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REVIEW

Life on the wire: on tensegrity and force balance in cells

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Abstract. Since cell mechanics has attracted the attention of a growing number of researchers, several models have been proposed to explain cell mechanical behavior, among which tensegrity is certainly the most convincing one. Originally developed by the architect Buckminster Fuller, tensegrity structures are based on the presence of discontinuous compression elements that balance the force generated by continuous tension elements, thus reaching an equilibrium that is completely independent of gravity. This model is a useful tool to predict cell spreading, motility and especially mechanotransduction, i.e. the capability to transform mechanical stresses into biochemical responses, a key process in homeostasis of many tissues that must continuously withstand mechanical forces, like bone, but which is still poorly understood.

Key words: Tensegrity, force balance, cell mechanics, mechanotransduction, biochemical responses

Everybody experiences forces in his lives. Living means to face forces that act on and surround everything and everybody. Living organisms have evolved in presence of mechanical forces and some tissues have specialized to withstand forces, like gravity, which otherwise would obstacle every movement and every action of living beings. Although biochemical pathways within cells have always attracted a great attention by researchers, just recently some light has been shed on the mechanical properties of eukaryotic cells, on how cells sense mechanical forces and on how cells react to them.

In facts, even if it has long been known that cells possess a highly organized internal scaffold, the cytoskeleton (1), cells have usually been depicted as a semi fluid membrane containing a liquid or jelly cytoplasm (2). It is now widely accepted that cell functions are regulated by mechanical forces (3-8), which influence cell differentiation, proliferation (9) and gene expression (10). Therefore understanding physical structure of cells has become more and more important, because it is the key factor to understand the profound link existing between cell shape and cell function, between physical forces and biochemical responses.

Among many biological models proposed over the years, the tensegrity theory has proved to be capable not only to explain observed properties of the cells but also to predict some of their complex behaviors.

Tensegrity ("tensional integrity") was first described by architect Buckminster Fuller (11) as structures composed by continuous tension elements and discontinuous compression elements. Since these structures do not rely just on compression bearing components, like a brick building or a stone arch would do, they are typically independent of gravity, and do not need as high a mass as a purely compression bearing structure would under an equivalent load, because compression elements (necessarily thick and bulky) are minimized, and the force is distributed to tension elements, that can be more slender and light. Probably

the most striking examples of tensegrity structures are Snelson's sculptures, one of Fuller's most brilliant students. His "stick and wire" creations clearly portray the physical principles underlying Fuller's theory (12), making tensegrity very easy to visualize (Fig. 1).

In this kind of structures, certainly the most popular ones, compression elements are basically compression resistant struts, like wood or iron sticks or bars, while tension elements are constituted by wires or elastic strings. An essential feature of tensegrity is the presence of pre-stress, an isometric tension balanced by compression struts within the structure (like in Snelson's masterpieces), by external elements (like a spider net attached to a tree's branches), or by a combination of both. According to an energetic principle formulated by mathematicians, all pre-stressed structures assume the configuration that minimizes their stored elastic energy (13).

Actually Fuller formulated his tensegrity theory while studying geodesic architecture, i.e. structures in which the elements are disposed along geodesic (minimum paths) lines. In facts geodesic domes (like the building la Geode, in Paris), although very different-looking from Snelson's sculptures, are a good example of tensegrity based on rigid, non extensible bars, with a triangular arrangement, to locally support either compressive or tensional forces. In this case the spatial arrangement of the elements and the load distribution, not the difference in the components' elasticity, determines the structure's stability. Good examples of geodesic structures are the molecule of fullerene, an allotropic state of carbon (14) (Fig. 2), or some viral capsides.

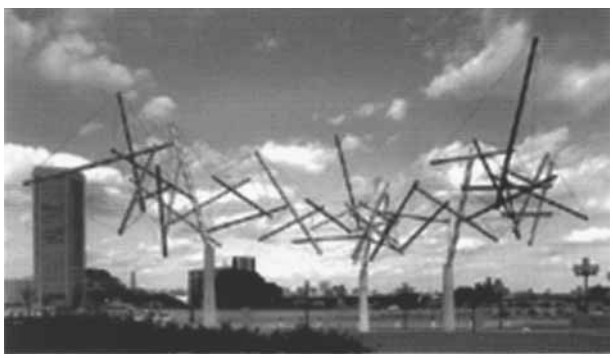

Figure 1. *Three Crowns*, a stick and bar tensegrity sculpture by K. Snelson (Baltimore, USA)

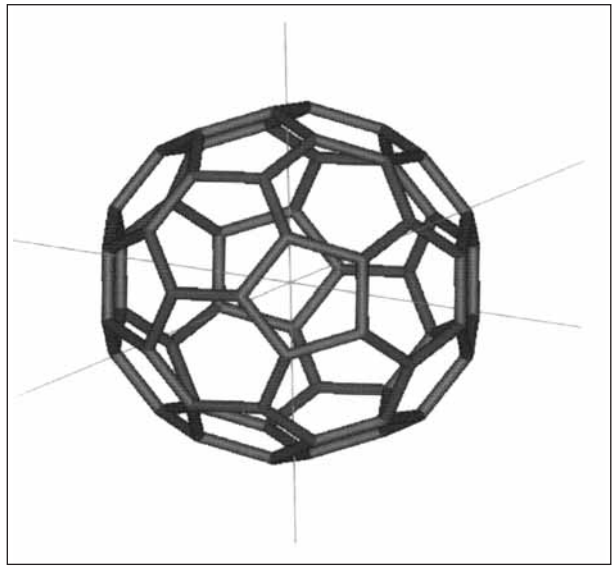

Figure 2. The Fullerene molecule shows a geodesic structure

Ingber and colleagues (15-17) first hypothesized that cell structure is actually based on a tensegrity architecture, that is, the cytoskeleton is formed by compression resistant components and tensional elements (Fig.3).

This theory fundamentally opposes the view of the cell as an elastic membrane surrounding a liquid cytoplasm, very popular to explain blood cell (18, 19)

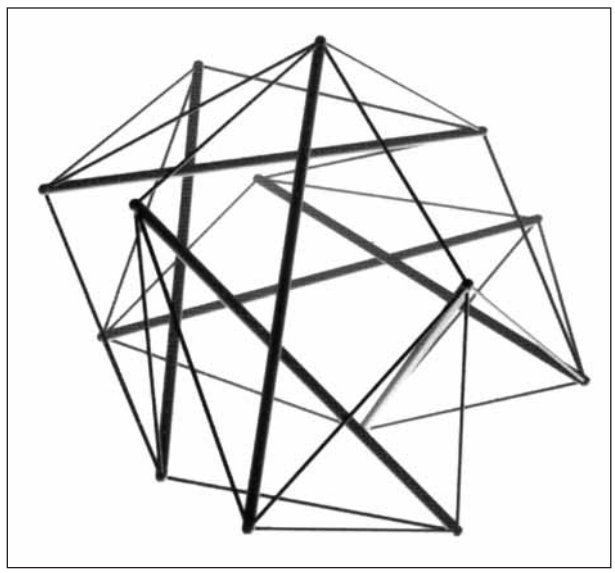

Figure 3. A six struts tensegrity model has been applied to cells to explain their mechanical behavior

and also attached cell (2, 20-23) mechanical behavior, but unable to provide a convincing explanation of mechanotransduction, because it assumes that mechanical stresses are homogeneously dispersed in the cytoplasm.

Tensegrity, as said, implies the presence of a prestress within the cell, generated by the acto-myosin molecular motors, carried throughout the cell by a continuous meshwork of actin filaments, and balanced by the extracellular matrix to which cells are attached, by load bearing microtubules, and by rigid actin bundles (24). Unattached cells possess an isotropic shape, round or circular, although they still possess a highly structured (1, 25) but loosely packed cytoskeleton (26), but they can rapidly convert their shape to an extended, oriented morphology, without changing the microfilaments number (27) or the internal content of F-actin (28). When cells attach to a substrate, they spread out, thanks to a cytoskeleton rearrangement, and form adhesion complexes to withstand the centripetal force internally generated by the actin filaments, thus transferring the tension to the extracellular matrix below (17). If the stiffness of the substrate is greater than the stiffness of the cytoskeleton, then the cell spreads and flattens, pulling against its focal adhesions (29). Using models made of sticks and elastic strings, Ingber clearly replicated not only cell shape changes but also nucleus polarization to the cell base during cell spreading (30). The tensegrity model predicts also that the axial tension between two focal adhesions determines the formation of bundles of parallel actin filaments (stress fibers) between the two adhesion complexes (31-33), as it is observed *in vitro*. The actin filamentous network can also rearrange in the apical region of the cells, to form polygonal nets, sometimes with a triangular assembly, resembling geodesic structures (34).

Many researchers have extensively investigated the role of the filaments, in cell responses to applied stresses, especially the role of microtubules and microfilaments, which seem to be more deeply involved in withstanding mechanical forces.

Microtubules are formed by the assembly of a base unit, the α-β tubulin heterodimer, and present a hollow structure (35), similar to a tube, with a higher second moment of inertia, that makes them a better candidate to withstand compressive loads (36). Microtubules present a longer persistence length (ξ) than actin filaments, where

$$\xi = \kappa_b/K_bT \quad (1)$$

(T = temperature, K_b = Boltzmann constant, κ_b = flexural rigidity of the filament). The persistence length is a measure of the filament stiffness: if the contour length of the filament (L) is much smaller than ξ, then this appears rigid and straight, but if L~ξ then the filament bends as the result of the energy exchanges with the surrounding environment (37). According to direct microscopic observations (38, 39) microtubules' ξ is in the range between 1-6 mm, that is hundred fold longer than microtubule length in living cells, and their Young's modulus is in order of GPa (40). This is in agreement with the observation that microtubules in solution appear straight[41], but they often appear curved and bent within cells[42], as if subjected to a compressive force (43).

On the contrary, actin filaments present a very different structure and different mechanical properties. F-actin (filamentous) appears as a 8 nm-wide coil formed by two strands of a globular protein, G-actin, although the two strands are not independently stable (44). Actin filaments are characterized by an exceptional elasticity (45-47), and a relatively low persistence length (~17 μm) (40, 48) , that makes them subject to bending fluctuations at cellular dimensions (49). Nevertheless they usually appear straight within cells, and specially abundant in structures associated with the leading edge of migrating cells, where they cooperate to pull the cell forward (44, 50). The role of actin in force generation and transmission in living cells has been intensively studied over the years. Actin is at the base of cell contractility, either through interactions with myosin (51), or through localized gel-sol transitions (52). Actin filaments distribute and support contractile stress within the cell, and their disruption determines reduction in cell stiffness, measured by Atomic Force Microscopy (53) and by cell populated reconstituted tissue models (54). Elasticity mapping of fibroblasts by Atomic Force Microscopy revealed the presence of tension lines, that coincided with actin filaments, observed at fluorescence microscopy (55, 56).

Microtubules seem to be extremely important for internal stability of cells presenting strong asymmetries, i.e. presenting elongated appendices, like neurons[17]. In these cells, microtubules disruption determines neurite retraction (33, 57-59), even if the extracellular matrix can provide an interchangeable load bearing role (60). According to some authors (61), microtubules balance just a small fraction of the internal pre-stress (~14%), although it has been shown that, under different conditions, microtubules can account for more than 50% of the pre-stress balance, in fibroblasts grown on collagen lattices (62). On the other hand, it has been shown that microtubule disruption by colchicine determines an increase in the tractional force exerted on the extracellular matrix through focal adhesions (63-66), because a higher fraction of the actin generated stress is necessarily balanced by the extracellular matrix, while F-actin depolymerization by cytochalasin is associated with a decrease in this force (64, 67). Some concerns have been raised about the possibility that the effect of microtubule disruption might be actually the result of an activation of myosin light chain kinase (65), with a subsequent increase in myosin actin contraction, or from a change in the intracellular calcium levels (68), rather than a tensegrity force balance, but recent findings seem to confirm this behavior also under conditions in which both the intracellular calcium level and the myosin light chains phosphorilation do not change (43).

Moreover, Pickett-Heaps and colleagues showed that if a microtubule was selectively severed by a laser beam, the neighboring microtubules appeared to buckle, as it was logic to expect, considering that the load was distributed among fewer compression bearing filaments (69), and Green-Fluorescent-Protein labeled microtubules appeared to buckle when they impinged onto surrounding cell structures (70), but straightened up when they passed the obstacle (71). In cells whose spreading was confined and the shape was influenced by ad-hoc designed micropatterned substrates, microtubules can balance up to 70% of the internal pre-stress (72).

The role of microtubules however seems to vary according to cell culture conditions and to cell type: a recent study conducted on chondrocytes revealed that viscoelastic properties of these cells before and after microtubule depolymerization by colchicine did not differ significantly (73).

On the other hand, intermediate filaments' role in cell mechanics remains quite elusive. Although encoded by one of the largest families of genes within the human genome (74) they are the least characterized and least known proteins of the cytoskeleton (75). *In vitro* studies revealed unique viscoelastic properties, which make them very resistant to breakage due to mechanical strain (76, 77), and a high elasticity, with a persistence length of 1000-1300 nm (78, 79): this makes them good candidates to stabilize cells and their internal compartments (80). Intermediate filaments are important elements of what some researchers call the nucleoskeleton (the structural proteins within the nucleus (81)), but they also surround the nuclear surface (82-84) and show associations with integrin rich focal contacts (85, 86), thus spanning from the nucleus to the cell surface (87). Their remarkably strong biochemical interactions with sequence-specific DNA and histones suggest the possibility that intermediate filaments might play an important role in coupling mechanical signals and gene expression (81, 88-90). It has been suggested that intermediate filaments may act as mechanical integrators (91), stabilizing nuclear form and cell structure (17, 92), holding separate parts of the cell (nuclei, microtubules) in place, opposing nuclear oscillatory expansion and contraction during DNA trascription (93). An important study by Eckes and colleagues (67) showed that vimentin deficient cells exhibited reduced mechanical stability: these cells were about 40% less stiff than wild type cells, and their cytoplasm could be easily torn under mechanical deformation. Moreover these cells, grown on collagen gels, presented a reduced contraction of the substrate, as a result of a decreased contractility. To explain microfilaments' role in cell mechanics, a six-strut tensegrity model has been used (94). According to this model, microtubules are rigid struts, bearing compression, actin microfilaments are elastic elements, initially under tension (pre-stress) and intermediate filaments are elastic elements, initially slack. This model predicts several properties observed in living cells: cell stiffness is reduced when intermediate filaments are disrupted by acrylamide (95) in comparison to untreated cells, and the difference

progressively increases with increasing stress. It is currently believed that intermediate filaments can carry tension but just at large applied mechanical strains, when they contribute to cell stiffness (72, 76, 95). Moreover it has been proposed that intermediate filaments provide a later support to microtubules, thus reducing their buckling under compression (94, 96).

The cytoskeleton is a complex scaffold in which all these structures actually cooperate and are intrinsically interconnected. The filamentous networks are interlinked so that the force is distributed among them (97).

The result of the high connectivity that characterizes every tensegrity structure is that applying a load on a point of the network generates an action at a distance, i.e. a structural rearrangement, due to the stress transmission along the tensional continuum, until a new equilibrium is achieved. As a consequence, cells possess preferential pathways for the distribution of stress within the cytoplasm, that transfer the mechanical action at a distance from the integrins to many cellular compartments, including the mitochondria, physically anchored to microtubules (98), and the nucleus (99), in a similar way to Snelson's sculptures, where pulling a strut determines a rearrangement of the components that propagates to the whole structure. In a famous study, Maniotis and colleagues (99) demonstrated that a mechanical stress applied to the cell surface bound integrins by a fibronectin coated micropipette determined an alignment of the nuclei along the tension lines and even a molecular rearrangement within nucleoli. Similar results could not be obtained if the force was applied in a parallel direction to the cell membrane (100), because in this case the main load bearing structure was the submembranous cytoskeleton (70). When mechanical stresses are applied to integrins, by surface bound microbeads, a greater force is required to deform the cell, than if the force is exerted on other kinds of membrane proteins, like metabolic receptors (101). Recently, Hu and colleagues used a new technique of intracellular stress tomography to visualize mitochondria displacement by mechanical stress applied by integrin bound ferromagnetic beads, and demonstrated that intracellular stress distribution pattern is modulated by pre-stress levels (102).

An extremely interesting aspect of tensegrity theory is the possibility to extend it to the whole body. When one stretches an arm, or takes a step, he contracts a series of muscles that transmit forces to tendons, ligaments and eventually to bones. The human and, more generally speaking, vertebrates' body is thus composed by rigid discontinuous elements, the bones, resembling the previously considered compression resistant struts, and a complex continuous network of contractile muscles and elastic ligaments, characterized by the presence of a pre-stress, the muscle tone. The spine would need a much bulkier structure if it were just a compression column, and the surrounding ligaments and muscles did not stabilize it. Muscles pulling the femur medially reduce the buckling of the bone under compression loads.

At a smaller size scale, cancellous bone structure optimizes its mechanical efficiency, minimizing the mass, by triangulating its small struts, the trabeculae, in a similar way to a geodesic structure, and the histological structure of the bone tissue itself is actually formed by hydroxyapatite crystals, that contribute to the compressive stiffness of the tissue and by a collagen network, that provides tensile stiffness. At a molecular level, a recent study analyzed proteins in terms of tensegrets, i.e. structural elements held together by attractive and repulsive forces. According to this hypothesis, α-helices or β-strands represent the rigid, compression bearing elements, while the attractive or repulsive atomic forces provide the stabilizing tension (103). Several studies seem thus to propose a hierarchical tensegrity structure for organisms at different size scales, with a sort of fractal perspective, in which a tensegrity structure is integrated within a larger and more complex structure possessing a tensegrity organization itself, creating a self maintaining and self balancing organism, in biochemical and mechanical equilibrium with the surrounding environment.

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