Biorheology 40 (2003) 213–220
IOS Press

Cytoskeletal architecture and mechanical behavior of living cells

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Abstract. Conventional continuum mechanics models considering living cells as viscous fluid balloons are unable to explain some recent experimental observations. In contrast, new microstructural models provide the desirable explanations. These models emphasize the role of the cell cytoskeleton built of struts-microtubules and cables-microfilaments. A specific architectural model of the cytoskeletal framework called "tensegrity" deserved wide attention recently. Tensegrity models particularly account for the phenomenon of linear stiffening of living cells. These models are discussed from the structural mechanics perspective. Classification of structural assemblies is given and the meaning of "tensegrity" is pinpointed. Possible sources of non-linearity leading to cell stiffening are emphasized. The role of local buckling of microtubules and overall stability of the cytoskeleton is stressed. Computational studies play a central role in the development of the microstructural theoretical framework allowing for the prediction of the cell behavior from "first principles". Algorithms of computer analysis of the cytoskeleton that consider unilateral response of microfilaments and deep postbuckling of microtubules are addressed.

1. Introduction

Biochemical transformation of mechanically distorted living tissues and cells is a commonly observed event [7]. The phenomenon underlying this transformation is called *mechanotransduction*. Though various scenarios have been proposed for explanation of mechano- electro- chemo- bio-transformations in cells, no reliable experimental evidence of mechanotransduction mechanisms has been reached and the whole picture still remains obscure. The final goal in the comprehension of the mechanotransduction phenomenon is setting up a general model where all kinds of interaction are coupled. To achieve this goal it may be crucial at the first stage to understand mechanical, electrical, chemical and biological cell behaviors independently. To this end investigation of the mechanical structure or architecture of living cells is among the meaningful problems. This knowledge will allow predicting mechanical response of living cells as the initial step of the mechanotransduction process.

Traditionally, living cells were thought to be viscous fluid balloons from the point of view of mechanics. This serene picture was smashed when the contractility of living cells was discovered. The latter was allowed by new experimental techniques based on the use of thin silicone rubber substrata. When affixed to such substrata cells contract and become more spherical [3]. Another piece of evidence that cells possess a complicated load bearing microstructure emerges from experiments with binding micropippets to adhesion receptors on the surface of living cells. In this case, pulling on receptors produces immediate structural re-organization deep inside the cell [5]. The aforementioned experimental results emphasize the role of cytoskeletal micro-structural frameworks of living cells comprising microtubules and microfilaments. It seems that the role of such frameworks is important in the mechanical response of living cells, what was ignored in the past. Ingber [1,4] assumed that cytoskeletal frameworks enjoy *tensegrity* architecture and particular cytoskeletal tensegrity model shown in Fig. 1 was the main subject of computational studies of different research groups [2,6,14]. The experimentally observed linear stiffening

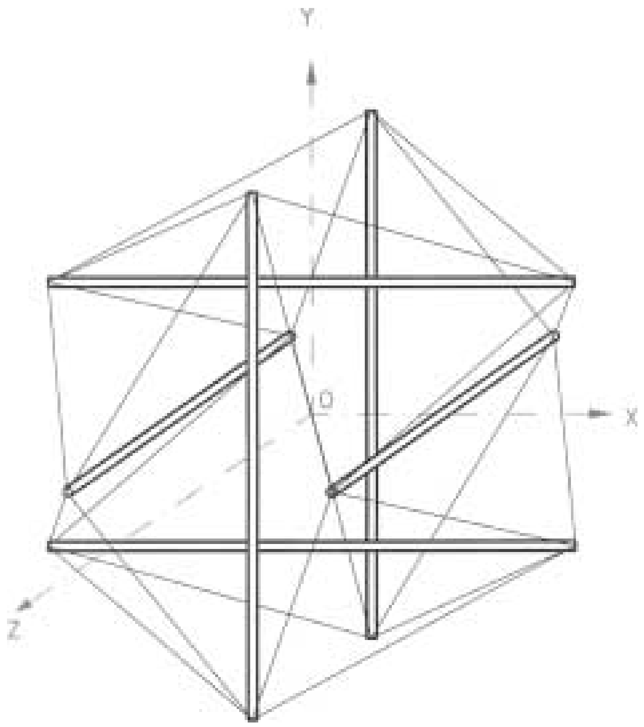

Fig. 1. Tensegrity cell model.

of living cells [8,16,17] and possible softening were explained by means of formal structural analysis within the frame of the considered tensegrity model.

The main feature of the mechanical response of living cells is non-linearity, which may be directly observed on cells experiencing large deformations. Stiffening as well as possible softening of cells are manifestations of this non-linearity. The goal of our note is revealing structural sources of cell non-linearity based on cytoskeletal framework modeling. It is shown that *geometrical degeneracy* and *buckling of microtubules* are main candidates responsible for the non-linearity. The meaning of tensegrity as the geometrically degenerate architecture is sharpened. The role and ways of computer modeling are strengthened.

2. Non-linearity in cell mechanics

Non-linearity of deformable solid bodies is defined, for instance, as a non-linear relation between the applied force and the corresponding displacement at the point of its application. The sources of non-linearity may be different. However, they generally belong to one of the two classes: material non-linearity and/or geometrical non-linearity. Since cytoskeletal structural frameworks are considered to be the main load bearing parts of living cells it is natural to confine attention to geometrical non-linearity only. The simplest kind of geometrical non-linearity is a large deformation of a ruler. Such deformation is typical for lengthy slender bodies. In the case of space frameworks, however, other kinds of deformation are representative. These are non-linear deformations caused by *geometrical degeneracy* or by *buckling*. Analysis of a simple two-member assembly shown in Fig. 2 shows principal features of the general behavior of spatial frameworks.

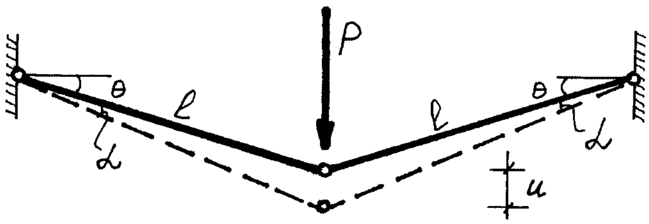

Fig. 2. Regular structure.

2.1. Governing equations

Let both members of the assembly shown in Fig. 2 be identical and possess the same modulus of elasticity E; cross-sectional area A; and length l. As a result of application of vertical force P axial member forces N appear and the vertical displacement of the central node is u. The member elongation, equilibrium condition, and material law take the following forms accordingly:

$$\Delta = \sqrt{(l\cos\theta)^2 + (l\sin\theta + u)^2} - l, \tag{1}$$

$$P = 2N\sin(\theta + \alpha) = \frac{2N(l\sin\theta + u)}{(l + \Delta)}, \tag{2}$$

$$N = \frac{EA\Delta}{l}. \tag{3}$$

Substituting (1) and (3) into (2), one derives equilibrium equation in terms of displacements. This equation is nonlinear in displacements. However, the non-linear analysis is generally superfluous. To better understand the role of non-linearity we expand Eqs (1) and (2) with account of (1) and (3) in power series about u:

$$\Delta = u\left\{\sin\theta + \frac{\cos^2\theta}{2}\left(\frac{u}{l}\right) - \frac{\cos^2\theta\sin\theta}{2}\left(\frac{u}{l}\right)^2 + \cdots\right\}, \tag{4}$$

$$P = u\frac{EA}{l}\left\{2\sin^2\theta + 3\cos^2\theta\sin\theta\left(\frac{u}{l}\right) + \frac{\cos^2\theta(5\cos 2\theta - 3)}{2}\left(\frac{u}{l}\right)^2 + \cdots\right\}. \tag{5}$$

Since relative displacements are small ($u/l \ll 1$), only the first term should be held in brackets in Eqs (4) and (5) while other terms are ignored. This simplification leads to the standard linear formulation of structural analysis:

$$\Delta = u\sin\theta, \tag{6}$$

$$P = u\frac{EA}{l}2\sin^2\theta = 2N\sin\theta. \tag{7}$$

Thus displacements and elongations are of the same order of magnitude $u \sim \Delta$ and zero elongations correspond to zero displacements. The linear equilibrium Eq. (7) may be interpreted as equilibrium for *undeformed* configuration; that is no displacements are taken into account when equilibrium is considered. Finally, the equilibrium is stable because the *tangent stiffness* is positive: $K = \partial P/\partial u = 2EA\sin^2\theta/l > 0$.

2.2. Geometrical degeneracy

A subtle situation occurs when $\theta = 0$. This specific initial configuration is shown in Fig. 3. In this case linear Eqs (6) and (7) take the following forms correspondingly:

$$0 \cdot u = \Delta, \tag{8}$$

$$0 \cdot N = P. \tag{9}$$

These equations are *singular* because of the zero coefficients. No definite solution is available.

Considering kinematic Eq. (8) it is possible to conclude that no displacement of the order of magnitude of the elongation is accessible to solve the equation. Another definition of singularity, more suitable for general formulation of the next section, states availability of a nontrivial solution for the homogeneous equation. The latter means that a displacement exists, which does not produce an elongation of the same order of magnitude. Stability is also lost for the degenerate configuration.

The physical meaning of the mathematical singularity of linear equations is impossibility to equilibrate the load in undeformed configuration. Deformed configuration and nonlinear equations should be considered. Particularly, kinematic and equilibrium Eqs (4) and (5) take the following form:

$$\Delta = \frac{u^2}{2l}, \tag{10}$$

$$P = \frac{EAu^3}{l^3}. \tag{11}$$

We arrive at non-linearity in both equations. The equilibrium is stable for nonzero displacements: $K = \partial P/\partial u = 3EAu^2/l^3 > 0$.

Availability of a nontrivial solution of the homogeneous equilibrium Eq. (9) means physical possibility of pre-stressing. Indeed, let both members possess initial axial forces N_0. Evidently, these forces are self-equilibrated: $0 \cdot N_0 = 0$. In this case, the equilibrium equation takes the following form:

$$P = 2(N_0 + N)\sin\alpha \cong \frac{2N_0u}{l} + \frac{(EA - N_0)u^3}{l^3}. \tag{12}$$

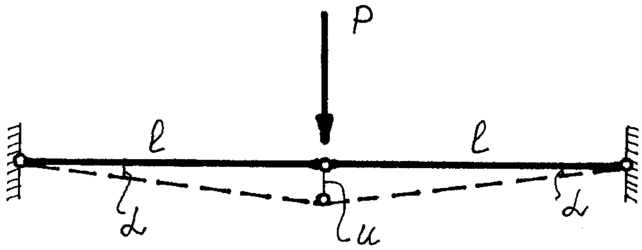

Fig. 3. Singular structure: Geometric degeneracy.

In principle, pre-stressing leads to appearance of the linear term in the equilibrium equation and affects its solution. Can we ignore the non-linear term in (12)? The general answer is no. The reason is that $N_0 \ll EA$ for the known materials. This means that both terms on the right hand side of (12) may be of the same order of magnitude.

Stability of the initial pre-stressed configuration is examined by using tangent stiffness: $K = \partial P/\partial u = 2N_0/l$, where $u = 0$. Thus pre-tensioning ($N_0 > 0$) stabilizes the initial configuration while initial compression ($N_0 < 0$) is unstable and can not be realized physically.

2.3. Buckling

Consider the two-member assembly with $\theta < 0$ as shown in Fig. 4. This assembly behaves linearly till a member buckles. When it occurs the force-displacement relation becomes non-linear. The latter is a result of strongly non-linear relations between the axial member force and the axial member shortening in the post-buckling stage. This post-buckling relation emerges from the solution of "elastica" problem. The implicit solution of this problem expressed in terms of elliptic integrals may be found in [9]. Particular numerical force-displacement relations may be found in [11]. These relations are highly non-linear.

3. Cytoskeletal architecture and computer modeling

3.1. Structural classification

In order to classify various pin-jointed frameworks the linearized multimember counterparts of kinematic and equilibrium Eqs (6), (7) are necessary:

$$\mathbf{Bu} = \mathbf{\Delta}, \tag{13}$$

$$\mathbf{B}^{\mathrm{T}}\mathbf{N} = \mathbf{P}. \tag{14}$$

Here $\mathbf{\Delta}$ and $\mathbf{N}$ are n by 1 column matrices assembled of axial elongations and forces of structural members; $\mathbf{u}$ and $\mathbf{P}$ are m by 1 column matrices assembled of nodal displacements and external loads; and $\mathbf{B}$ is the linearized kinematic matrix while its transpose $\mathbf{B}^{\mathrm{T}}$ is the equilibrium matrix.

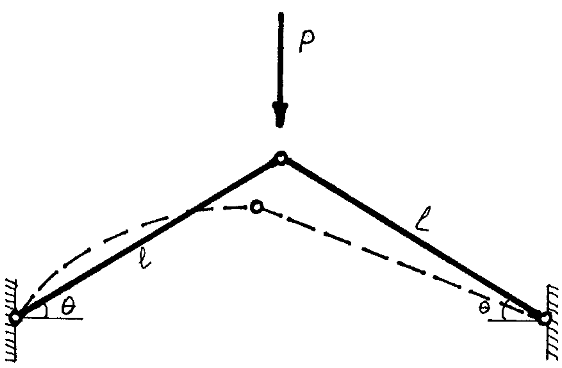

Fig. 4. Buckling of the compressed member.

We face the geometrical degeneracy when homogeneous kinematic Eq. (13) possesses a nontrivial solution. The mathematical indication of existence of such nontrivial solution is a positive difference between the number of nodal degrees of freedom (displacements) m and the rank r of kinematic matrix **B**; this difference is called *degree of kinematic indeterminacy*. It may be observed after some computation that the tensegrity model given in Fig. 1 is geometrically degenerate. Indeed the number of elements is $n = 30$ and the number of nodal degrees of freedom excluding rigid body motions is $m = 36 - 6 = 30$. Rank of matrix **B** is $r = 29$ and the degree of kinematic indeterminacy $m - r = 1$ is positive.

Structures with positive degree of kinematic indeterminacy are called *kinematically indeterminate* or *underconstrained* (lack of constraints). Another name of these degenerate structures is *infinitesimal mechanisms*. Structures with zero degree of kinematic indeterminacy are called *kinematically determinate*. Tensegrity structures are a sub-class of underconstrained structures where one compressed member meets an arbitrary number of tensioned members at every node. Specific features of underconstrained structures as well as appropriate references may be found in [12,13].

In case where *degree of static indeterminacy* $(n - r)$ is positive, that is a nontrivial solution of the homogeneous equilibrium Eq. (14) exists, the structure is called *statically indeterminate* and it allows pre-stressing. If *degree of static indeterminacy* $(n-r)$ is zero the structure is called *statically determinate*.

Kinematically determinate structures are generally stable while kinematically indeterminate structures are generally unstable. It may happen, however, that a structural assembly is both kinematically and statically indeterminate. Pre-stressing may stabilize such structure. This is the case in Fig. 4.

It is important to emphasize that nothing prevents cytoskeletal frameworks from enjoying any kind of architecture in accordance with the classification given above. The non-linear mechanical behavior may result from geometrical degeneracy of kinematically indeterminate structures as in the case of tensegrity models. The non-linearity can appear in case of kinematically determinate regular structures where local buckling of microtubules occurs [15] or it may be a combination of geometrical degeneracy and buckling [14].

3.2. Computer modeling

Static or quasi-static mechanical responses of cytoskeletal frameworks, where inertia effects are negligible, can only be modeled numerically. No other approach is available to analyze equilibrium of cells because of their non-linear behavior. General equilibrium path following procedures should incorporate possible buckling of microtubules and "switching off" of microfilaments, which do not resist compression and behave like cables. Constitutive relations for microtubules and microfilaments may be written in the following forms accordingly:

$$N = \beta\frac{EA}{l}\Delta + (1-\beta)\frac{\pi^2 EI}{l^2}\begin{pmatrix} -1 + 0.470935\left(\dfrac{\Delta+\Delta_{\mathrm{cr}}}{l}\right) - 0.530524\left(\dfrac{\Delta+\Delta_{\mathrm{cr}}}{l}\right)^2 \\ -0.477617\left(\dfrac{\Delta+\Delta_{\mathrm{cr}}}{l}\right)^3 - 0.65546\left(\dfrac{\Delta+\Delta_{\mathrm{cr}}}{l}\right)^4 \end{pmatrix}, \tag{15}$$

$$\beta = \begin{cases} 1, & \text{if } \Delta \geqslant -\Delta_{\mathrm{cr}}, \\ 0, & \text{if } \Delta < -\Delta_{\mathrm{cr}}, \end{cases} \qquad \Delta_{\mathrm{cr}} = \frac{\pi^2 EI}{EAl^2}.$$

$$N = \beta\frac{EA}{l}\Delta, \qquad \beta = \begin{cases} 1, & \text{if } \Delta \geqslant 0, \\ 0, & \text{if } \Delta < 0. \end{cases} \tag{16}$$

Here, "switch functions" β control the stage of deformation; Δ_{cr} corresponds to the buckling load; and the polynomial in parentheses of Eq. (15) describes the post-buckling of microtubules. This approximation allows for the post-buckling bending of a microtubule into a ring. Constitutive Eqs (15), (16) have to be slightly modified in presence of pre-stressing forces and further included into Newton–Raphson and arc-length continuation algorithms allowing for turning and bifurcation points as well as tracing non-stable equilibrium paths [11].

4. Closure

Recent experimental results emphasize the role of the cytoskeletal framework, comprising struts-microtubules and cables-microfilaments, as the main load bearing part of the cell. Non-linear cell response to mechanical loads may be explained by the geometrically non-linear behavior of the cytoskeletal framework. Two possible sources of this non-linearity are geometrical degeneracy (tensegrity models) and/or buckling of microtubules. Though models of microstructural cytoskeletal frameworks may comprise a modest number of structural elements, the full-scale non-linear numerical analysis seems to be unavoidable. Computer modeling of cytoskeletal frameworks should account for flexibility and post-buckling of microtubules and unilateral (with no compression) behavior of microfilaments. It is believed that development of mechanical models of living cells further hopefully coupled with biochemistry will better approach our understanding of the mechanotransduction phenomenon.

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