A Microstructural Approach to Cytoskeletal Mechanics based on Tensegrity

DIMITRIJE STAMENOVIC†, JEFFREY J. FREDBERG‡, NING WANG‡, JAMES P. BUTLER‡ and DONALD E. INGBER§

† Department of Biomedical Engineering, Boston University, Boston, MA 02215, ‡ Physiology Program, Department of Environmental Health, Harvard School of Public Health and § Departments of Surgery and Pathology, Children’s Hospital and Harvard Medical School, Boston, MA 02115, U.S.A.

(Received on 19 October 1995, Accepted in revised form on 9 February 1996)

Mechanical properties of living cells are commonly described in terms of the laws of continuum mechanics. The purpose of this report is to consider the implications of an alternative approach that emphasizes the discrete nature of stress bearing elements in the cell and is based on the known structural properties of the cytoskeleton. We have noted previously that tensegrity architecture seems to capture essential qualitative features of cytoskeletal shape distortion in adherent cells (Ingber, 1993a; Wang et al., 1993). Here we extend those qualitative notions into a formal microstructural analysis. On the basis of that analysis we attempt to identify unifying principles that might underlie the shape stability of the cytoskeleton. For simplicity, we focus on a tensegrity structure containing six rigid struts interconnected by 24 linearly elastic cables. Cables carry initial tension ("prestress") counterbalanced by compression of struts. Two cases of interconnectedness between cables and struts are considered: one where they are connected by pin-joints, and the other where the cables run through frictionless loops at the junctions. At the molecular level, the pinned structure may represent the case in which different cytoskeletal filaments are cross-linked whereas the looped structure represents the case where they are free to slip past one another. The system is then subjected to uniaxial stretching. Using the principal of virtual work, stretching force vs. extension and structural stiffness vs. stretching force relationships are calculated for different prestresses. The stiffness is found to increase with increasing prestress and, at a given prestress, to increase approximately linearly with increasing stretching force. This behavior is consistent with observations in living endothelial cells exposed to shear stresses (Wang & Ingber, 1994). At a given prestress, the pinned structure is found to be stiffer than the looped one, a result consistent with data on mechanical behavior of isolated, cross-linked and uncross-linked actin networks (Wachsstock et al., 1993). On the basis of our analysis we concluded that architecture and the prestress of the cytoskeleton might be key features that underlie a cell’s ability to regulate its shape.

Introduction

Mechanical stresses on cells, such as those imposed by hemodynamic forces, gravity or cell-generated tension, are known to regulate tissue growth and development and to alter cell form and function (Ingber, 1991; Davis & Tripathi, 1993). For example, when adherent endothelial cells are exposed to flow-induced shear stresses, the cytoskeleton (CSK) undergoes major structural reorganization, the topological profile of cell height changes, ion channels become activated, acetylcholine and substance P are released, and changes in gene expression occur (Davis & Tripathi, 1993). Since many elements of the cell's metabolic machinery appear to be immobilized on insoluble support scaffolds, changes in cell function may result from CSK remodeling and structural rearrangement (Ingber, 1993b). As such, the mechanical basis of CSK deformability becomes of central interest.

† Author to whom correspondence should be addressed. E-mail: dimitrije@enga.bu.edu

© 1996 Academic Press Limited
The standard approach in cell mechanics is based upon the continuum hypothesis. It views the cell as a continuous elastic cortical shell surrounding a continuous viscous or viscoelastic core (cf. Elson, 1988; Evans & Yeung, 1989; Fung & Liu, 1993). Shape distortion of the cell is assumed to result primarily from stresses distributed over the cell membrane and transmitted throughout the cytoplasm following the laws of continuum mechanics. This view of CSK deformability has been fruitful. The continuum hypothesis rests on the premise that a scale which is small compared with the cell, but large compared with the distance between microstructural elements, the microstructure itself need not be taken into account explicitly. The physical attributes (mass, force, stiffness, strain energy, friction) and deformation within a given small volume are assumed to be spread continuously throughout that volume rather than being concentrated in a small fraction of it.

The purpose of this report is to consider the implications of an alternative viewpoint that emphasizes the discrete nature of stress bearing elements in the cell and is based on known properties of the CSK. Our rationale is as follows. In contrast to the continuum perspective, it is now firmly established that force transmission between the cell and the extracellular milieu occurs at focal adhesion sites and is mediated by specific trans-membrane receptors, such as integrins, that form discrete molecular bridges that interlink intracellular CSK filaments with extracellular matrix anchoring scaffolds (Ingber, 1991; Schiro et al., 1992; Schmidt et al., 1993; Wang et al., 1993; Scott-Burden, 1994). It is also well established on structural grounds that the CSK is an interconnected lattice comprised of discrete microfilaments, microtubules, and intermediate filaments (cf. Amos & Amos, 1991; Ingber, 1993a). Although much is known about the molecular constituents of the adhesion complexes and the CSK matrix, there is little understanding of how these components are organized architecturally or how they act to resist shape distortion. To our knowledge, no attention has been focused on the issue of the degree to which deformation of this lattice might conform to the tenets of the continuum hypothesis or on the implications of departures therefrom.

We have previously noted that eukaryotic cells display both CSK structure and elastic deformability that appear to be consistent with so-called tensegrity (tensional integrity) architecture as first described by Buckminster Fuller (Fuller, 1961; Ingber & Jamieson, 1985; Ingber, 1993a; Wang et al., 1993; Ingber et al., 1994). In its simplest representation, Pugh (1976) defined tensegrity structures as the interaction of a set of discontinuous (isolated) compression elements (e.g., struts) with a set of continuous tension elements (e.g., cables) in the aim to provide a stable volume and shape in the space. The tension elements carry “prestress” (i.e., initial stress), conferring load-supporting capability to the entire structure. The role of the compression elements is to provide prestress in the tension elements. Together, they form a self-equilibrating, stable mechanical system.

A distinguishing characteristic of the tensegrity structure is that in order to express a resistance to distortion of shape it requires a prestress in its members even before the external load is applied. Examples of tensegrity structures in nature include spider webs, gas-liquid foams, plant leaves, and mammalian lungs. In the case of foams, leaves, and lungs the prestress is provided by the pressure of the inflating fluid (the compression element in lieu of rigid struts), and is carried by lattice tension elements (e.g., liquid films in foams). In the case of spider webs, the prestress is provided by discrete attachments to surrounding objects, such as tree branches, and is balanced by tension in web threads. Even though they are external to the web itself, the tree branches may be viewed as the compression elements in a tensegrity structure, because they are an integral part of the mechanically stable whole. In the absence of the prestress, the intrinsic resistance to shape distortion is lacking in these structures because their internal degrees of freedom of motion are not fully constrained. In the presence of a prestress, however, the structural elements move relative to one another until they attain a configuration which provides equilibrium between external shear forces and those carried by the structural elements. The larger the initial forces carried by those elements (i.e., the larger the prestress), the smaller the deformation those structures would undergo at a given shear stress before they attain a new equilibrium configuration.

A key feature of any tensegrity structure is the interconnectedness of its elements, i.e., the manner in which structural elements are mutually connected and the degree of relative motion between interconnecting elements at their junctions. For example, interconnection between liquid films in foams is such that at equilibrium surface forces carried by those films are equal. This type of interconnectedness yields minimal shear stiffness (Stamenović, 1991).

It is likely that the CSK together with the extracellular matrix form a tensegrity structure (Ingber, 1993a; Ingber et al., 1994). For example, the regions of extracellular matrix that stretch between focal contacts represent local compression-resistant elements which resist the tension exerted by
stress-fibers inside the cell (Harris et al., 1980; Ingber, 1993a). Within the CSK of living cells, microtubule bundles act as compression-resistant struts and stabilize cell shape by resisting the pull of the contractile actin lattice (Joshi et al., 1985; Dennerl et al., 1988; Danowski, 1989; Kolodney & Wysolmerski, 1992; Heidemann & Buxbaum, 1994).

Model structures built of sticks and elastic strings according to the rules of tensegrity architecture qualitatively mimic many of the phenomena that have been observed in living cells including the effects of substrate adhesion on cell shape, cell polarity, and CSK remodeling (Ingber & Jamieson, 1985; Ingber, 1993a; Ingber et al., 1994). These tensegrity structures also exhibit a nearly linear dependence between the stiffness of the entire structure and the applied stress, over a wide range of stresses (Wang et al., 1993). Importantly, this peculiar “linear stiffening” response appears to be a fundamental property of living cells (Wang et al., 1993; Wang & Ingber, 1994) as well as tissues (cf. Fung, 1981). While the characteristic linear stiffening can be predicted by empirical relationships (Mow et al., 1992) or phenomenological models (Frisén et al., 1969), these approaches have not been able to explain the observed phenomena based on first principles (McMahon, 1984).

In the sections below we extend these qualitative notions into a formal microstructural analysis. On the basis of that analysis we attempt to identify unifying principles that might underlie shape stability of the CSK. For simplicity, we focus on a tensegrity structure containing six rigid struts interconnected by 24 linearly elastic cables; such a simple tensegrity structure embodies the same essential features observed in structures with different arrangements and numbers of structural elements as well as in hierarchical arrangements of different sized tensegrity arrays. Most importantly, we do not view this six-strut structure as a direct, one-to-one model of some part of the CSK. Rather, it is a plausible description of the mechanisms that regulate cell shape stability for which a quantitative basis has been lacking in the past. The six-strut tensegrity structure was subjected to uniaxial stretching and corresponding force vs. extension relationships were calculated for different prestresses and for different types of interconnectedness of structural elements, starting from first principles of mechanics. This approach elucidates how simple tensegrity structures naturally come to express many of the seemingly complex behaviors observed in living cells exposed to shearing forces. This does not at all preclude the numerous chemically mediated mechanisms which are known to regulate CSK filament assembly. Rather, it elucidates a higher level of organization in which biochemical remodeling events function and also may be regulated.

**Method**

**DESCRIPTION OF THE PROTOTYPE TENSEGRITY STRUCTURE**

The CSK is assumed to be organized as a tensegrity structure (Ingber & Jamieson, 1985; Ingber et al., 1994). Thus, to simplify our approach, a six-strut tensegrity structure (Fig. 1) was considered as a first step in implementing tensegrity architecture in studies of cell mechanics.

The six-strut tensegrity structure is composed of 24 cable segments and six struts. In this study, the cables are viewed as elastic elements which support only tension forces whereas the struts are viewed as rigid bars under compression. The struts are slender and support no lateral load. At the reference (initial) state, compression forces in the struts balance tension forces in the cables. The initial tension is referred to as a prestress. Within the CSK, microfilaments and intermediate filaments may play the role of cables...
struts from changes in the distances between the pairs of parallel
relationships are obtained
model geometry, as described in the Appendix. The
assumption are: (i) the tension force in the cables
slide relative to the struts. Consequences of this
junctions ("looped structure") and thus, they can
case the cables run through frictionless loops at the
length, and (ii) forces acting at each end of a strut or
the tension force in each cable segment depends on its
structure"). Consequences of this assumption are: (i)
intermolecular binding interactions (e.g., microtubules
in contrast, the looped structure could correspond to the case
when those filaments are not cross-linked and can slide relative to each other (e.g., intermediate
filaments across actin stress fibers).

GEOMETRIC CONSIDERATIONS

For convenience, the struts are assumed to be of
unit length. Thus, at the reference state the length of
each cable segment is \( l_0 = 0.375 \) and the distance
between parallel struts \( s_0 = 0.5 \) (Kenner, 1976).

The structure is stretched in the direction of the
Ox-axis (axial direction) by pulling the struts \( \overline{AA} \) and
\( \overline{A'A'} \) apart by forces of magnitude \( T/2 \) applied at each
endpoint of these two struts. The resultant pulling
(stretching) force is, therefore, \( T \) (Fig. 1). This causes
changes in the distances between the pairs of parallel
struts from \( s_0 \) to \( s \), for the struts \( \overline{AA} \) and \( \overline{A'A'} \), \( s_i \), for
the struts \( \overline{BB} \) and \( \overline{B'B'} \), and \( s_i \), for the struts \( \overline{CC} \) and
\( \overline{C'C'} \), and changes in the cable lengths from \( l_0 \) to:
\( l_i = \overline{AB} = \overline{A'B'} = \overline{A'B'} = \overline{A'B'} \), \( l_i = \overline{BC} = \overline{B'C'} = \overline{B'C'} \), and
\( l_i = \overline{AC} = \overline{A'C'} = \overline{A'C'} \) (Fig. 1). Changes in the distances between parallel
struts, \( \Delta s_i = s_i - s_0 \) (\( z = x, y, z \)), are referred as
extensions. Relationships between distances \( s_i \), \( s_i \), and \( s_i \) and cable lengths \( l_i \), \( l_i \), and \( l_i \) are derived from
model geometry, as described in the Appendix. The following relationships are obtained

\[
\begin{align*}
  l_i &= 0.5 \sqrt{s_i^2 + s_i^2 - 2s_i} + 2, \quad (1) \\
  l_i &= 0.5 \sqrt{s_i^2 + s_i^2 - 2s_i} + 2, \quad (2) \\
  l_i &= 0.5 \sqrt{s_i^2 + s_i^2 - 2s_i} + 2. \quad (3)
\end{align*}
\]

INTERCONNECTEDNESS

Two cases of interconnectedness are considered.
One, where cables and struts are connected by
frictionless pin joints at their junctions ("pinned structure"). Consequences of this assumption are: (i)
the tension force in each cable segment depends on its
length, and (ii) forces acting at each end of a strut or
a cable reduce to a single force (tension for cables and
compression for struts) and no couples. In the other
case the cables run through frictionless loops at the
junctions ("looped structure") and thus, they can
slide relative to the struts. Consequences of this
assumption are: (i) the tension force in the cables
depends on the overall cable length, not the length of
an individual segment, and (ii) loops joints transmit
only tension and compression forces to cables and
struts, respectively, no couples. At the molecular
level, the pinned structure could correspond to the
case where different CSK filaments are cross-linked or
physically bound to one another through intermolec-
ular binding interactions (e.g., microtubules and
intermediate filaments through kinesin). In contrast,
the looped structure could correspond to the case
when those filaments are not cross-linked and can slide relative to each other (e.g., intermediate
filaments across actin stress fibers).

EQUILIBRIUM EQUATIONS

Pinned structure

From the principle of virtual work it follows that
the work of stretching force \( T \) during an incremental
axial extension of the structure (\( \Delta s_i \)) is equal to the
work of tensile forces in the cables \( (F_1, F_2, \text{ and } F_3) \)
during corresponding changes of cable lengths
(\( \Delta l_i \), \( \Delta l_i \), and \( \Delta l_i \)).

\[
T \Delta s_i = 8 \sum_{i=1}^{3} F_i \Delta l_i. \quad (4)
\]

By substituting eqns (1–3) into eqn (4), the following
relationships are obtained

\[
T = 2 \left( F_i \frac{s_i}{l_i} + F_3 \frac{s_3 - 1}{l_3} \right) \quad (5)
\]

and

\[
\begin{align*}
  (a) \quad F_1 \frac{1-s_i}{l_i} &= F_2 \frac{s_i}{l_2} \\
  (b) \quad F_3 \frac{1-s_3}{l_3} &= F_1 \frac{s_3}{l_1}. \quad (6)
\end{align*}
\]

Equation (5) describes the balance of forces at the
joints \( A \) and \( A' \) along the \( Ox \)-axis (Fig. 1). Equation
(6a) describes the balance of forces at the joints \( B \) and
\( B' \) along the \( Oy \)-axis and eqn (6b) describes the
balance of forces at joints \( C \) and \( C' \) along the \( Oz \)-axis
(Fig. 1). The balance of forces at the joints in all other
directions are satisfied by the symmetry of model
geometry.

Looped structure

In this case, forces in each cable are equal
throughout deformation \( (F_1 = F_2 = F_3 = F) \) and
depend on the overall cable length \( L = 8(l_1 + l_2 + l_3) \). Taking these into account, it follows from eqns (5) and
(6) that

\[
T = 2F \left( \frac{s_i}{l_i} + \frac{s_3 - 1}{l_3} \right) \quad (7)
\]

whereas microtubules or cross-linked actin bundles
may play the role of struts (Ingber, 1993a).

The origin \( O \) of a rectangular Cartesian coordinate
system \( Oxyz \) is placed at the center of the structure,
with the \( Ox \)-axis parallel to the struts \( \overline{CC} \) and \( \overline{C'C'} \),
\( Oy \)-axis parallel to the struts \( \overline{AA} \) and \( \overline{A'A'} \), \( Oz \)-axis
parallel to the struts \( \overline{BB} \) and \( \overline{B'B'} \) (Fig. 1).
and

\[
\begin{align*}
(a) \frac{1-s_i}{l_i} &= s_{i-1} \quad \text{and} \quad (b) \frac{1-s_i}{l_i} &= s_{i+1}.
\end{align*}
\]

Equations (7) and (8) represent balance of forces at the joints as described above.

**CABLE ELASTICITY**

In this simple starting case, it is assumed that the cables are linearly elastic (i.e., Hookean) and carry only tensile forces. Hence, their force vs. length relationships are given as following:

\[
F_i = \begin{cases} 
  k(l_i - l_R) & \text{if } l_i > l_R \\
  0 & \text{if } l_i \leq l_R 
\end{cases} \quad (i = 1, 2, 3) \quad (9)
\]

for the pinned structure, and

\[
F = \begin{cases} 
  K(L - L_R) & \text{if } L > L_R \\
  0 & \text{if } L \leq L_R 
\end{cases}
\]

(10)

for the looped structure. Here \( k \) and \( K \) denote cable stiffnesses, \( l_R \) is the resting (unstressed) length of the cable segment, \( l_e \leq l_0 \), and \( L_R = 2l_R \) is the overall resting length of the cable. Since the cable length \( l_0 = \sqrt{0.375} \) is well defined, it was used as the reference length in calculating cable strains instead of the resting length \( l_R \), which can take any value between 0 and \( l_0(0 < l_R \leq l_0) \).

Stretching force vs. axial extension \((T \text{ vs. } \Delta s_x)\) relationships for the model are obtained as following. For a given cable stiffness, and a given initial cable strain \( \xi \), lateral distances between struts, \( s_y \) and \( s_z \), are computed from eqns (6a) and (6b) (pinned) and eqns (8a) and (8b) (looped) for a series of values of axial distances, \( s_x \). Computed values are used to obtain stretching force \( T \) vs. axial extension \( \Delta s_x \), relationships from eqn (5) (pinned) and eqn (7) (looped). Structure stiffness is obtained as the ratio \( E \equiv T/\Delta s_x \). Note that the units of stiffness \( E \) are in spring equivalents, i.e., force per unit length. Computations were done numerically, using Mathematica software.

**Results**

We began our quantitative analysis of the architectural basis of cell shape stability by varying prestress in the pinned six-strut tensegrity structure. This was accomplished by numerically varying the initial cable strain \( \xi \), at a given cable stiffness \( k \). Results were obtained for the unit cable stiffness, \( k = 1 \). The stretching force \( T \) increased with increasing axial extension \( \Delta s_x \), and, in general, this dependence was nonlinear [Fig. 2(a)]. This nonlinearity, however, decreased with increasing prestress (i.e., increasing \( \xi \)) and became linear when the prestress was maximal (i.e., \( \xi = 1 \)). In other words, structural stiffness \( E \) increased with increasing stretching force \( T \) (stiffening response) [Fig. 2(b)]. This indicates that the resistance of the structure to shape distortion increases with increasing stretching force. The magnitude of the stiffening response decreased with increasing prestress [i.e., the dependence of structural stiffness \( E \) on stretching force \( T \) decreased with increasing \( \xi \); Fig. 2(b)]

The lateral extension \( \Delta s_x \), increased [Fig. 3(a)] whereas the lateral extension \( \Delta s_y \) first increased and
then decreased with increasing axial extension $\Delta s_x$ [Fig. 3(b)]. Furthermore, with increasing prestress (i.e., increasing $\xi$), these dependencies diminished such that in the limit where the prestress approached maximum, lateral extensions vanished (i.e., $\Delta s_y, \Delta s_z \to 0$ as $\xi \to 1$). The asymmetry in the dependences of lateral extension $\Delta s_y$ and $\Delta s_z$ on axial extension $\Delta s_x$ implies that the model is not isotropic.

Fractional changes in cable lengths (cable strains) $\Delta l_i/l_0 \equiv (l_i - l_0)/l_0 (i = 1, 2, 3)$ were much smaller than the fractional change of the structure length in the uniaxial direction (uniaxial strain) $\Delta s_x/s_0$ (Fig. 4). In other words, as the entire structure stretches uniaxially, it extends to a much greater degree than its individual cables elongate.

For the looped structure, in order to have the same prestress at a given initial cable strain $\xi$ as in the pinned case, it is assumed that the overall cable stiffness $K = k/24$. Results were obtained for the cable stiffness $K = 1/24$ and for the same values of the initial cable strain $\xi$ and the axial distance $s_x$ as in the pinned case. Stretching force $T$ increased nonlinearly with increasing axial extension $\Delta s_x$ [Fig. 5(a)]. Unlike the pinned structure, structural stiffness $E$ exhibited a “softening” effect at higher values of prestress (i.e., higher $\xi$); stiffness $E$ decreased after an initial increase in response to increasing stretching force $T$ [Fig. 5(b)]. However, for smaller values of prestress (i.e., smaller $\xi$), the model predicts a stiffening effect [Fig. 5(b)]. In comparison with the pinned structure, the looped
structure is more compliant at a given prestress [Figs 2(b) vs. 5(b)].

For the cable stiffness $K = 1/24$ and for the same values of the initial cable strain $\bar{\varepsilon}$ and the axial distance $s$, as in the pinned case, lateral extension $\Delta s_y$ decreased, whereas lateral extension $\Delta s_z$ first decreased and then increased with increasing axial extension $\Delta s_x$ (Fig. 6). These relationships are opposite to those in the pinned structure [Figs 3(a) and 3(b)] and are independent of prestress (i.e., independent of $\bar{\varepsilon}$). Again, the asymmetry in the dependences of lateral extensions $\Delta s_y$ and $\Delta s_z$ on the axial extension $\Delta s_x$ implies that the model is not isotropic. In addition, when stretched uniaxially, the looped structure extends much more than the cable elongates, and this effect is independent of prestress (i.e., independent of $\bar{\varepsilon}$; Fig. 7).

Schematic depictions of structural rearrangements during uniaxial stretching is shown in Fig. 8(a), for the pinned structure, and Fig. 8(b), for the looped structure.

**Discussion**

**MECHANISTIC CONSIDERATIONS**

The simple architecture of the six-strut tensegrity structure and the deformation it undergoes during uniaxial stretching do not directly correspond to that of the CSK lattice of living cells or the deformation that the CSK undergoes when mechanically stressed. Nevertheless, these simplistic structures have been shown to qualitatively mimic many properties...
expressed in living cells (Ingber & Jamieson, 1985; Ingber, 1993a; Ingber et al., 1994). Thus, there is reason to suppose that they may share essential principles of design and a common basis of mechanical stability.

One of the most important results of this study is that the predicted stretching force vs. axial extension \((T \text{ vs. } \Delta s)\) relationships of the structure were nonlinear [Figs 2(a) and 5(a)], even though the force vs. extension relationship of the cables was linear [eqns (9) and (10)]. This indicates that mechanical properties of individual structural elements are not the sole determinants of mechanical properties of the integrated structure during its shape distortion. Instead, prestress and architectural features of the structure were found to contribute importantly to its mechanical properties, a finding consistent with results obtained in studies with living cells (Wang et al., 1993). This point can be seen by rewriting eqn (5) as follows

\[
T = (k \xi) \times \left[ \left( \frac{1 + \Delta l/\xi l_0}{1 + \Delta l/\xi l_0} - \frac{1 + \Delta l/\xi l_0}{1 + \Delta l_0/\xi l_0} \right) + 2 \left( \frac{1 + \Delta l/\xi l_0}{1 + \Delta l_0/\xi l_0} + \frac{1 + \Delta l_0/\xi l_0}{1 + \Delta l_0/\xi l_0} \right) \Delta s \right].
\] (11)

There are two distinct terms on the r.h.s. of eqn (11).

The term \(k \xi\) equals prestress divided by the reference cable length \(l_0\). The term in the angular bracket represents the contribution of the structure’s architecture (i.e., the geometry of the structure and the manner in which structural elements are interconnected). The prestress determines the initial structural stiffness whereas the architecture determines how the structural stiffness changes during deformation. The only parameter that is common in both terms is the initial cable strain \(\xi\). Thus, the degree to which the cables are initially extended contributes to both initial stiffness and change of stiffness during deformation. On the other hand, the cable stiffness \(k\) appears only to influence the prestress. Thus, the cable stiffness \(k\) only affects the initial stiffness of the entire structure; it does not contribute to the change in stiffness the structure exhibits during deformation. The reason for the latter is that cable elasticity is defined in terms of a single elastic coefficient, \(k\) [eqn (9)].

According to the above, it appears that the contribution of the initial cable strain \(\xi\) to the structural stiffness \(E\) is both through prestress \((k \xi)\) and through architecture of the structure whereas the contribution of the cable stiffness \(k\) to the structural stiffness \(E\) is only through prestress. To illustrate this, we consider the following cases.

In the absence of prestress (i.e., \(\xi = 0\)) the structure has not initial (i.e., no intrinsic) stiffness (i.e., \(E = 0\)
when $\Delta s = 0$) [Fig. 2(b)]. The structure is very deformable as indicated by lateral extensions $\Delta s$ and $\Delta L$ which are larger than in any case of a non-zero prestress.

If the prestress were maximal (i.e., $\xi = 1$ for a given $k$), then the structural stiffness would be constant throughout deformation ($E = 4k$ for all $\Delta s$, $\Delta L$) [Fig. 2(b)]. The structure is also not easily deformed as indicated by zero lateral extensions $\Delta s$ and $\Delta L$.

Note that $\xi = 0$ and $\xi = 1$ define the lower and the upper bounds of prestress. It is unlikely that the prestress in the CSK of living cells would correspond to either one of these limits, but more likely would fall between these limits.

If the prestress is varied isometrically, i.e., if the initial cable strain $\xi$ is fixed and cable stiffness $k$ is varied, the initial structural stiffness will vary proportionately, but the manner in which the stiffness of the entire structure changes during stretching will not. In other words, graphs of structural stiffness vs. stretching force would be mutually parallel with higher stiffnesses corresponding to higher prestress (i.e., higher $k\xi$). This type of response to isometric changes in prestress occurs, for example, in airway smooth muscle cells that are stimulated by contractile agonists (Hubmayr et al., 1995).

This analysis also revealed that at a given prestress the looped structure is more compliant than the pinned one [Figs 2(b) vs. 5(b)]. The reason for this increased deformability is that when looped connections are utilized, there are more unconstrained internal degrees of freedom of motion due to sliding of the cables through the frictionless loops. Thus, under a given stretching force $T$, the looped structure undergoes larger deformation than the corresponding pinned structure and consequently, it is more compliant. Another distinct feature of the looped structure is that during stretching it undergoes the same shape distortion regardless of the prestress (Fig. 6). This behavior is due to the fact that the equilibrium requirements at the joints $B$ and $B'$ [eqn (8a)] and joints $C$ and $C'$ [eqn (8b)] are independent of the prestress (i.e., independent of $\xi$).  

**ENERGETIC CONSIDERATIONS**

An explanation based on energetics for why the six-strut tensegrity structure has no intrinsic stiffness unless prestressed is rooted in the fact that at the reference state the total cable length $L$ (and hence elastic energy stored) attains a minimum (Kenner, 1976). This can be easily seen in the looped structure and therefore, it is considered first. For small axial extensions, structural stiffness equals the initial slope of the force vs. extension relationship (i.e., $E \rightarrow dT/d\xi$, as $\Delta s, \Delta L \rightarrow 0$). Thus, it follows from eqn (7) that

$$ E_0 = \left. \frac{dT}{d\xi} \right|_0 = \left( \frac{dF}{d\xi} \frac{\partial L}{\partial \xi} \right)_0 + F_0 \frac{d}{d\xi} \left( \frac{\partial L}{\partial \xi} \right)_0, $$

(12)

where subscript 0 denotes the function evaluated at the reference (initial) state. Since the total cable length $L$ attains minimum at the reference state, $\partial L/\partial \xi|_0 = 0$ and hence the first term on the r.h.s. of eqn (12) vanishes. Since in the absence of prestress the cables carry no initial force (i.e., $F_0 = 0$), the second term on the r.h.s. of eqn (12) vanishes and hence, the initial structural stiffness $E_0 = 0$. Thus, prestress is necessary for the structure’s intrinsic ability to resist shape distortion (i.e., $E_0 > 0$).

The pinned case is considered next. It follows from eqn (5) that

$$ E_0 = \left. \frac{dT}{d\xi} \right|_0 = 8 \sum_{i=1}^{3} \left[ \frac{dF_i}{d\xi} \frac{\partial L_i}{\partial \xi} \right]_0 + F_0 \frac{d}{d\xi} \left( \frac{\partial L_i}{\partial \xi} \right)_0. $n(13)

By differentiating eqns (1–3) and (6) with respect to $x$, it is obtained that $dL_i/d\xi|_0 = 0$ (i = 1, 2, 3) when the initial cable strain $\xi = 0$. Thus, the first term on the r.h.s. of eqn (13) vanishes. Since in the absence of prestress (i.e., $\xi = 0$) the cables carry no initial forces (i.e., $F_i|_0 = 0$, i = 1, 2, 3), the second term on the r.h.s. of eqn (13) also vanishes and hence, the initial structural stiffness $E_0 = 0$. Thus, as in the case of the looped structure, prestress is required for the structure’s intrinsic ability to resist shape distortion (i.e., $E_0 > 0$).

In summary, the fact that the total cable length (and hence elastic energy) attains a minimum at the reference state implies that the six-strut tensegrity structure is stable and does not collapse in the absence of prestress. However, prestress provides the initial stiffness $E_0$ to the structure whereas the absence of prestress causes the structure to lack intrinsic ability to resist shape distortion.

**PHENOMENOLOGICAL CONSIDERATIONS**

Instructive parallels are evident between the behavior of tensegrity structures and observations in living cells. First, the tensegrity structure exhibits structural stiffness $E$ that increases with increasing level of prestress, for example, due to increased initial strain $\xi$ in the cables [Figs 2(b) and 5(b)]. This result is consistent with the observation that CSK stiffness measured in living endothelial cells increases with
increased cell spreading (Wang & Ingber, 1994) which is, in turn, mediated by the cell extension and the CSK reorganization (Mooney et al., 1995). Thus, it is not unreasonable to postulate higher levels of prestress in more highly spread cells, although this remains to be demonstrated experimentally. Second, the tensegrity structure exhibits initial stiffness (stiffness at the reference state) that increases with increasing prestress [Figs 2(b) and 5(b)]. Interestingly, the initial CSK stiffness (i.e., the value extrapolated for CSK stiffness at zero applied stress) in spread endothelial cells has been shown to be higher than that in round cells (Wang & Ingber, 1994), a finding consistent with the possibility that the initial cell stiffness is provided by the prestress in the CSK. Third, the pinned tensegrity structure exhibits stiffness greater than that of the looped structure [Figs 2(b) vs. 5(b)]. This result is consistent with the observation that cross-linking in isolated actin filament networks increases their ability to resist shape distortion (Wachsstock et al., 1993) and with the finding that CSK stiffness increases when ATP is depleted and actomyosin cross-bridges are fixed (Wang & Ingber, 1994). Fourth, the tensegrity structure undergoes larger fractional changes of length than its cables when it stretches uniaxially (Figs 5 and 7). Living cells, such as neurites can similarly elongate when mechanically stressed (Dennerl et al., 1988) even though individual actin filaments and microtubules are not very extensible (Gittes et al., 1993). Finally, the tensegrity structure exhibits stiffening. Although it is not linear over the entire range of observed prestresses (i.e., for $0 \leq \xi \leq 1$), the stiffening response is close to linear for some prestresses within this range [Figs 2(b) and 5(b)].

This specialized form of stiffening behavior is exhibited by living endothelial cells (Wang et al., 1993; Wang & Ingber, 1994) as well as many biological tissues (cf. Fung, 1981; Mow et al., 1992). Thus, our results support the concept that CSK architecture and the prestress rather than the mechanical properties of its individual filaments, are the primary determinants of cell deformability.

On the other hand, some of the responses of the simple tensegrity structure to uniaxial extension that we observed in the present study do not correspond directly with observations in cells. For example, the structure exhibited a stiffening response that decreased with increasing prestress [Figs 2(b) and 5(b)]. In contrast, data obtained in living endothelial cells (Wang & Ingber, 1994) indicate that the stiffening response increases with increasing cell spreading (i.e., increasing prestress). There are many possible reasons for this discrepancy. First, in the structure treated in this report the reference configuration was always spherical (round) and the interconnectedness did not change when the prestress was altered. This is not likely the case in living cells; as the cell spreads, CSK architecture transforms from a round to a flat configuration. Moreover, cell spreading may cause changes in prestress that are not entirely related to the extent of spreading but also reflect alterations in interconnectedness or density of the CSK (e.g., altered CSK polymerization) or to biochemical changes in the cell (e.g., calcium influx, changes in ATP levels, protein phosphorylation, etc). Second, the tensegrity structure underwent lateral expansions when it was stretched uniaxially (lateral extension $\Delta_s$, and/or $\Delta_s$, increase with increasing axial extension $\Delta_s$, over a portion or over the entire range of $\Delta_s$; Figs 3 and 6). This result is the opposite from that exhibited by common materials, including tissues and cells, which undergo lateral contraction when stretched uniaxially. Third, for simplicity in this first attempt at quantitatively modeling the CSK, cables were assumed to be linear elastic (i.e., Hookean) and the struts to be rigid. In contrast, CSK filaments are known to exhibit nonlinear and viscoelastic behavior (Janmey et al., 1991; Wachsstock et al., 1993). Finally, the model experiences both shape and volumetric deformation during uniaxial stretching (as employed in this study) whereas cells exposed to shear stresses only experience isovolumetric shape deformation (Wang et al., 1993; Wang & Ingber, 1994).

The parallel drawn between mechanical behaviors of the pinned vs. looped structures on one hand, and of cross-linked actin networks vs. uncross-linked actin gels (Wachsstock et al., 1993) on the other, should also be taken with reservation. One reason is that the model depicts the effect of interconnectedness between two types of structural elements, cables and struts, whereas the published data depict the effect of cross-linking between filaments of a single biopolymer, actin. Another reason is that those data were collected in isolated actin networks, in the absence of prestress. Importantly, these actin gels also do not exhibit linear stiffening behavior (Janmey et al., 1991; Wang et al., 1993).

In this study we only considered the possible contributions of static tensegrity CSK mechanism to the observable mechanical behavior of living cells exposed to shear stresses. The contributions of cross-link dynamics was not considered. Cross-link dynamics by itself cannot explain linear stiffening. For example, we have shown previously that membrane permeabilized CSK preparations that probably do not undergo significant CSK remodeling still exhibits linear stiffening behavior, although other CSK behaviors (e.g., stiffness, permanent defor-
mation, apparent viscosity) differ significantly (Wang & Ingber, 1994). Furthermore, if dynamic changes in the CSK (e.g., polymerization, geometrical rearrangements, and cross-linking) are stress sensitive (as suggested by the works of Hill & Kirschner, 1981; Dennerl et al., 1988; and Ingber, 1993a), then local dynamic remodeling of the CSK would occur in response to local static stress so as to minimize those stresses. However, at any instant of time, those stresses could be stabilized via tensegrity architecture.

Conclusions

Despite these reservations, this analysis reveals the quantitative basis of several phenomena that tensegrity architecture expresses. Many of the features are expressed in endothelial cells and other eukaryotic cells as they resist shape distortion. This is also expressed in endothelial cells and other cell types (cf. Elson, 1988; Evans & Yeung, 1989; Fung & Liu, 1993). By considering the discrete nature of the CSK it is possible to obtain forces and deformations that mediate cell shape distortion starting from the first principles of mechanics (e.g., from equilibrium of the adjacent structural elements) as we did in this analysis. The overwhelming weight of morphological evidence indicates that the complex lattice of CSK microfilaments, microtubules, and intermediate filaments (Amos & Amos, 1991; Ingber, 1993a) stretches through the cytoplasm from the cell surface to the nucleus (Fey et al., 1984). Furthermore, the shape stability of many cells has been shown to depend on a balance between tension generated in contractile microfilaments and resisted by internal microtubules (Dennerl et al., 1988; Danowski, 1989; Kolodney & Wysolmerski, 1992; Ingber, 1993a; Heidemann & Buxbaum, 1994). Thus, tensegrity architecture is not an unreasonable starting point for modeling the living cell CSK in adherent cells. Nevertheless, the six-strut tensegrity model must be regarded only as a crude representation of the mechanics of the CSK. Even so, our analysis identified prestress and architecture of the CSK as key features that might underlie a cell’s ability to regulate its shape.

We thank Mr. Radoslaw Kosior (Boston University) for graphical work. This work was supported by grants from NIH (HL-33009 and CA45548) and NASA (NAG-9-430). Dr. Ingber is a recipient of Faculty Research Award from the American Cancer Society.


APPENDIX

A derivation of the expression for \( l_i \) as a function of \( s \) and \( s_i \), eqn (1), is given below. It is based on the derivation of Kenner (1976).

Figure A1 depicts the portion of the six-strut tensegrity structure from Fig. 1 inside the first quadrant of the coordinate system \( Oxyz \); \( \overline{AB} = l_i \), \( \overline{OA}_j = s_j/2 \), \( \overline{OB}_j = s_j/2 \), \( \overline{AA}_i = 1/2 \), and \( \overline{BB}_i = 1/2 \). Thus,

\[
l_i = \sqrt{\overline{AB}_i^2 + \overline{BB}_i^2} = \sqrt{\overline{OA}_i^2 + (\overline{AA}_i - \overline{OB}_i)^2 + \overline{BB}_i^2} = 0.5\sqrt{s_i^2 + s_i^2 - 2s_i^2 + 2}.
\]

The expressions for \( l_i \) and \( l_j \) given by eqns (2) and (3) can be obtained in a similar manner.