classic tensegrity structures because their axonal microtubules appear to be dispersed

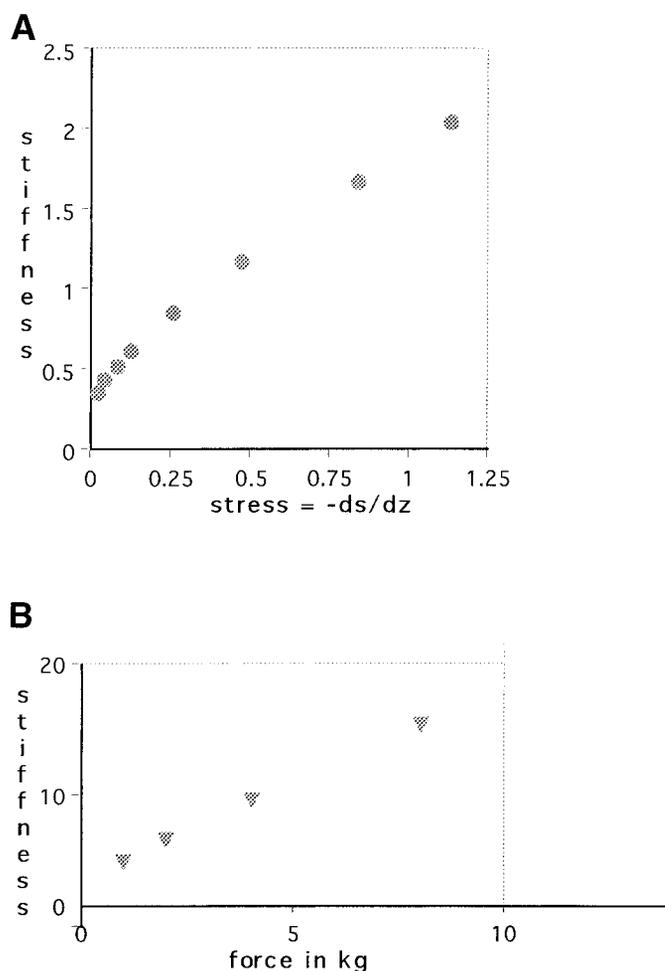


Fig. 5. Stress hardening of liquid drops and of rubber balls. *A*: stress hardening of a liquid drop; analysis of values from Table VI in the model of Yoneda et al. (33) of the mechanical properties of a liquid drop. As height of the drop (z) is decreased by external compression, surface area (s) increases. Because of the surface tension, increased area corresponds to increased energy. Thus the change in surface area (ds , effectively change in energy) with respect to relative height ($-ds/dz$) is a force (because energy is force acting through a distance). We calculated values of this force change at 8 drop heights down to 80% of its original height and calculated values for stiffness (stiffness = force change/%height change), which are plotted here as a function of the 8 corresponding forces. *B*: stress hardening of a rubber ball; analysis of Fig. 8 in Hiramoto (15) in which empirical measurements were taken on an inflated rubber ball. Weights were sequentially added to compress a rubber ball whose relative height was measured after application of 5 different forces from 0 to 8 kg. At each such step of deformation, the difference in force was divided by the difference in height to provide a stiffness between deformations, plotted here as a function of the compressing force on the rubber ball.

and under compression and because they show cytoskeletal integration in the form of action at a distance in response to local mechanical disturbances. For example, Fig. 6 shows a reproducible shape-change phenomena in which towing of a chick forebrain axon at the distal end causes a significant migration of the cell body cytoplasm, including the nucleus, into the axon shaft. When tension is relieved, the nucleus and cytoplasm then migrate back to the original position among the dendrites. At this time, we have no other

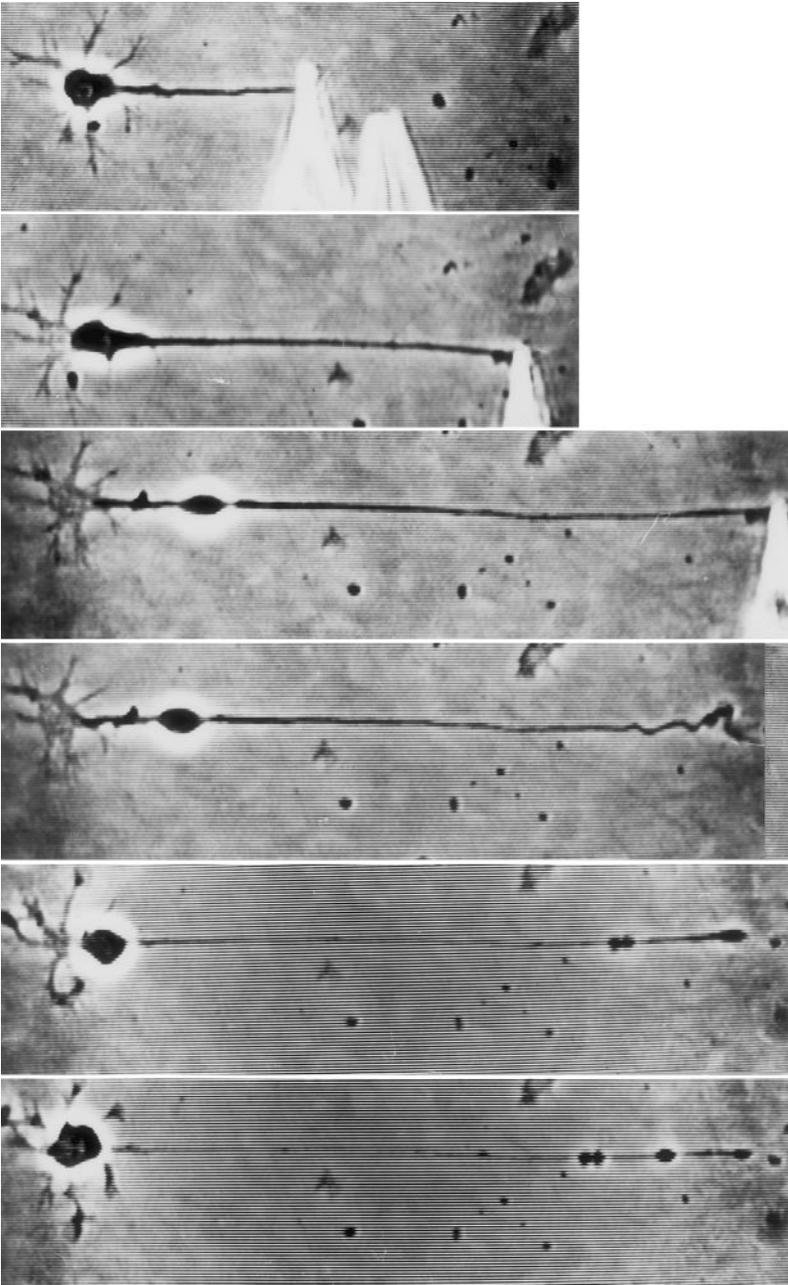


Fig. 6. Cytoskeletal action at a distance in cultured chick forebrain neurons. Experimental tension was applied to a chick forebrain neuron with an attached calibrated glass needle. As previously reported for this and other neuronal types, this causes the axon to elongate. Of particular relevance to tensegrity, at relatively high tensions, the cytoplasm of the soma, including the nucleus, migrates into the axon during towing to leave a somatic "ghost" at the original site. When the needle was pulled free, after ~ 2.5 h, the somatic cytoplasm returned to its original location by 5 h. Particularly intriguing was that, throughout the 5-h observation, "dendrites" from the cell body remained motile whether or not the soma contained the nucleus and phase-dense cytoplasm. The movement of cytoplasmic mass shown here to change cell shape at one end of the neuron in response to tension applied at the other end is highly reproducible, if technically demanding. Specifically, this cytoplasmic migration requires placing the neurite under tension just below that at which it would detach from the needle, i.e., pulling hard but not too hard.

interpretation of these data except that it is the sort of generalized shape change typical of classic tensegrity: pulling on one end caused significant changes at the other end of the structure. Furthermore, although some compressive support for neurite tension is clearly provided by dish attachment (neurites retract when growth cones are dislodged from substratum), this shows the structure is under tension. In addition, our older work (outlined above) continues to suggest that substantial compressive support may also be provided internally by microtubules that are dispersed and integrally connected to the tension structure.

In summary, our view of tensegrity is that it should denote a quite specific type of architecture and must be carefully distinguished from other tensile structures.

As we have discussed here, most cell models are fundamentally tensile and share mechanical properties with tensegrity architecture, such as prestress and stress hardening. Therefore, it will be important to develop clear predictions that distinguish tensegrity structures from other tensile structures. Interest in mechanotransduction is increasing rapidly; the format here is too brief to allow us to cite the reviews on the role of forces and mechanical properties in sensory transduction, ventilation and other organ function, growth and development of plants and animals, and cellular differentiation and morphogenesis. However, unlike chemical signaling, for which textbooks and professional articles are adorned with elaborate, Rube-Goldberg-like sequences of molecular cause and effect

(epinephrine activates the β -receptor that activates G_s , activating adenylate cyclase, and so forth), there are few detailed models for mechanotransduction. Like the diagrams for chemical signaling, architectural models such as tensegrity will help in visualizing and comprehending mechanotransduction, but only if they are approached critically and skeptically.

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REBUTTALS

Donald E. Ingber: I believe that most of the concerns raised by Dr. Heidemann and co-workers were addressed in my original editorial; however, there are a few points that deserve further clarification and emphasis. The major issue is the definition of tensegrity. I used Fuller’s own formal definition, which can be found in the definitive text of his life’s work (*Synergetics*). A more thorough definition of tensegrity that closely matches my own can be found in layman’s terms in *A Fuller Explanation* by Amy Edmondson, a close associate of Fuller’s (3). The fact that tents, spider webs (e.g., stabilized by attachment to compression-resistant tree branches), and ship’s riggings (which Fuller often described in terms of tensegrity) existed for years before Fuller’s birth is of no import. Fuller did not invent this architectural method; he discovered the universality of its use and inspired its application by others (e.g., Snelson). To arbitrarily narrow and make concrete Fuller’s definition and then to cite a random Fuller quote out of context, which was written for an artist’s journal, seems unreasonable to me; however, I leave that to the reader. Perhaps most befuddling is that Dr. Heidemann and colleagues then ignore their own new narrowed definition when they “see” tensegrity in their own experimental system: they admit that “some compressive support for neurite tension is clearly provided by the dish attachment” (see above).

Another point of confusion is the concept that local mechanical inputs must result in action at a distance in a tensegrity structure. The point is that action at distance (global structural rearrangements) can occur in tensegrity structures; however, this is not always the case and especially so in multimodular and hierarchical arrays, as observed in living cells, tissues, and organisms. For example, stress transmitted through the network will dissipate locally if it is passed to a support element that is highly flexible; in essence, this is why forces dissipate at the highly compliant lipid bilayer/submembranous cytoskeleton, whereas they pass deep into the cell across stiffer integrin connections. In fact, local variations in the compliance of different cytoskeletal support elements may be how stresses are selectively transmitted to and focused on particular transducing molecules (e.g., stress-sensitive ion channels) during the process of cellular mechanotransduction. Local accommodation and dissipation of force may also be observed if the multimodular cytoskeleton is tethered at many points to a fixed ECM; responses may only extend between neighboring adhesions and progress no farther. Multimodularity and the existence of multiple tethers to extracellular scaffolds also may permit the cell to remove or dynamically rearrange a local support element without loss of mechanical integrity in the larger structure. This form of structural memory could play an important role in maintenance of cell form as well as tissue regeneration.

Yet another misconception by some is that tensegrity is only relevant for describing static behaviors. All tensegrity structures exhibit characteristic dynamic (frequency dependent) behaviors; in fact, we have recently shown that a priori predictions from the tensegrity model relating to dynamic responses match nicely with experimental results (N. Wang, K. Naruse, D. Stamenovic, J. J. Fredberg, S. M. Mijailovich, G. Maksym, T. Polte, and D. E. Ingber, unpublished observations). Furthermore, in the cell, it is the three-dimensional arrangement of support elements within the tensegrity-stabilized array that channels and focuses mechanical energy on the cytoskeleton-bound molecules that mediate its remodeling. Thus tensegrity is also critical for slower time-dependent responses because it guides how one instantaneous "hard-wired" tensegrity configuration will be transmuted into the next; its absence, pattern integrity would be lost over time. Finally, it is well known that the different cytoskeletal filament systems exhibit their own time-dependent (viscoelastic) responses (12); however, these properties are not sufficient to explain complex cell behaviors, unless architecture and prestress (and hence, tensegrity) are also taken into account (Refs. 15 and 19 and Wang et al., unpublished observations).

My colleagues' major claim that negative results obtained in one study (7) using a single cell type and a poorly characterized method (pulling on cell membranes with laminin-coated micropipettes) are sufficient to disprove tensegrity and to discount the results from the various publications I cited in my editorial is absurd to say the least. I also do not understand why

these authors did not consider that there might be possible caveats in their work, given that we had previously demonstrated action at a distance when we pulled on fibronectin receptors that we knew formed intact focal adhesions and not when we pulled on other receptors that only connected to the submembranous actin cytoskeleton (15). Action at a distance also has been observed by other groups (16), including in a recent study using GFP-labeled intermediate filaments (8). Furthermore, buckling of microtubules was actually demonstrated in the study by Dr. Heidemann et al. in which the cells' adhesions were quickly detached (Fig. 6 in Ref. 7). However, so far, they have ignored this point. Another caveat not considered that was raised in a prior publication (15) is that the flexibility of the cytoskeleton becomes greatly reduced when cells are cultured on rigid dishes coated with high densities of matrix molecules that promote formation of increased numbers of focal adhesions along the cell base. As described above, only microdomains of the multimodular cytoskeletal lattice between the fixed focal adhesions would be expected to rearrange or significantly deform in response to a local mechanical manipulation under these conditions. In fact, this is exactly what Dr. Heidemann's team showed in well-spread fibroblasts: a local incision in the cell resulted in a local retraction response (7). This was used as additional evidence to claim the absence of "action at a distance" in cells and thus to invalidate the tensegrity model. We observed similar local responses when we cut highly adhesive cells (17); however, we found that we could obtain more global responses by first using a micropipette like a spatula to loosen the basal adhesions beneath the cell body before application of a similar incision. Interestingly, the nerve cells that Dr. Heidemann studies form relatively few substrate adhesions beneath the cell body; this may be why he can more easily visualize action at a distance at the whole cell level in that system.

The most important point of this discussion is that my critics are correct in that there are alternative explanations and models that can explain the results from any single experiment. However, only the tensegrity model is consistent with all of these findings. Furthermore, only tensegrity can also predict many of these results a priori. For example, reconstituted gels of intermediate filaments can also exhibit strain hardening (12); however, living cells still exhibit strain hardening after intermediate filaments are chemically disrupted or knocked out genetically (20). It is also true that others structures, including rubber balls, liquid drops, tensed cable networks, and tensed cortical membrane/viscous cytosol models, may exhibit strain-hardening behavior and approximately linear dependences of stiffness on prestress. However, these other tensed models are not consistent with many other experimental results (as I described in my original discussion above) or with the microarchitecture that we observe in living cells (dense cytoskeletal networks throughout the cytoplasm, straight microfilaments, curved microtubules). More importantly, Dr. Heidemann's pre-

ferred elastic membrane-viscous cytosol-elastic nucleus model clearly does not fit with the structural complexity that cell biologists know exist at the molecular level in the cytoplasm of living cells (4,9). It also is not consistent with experimental results from many laboratories including my own, which showed that intermediate filaments connect nuclei to surface receptors, that microtubules and other cytoskeletal filaments play a key role in cell and nuclear shape control, and that there are filamentous load-bearing elements within the depth of the nucleus (2, 5, 14, 15, 19). The other established continuum models of cell mechanics, although useful at the whole cell level in particular situations, similarly offer no handle on the mechanical role of specific molecular structures or any mechanistic basis for complex mechanical behavior in cells. Only tensegrity satisfies all of these requirements.

Finally, I agree with Dr. Heidemann and his coauthors when they state that “cellular stiffening is fascinating to us because it appears at every scale of force, length, and time.” However, again, only tensegrity can explain why this behavior is observed at these different size scales. Clearly, our bodies and tissues are not constructed like a liquid droplet, a rubber ball, or even a worm-like polymer; rather, they are prestressed hierarchical networks composed of contractile cells and extracellular matrices that can bear tension or compression locally. The finding that both the musculoskeleton and mitotic spindle gain their stability through use of tensegrity and that regions of the actin cytoskeleton (geodomes), organelles (clathrin-coated vesicles), enzyme complexes, viruses, and protein filaments all exhibit tensegrity-based geodesic architecture (1, 11, 13, 18) provides perhaps the strongest argument that this model is the most robust theoretical formulation of biological structure available at the present time.

FINAL STATEMENT

The critics of any new paradigm in science will always bring up new problems and continue to “raise the bar.” However, a new theory will succeed if it is found to be useful and if it provides new mechanistic insights to the wider community. Although the current embodiment of the tensegrity model may not incorporate all of the features one might assume to be critical, experimental results confirm that it apparently does incorporate the subset of features that are sufficient to predict many complex cell mechanical behaviors, in fact, many more and diverse responses than any other existing model. More importantly, the introduction of the tensegrity model has also changed the way we view cell regulation and has led to the recognition of the critical importance of cytoskeletal prestress for control of cell shape stability (Ref. 17 and Wang et al., unpublished observations) as well as for regulation of biochemical functions, including dynamic force-dependent remodeling of the cytoskeleton (6) and shape-dependent control of cell cycle progression (10). Like any theoretical model, tensegrity is a work in progress that

will need to be continually refined as we gain more information about the complex system we call the cell. However, in the end, it will be in the forging of a new understanding of the relation between mechanics, molecular structure, and biochemical function that the importance of higher order architecture and prestress will become most clear and in which tensegrity will provide its greatest value.

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Steven R. Heidemann, Phillip Lamoureux, and Robert E. Buxbaum: Our distinguished counterpart in this debate is, of course, an eloquent and forceful advocate for tensegrity and cytomechanics generally. In addition, there is a great deal in his “pro” discussion with which we wholeheartedly agree. Indeed, we have the feeling that our behavioral model for the mechanics of the cell is not much different. Even so, we continue to have strong doubts about tensegrity, both as a generally useful model for these behaviors and as a clearly defined word. We seem to agree that fluid vs. solid behaviors by the cell is the key to this debate. Does the cell respond mechanically with elastic, sustained equilibria between force and amount of deformation? Or does the cell respond with viscous dissipation of simple deformations and force transmission by flowing? Dr. Ingber writes of viscous cytoplasm penetrating the cytoskeleton and he notes that his laboratory has seen the same type of fluid behaviors that we recently published. Thus we all seem to agree that cells show both basic kinds of behaviors, although that is hardly an original insight by any of us (10). More crucially, we agree that “transmission of tension across molecular connections within the cytoskeletal network influences shape stability throughout the entire cell.” We recently pointed to the importance of such connections, demonstrated in experiments on growth cone crawling in *Aplysia* (6). These experiments (11) quite directly showed transmission of actomyosin-generated tension from the cell surface to the microtubule-rich, central cytoplasm. Dr. Ingber’s view also implicitly accepts that these behaviors change with time. Cells show tensegrity behaviors only “if the correct series of molecular couplings are formed” and “the elastic cortex-viscous cytosol model . . . will never exhibit directed action at a distance” (see above). Notwithstanding our disagreement that the viscous model suggests any such thing (see discussion of viscoelasticity below), there is a temporal dimension contained within “if” and “never.” Thus we probably agree that sometimes the cell responds fluidly and at other times elastically. These agreements paradoxically lead to one of our two principal disagreements. From the standpoint of physical behaviors and analysis, the lack of a temporal dimension to the ideas and mental images surrounding architectural tensegrity seriously compromises its value for biology. Whether one points to the Dymaxion house, geodesic domes, or string-and-strut models as illustrative tensegrity structures, their mechanical properties do not change with time. The lack of a temporal dimension to tensegrity has important implications both for its utility in cell modeling and in the physical evidence used to distinguish between it and other models.

From the standpoint of physical evidence used to support or falsify tensegrity (or any other cell model), we think the time scale of this evidence is crucial to a clearer understanding of “what’s going on.” This arises

from another likely area of agreement: that cytoplasm in its fluid responsiveness is a viscoelastic fluid, not Newtonian like water. As anyone who has played with Silly Putty knows, whether it behaves like a solid or a fluid depends on the rapidity or abruptness of the input. Silly Putty can both bounce like a rubber ball and flow slowly over the edge of a desk: pull it abruptly and it breaks; pull it steadily and it flows. Similarly, both actin and microtubule suspensions, though liquid at long time scales and high stresses, behave as a solid at short time scales or small stresses (2, 7). Figure 3 of our esteemed counterpart’s initial position piece is an ideal example of the importance of such viscoelastic phenomena in this debate. This figure shows an uncontested example of elastic cellular response and evidence of connectedness between the cell surface and the underlying cytoskeleton. If the time scale of this observation was 10 min, we would agree that it represents the sort of solid interconnections envisaged by tensegrity and provides support for it. (We would argue that our Fig. 6 in our original discussion above is just such an example.) On the other hand, if the time scale of this observation is 10 s or less, we would bet our money on viscoelasticity. Contrary to Dr. Ingber’s assertions concerning viscous models, which apply to Newtonian fluids, viscoelastic fluids manifest both substructure of filaments within the fluid (e.g., Ref. 3) and interconnectedness due to passive entanglements of polymer filaments (4). On the basis of Maniotis et al (8), which is also the basis of Dr. Ingber’s Fig. 3 (as noted), it would seem that this response occurred in 2 s or less, much shorter than the 5- to 10-min time scale over which fibroblasts maintain cell shape and crawl. Given the images and the time scale, our honest assessment of Fig. 3 is that it is most likely due to viscoelastic behavior, like threads embedded in Silly Putty becoming aligned by pulling on the putty. Whether or not cytosolic viscosity is a better model than tensegrity structures for what is happening in Fig. 3, we think viscoelastic structure, the possibility of fluid interconnectedness, and general time dependence of mechanical behaviors have all been completely overlooked by the tensegrity model.

If we agree that solidlike, cytoskeletal connections come and go, then we would argue that such temporal aspects may be the most important for modeling of cell mechanics. Our recent analogy of cytoskeletal and cell surface connections to that of an automobile transmission with clutches that engage and disengage an actomyosin motor was intended to highlight this engagement-disengagement aspect of cell mechanics (6). In our view, the ideas and images surrounding tensegrity do not help at all in thinking about this “now you see it, now you don’t” aspect of cytomechanics. Tensegrity structures behave purely elastically, all the time, and the stability of the interconnections fundamentally underlies tensegrity’s beauty as a physical model and as aesthetic objects. At the very least, if the come-and-go connectedness of a solidlike transmission is closer to reality than the temporally stable connections and behaviors of tensegrity structures, then cellular

tensegrity must incorporate force, frequency, or time-dependent transmission criteria. Do cells respond elastically 10 or 90% of the time, in response to 10 or 90% of experimental deformations? Do 10 or 90% of mechanical adhesions at the surface produce long-term (>1 min) connections to the underlying cytoskeleton? If not, one is left in the untenable position that when the cell behaves in an interconnected fashion it is tensegrity, but the rest of the time and the remainder of the cellular responses are meaningless.

In addition to the absence of a temporal dimension inherent in tensegrity, our other major disagreement is indeed with the meaning of the word tensegrity and our "overly concrete definition." We continue to find Dr. Ingber's account of tensegrity far too broad to be of real use. That is, a "structural framework" that includes balloons, the human body, geodesic domes, and Snelson sculptures is an accommodating framework indeed. We wonder aloud whether the simple word "solid" isn't a better description for the nonfluid behaviors of the cell. As argued above by us, the solid state is the simplest representation of durable interconnections among elements. We pointed out in our initial discussion above that a wide variety of different structures manifest prestress, local compression and are stress/strain hardening, important features of tensegrity structures. In Dr. Ingber's discussion above, he states, "One of the most important features of the tensegrity model, as opposed to the viscous cytosol model, is that it predicts that applied mechanical forces will not be transmitted into the cell equally at all point on the cell surface." In fact, most complex solid structures do not transmit forces equally to/at all points on the surface. A particularly dramatic example, as long as we are talking about architecture, is the photoelastic analysis of stresses in Gothic cathedrals (9). These show forces at one location (the vault weight and clerestory wind loads) transmitted by the flying buttresses to the pier buttresses at a distance. Surely, Notre Dame de Paris is not a tensegrity structure!

With time, we have found tensegrity to be a less and less useful model or mental image to generate original predictions about cytomechanics. We repeatedly found that the qualitative mechanical predictions of tensegrity (such as surface prestress, strain hardening, local compression, and nonuniform stress transmission) are widespread among solid objects and that time-dependent behaviors are not modeled at all by man-made tensegrity structures. Is tensegrity a good metaphor if we only mean "transmission of tension across molecular connections within the cytoskeletal network" and "long-distance force transfer" when this is commonplace in solids? Tensegrity is attractive largely because

of its rare and innovative structural features (1, 5). (How frequently have you seen geodesic domes, and did you mistake them for typical architecture?) If cellular tensegrity does not share at least some unique features of man-made tensegrity objects, but only common, solid properties, then we ask whether tensegrity, among the myriad of man-made structures to potentially describe cytomechanics, is the best or even a generally useful model and metaphor.

FINAL STATEMENT

As we noted in our original argument above, we are keeping an open mind about tensegrity as a model for cellular mechanics. However, for us at the moment, it does not seem to be general enough for cellular responses or specific enough to experimentally distinguish from elastic, and even viscoelastic, models. We think it is unlikely that any cell will correspond with a strict definition of tensegrity, but some aspects of tensegrity may well be applicable to some cells, at some times, under some conditions. Too broad a definition of tensegrity eliminates its usefulness and originality as a model. An important advance would be to identify and agree on mechanical properties of man-made tensegrity structures that are not widespread among elastic structures so that we can look for these behaviors in cells.

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