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Mechanical Properties of Living Cells and Tissues Related to Thermodynamics, Experiments and Quantitative Morphology – A Review

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1. Introduction

Physics and living matter

Although Galileo Galilei laid down the fundamentals for a mathematical description of natural processes, a long time passed before living organisms became a focus in physics. Given the high-spirited concept from René Descartes, living organisms were considered subject to the same universal laws as all objects. However, this approach did not provide scientists with an apparatus for systematically describing the physics of living matter. Isaac Newton's examination of physical reality was based on analysis of the forces that cause movement of objects. Although he developed a very efficient and fruitful mathematical apparatus, living organisms appeared as "black boxes" therein. Terms such as "trajectory" and "force" became highly problematic when attempting to describe such phenomena as the growth, assembly, and physical activities of complex biological systems. These problems became apparent during the 20th century with advances in thermodynamics, molecular physics, computer simulations, and highly sophisticated experimental and imaging methods.

From the perspective of physics, living cells are considered physical systems, i.e., material structures composed of atoms and molecules governed by the laws of physics. A living cell becomes material with certain rheological properties. Its molecular structure is described using terms from macromolecular chemistry, polymer physics, statistical mechanics, and thermodynamics. Most of the biological attributes for living matter are reduced or neglected on purpose, as they are typically undefined using these physical methods. However, even physicists must reflect the enormous complexity of biological systems. When considering a living cell as a "material", we cannot ignore that the phenomena, such as the "material", is able to move spontaneously, and cells can migrate through pores and avoid barriers while simultaneously changing their shape as well as their viscoelastic and rheological properties. Moreover, these physical objects of interest are growing and multiplying in number, and

they can even undergo cell death (e.g., apoptosis). Therefore, physicists must be careful when interpreting results from highly idealized models. This is an issue especially when confronting models with real experiments, as living systems typically do not behave as passive objects during an experiment. They are able to actively respond to the experimental conditions by changing their mechanical properties.

2. Thermodynamics of living structures

By publishing his "Reflections on the Motive Power of Fire" (Carnot, 1824), Sadi Carnot provided not only a theory and description useful for machine and design engineers, but he also laid the foundations for the second law of thermodynamics. At first, this theory was unnoticed as a universal natural law that cannot be simply deduced from physics based on Newtonian mechanics. This led Rudolf Clausius to become one of the founders of a new discipline: thermodynamics. Thermodynamics was based on physical quantities that were unknown until then, i.e., internal energy (U) and entropy (S). Both of these quantities contribute to the Helmoltz *free energy*, *F (F=U-TS)*, where T is the absolute thermodynamic temperature. Free energy is a consideration for systems interchanging heat with the surrounding matter, which is exactly the case for living cells and tissues in organisms.

Let us consider a sample material at a resting state without any unbalanced forces between the system and adjacent systems and in a state of thermodynamic equilibrium with its surroundings. The macroscopic conditions in such a system may be described using a set of working parameters $(\boldsymbol{A}^{(0)})$. These parameters can be set by mechanical, electrical, or magnetic actions, thus characterizing, for example, the shape or volume of the sample, its surface, and electrical polarization. Let us consider a change in these parameters caused by an external action, where surrounding the system is a heat reservoir at a steady temperature. The work done by these external forces (W) depends on the structure of that material and realization of such a change. As long as the change is *reversible, W* equals the free energy difference between the initial and the final state $\Delta F=F(\boldsymbol{A})-F(\boldsymbol{A}^{(0)})$. According to the second law of thermodynamics, relationship (1) applies to *any* process:

$$W \geq \Delta F \,. \tag{1}$$

The relationship between free energy and the parameters **A** is extremely important when describing our sample materials. Obviously, it is a measure of the *minimum amount of work* required to change the parameters of the material. However, the condition of reversibility is non-trivial. Changing the macroscopic configuration of most materials inevitably leads to a number of irreversible processes, such as the initiation and propagation of lattice defects in polycrystalline materials. In living tissues, it is logical to assume that certain processes are reversible, as tissues are clearly able to change their configuration across a broad range without any irreversible structural changes. However, the processes must be performed slowly to eliminate the effects from viscosity.

Free energy appears to be a key quantity in characterizing the rheological properties of living cells and tissues. When applying the laws of physics to living systems, it is a great challenge to describe the relationship between free energy and the working and structural parameters of the living system. However, both U and TS contribute to free energy, i.e., free energy is determined by energetic and entropic factors. This renders the task extremely difficult, which can be demonstrated using the biopolymer networks responsible for most

mechanical properties in tissues. These networks are *semiflexible*, i.e., the persistence length *(l_p)* is comparable to the typical distance between adjacent nodes (the contour length, L_C); see Fig. 1.

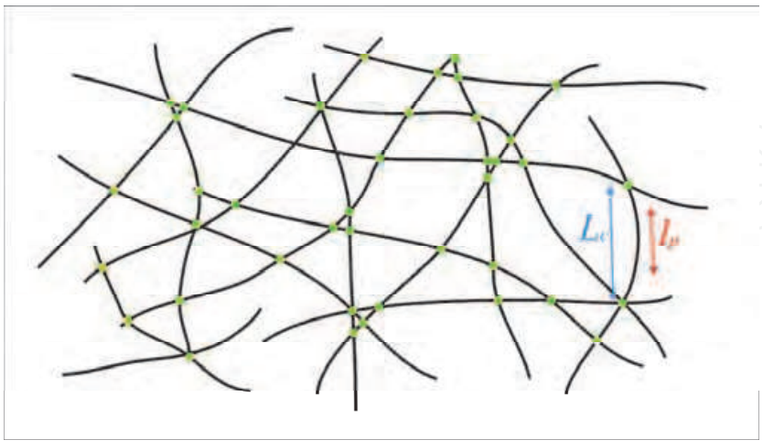

Fig. 1. Schematic for a network of semiflexible cytoskeleton biopolymer chains responsible for biomechanical properties in cells. The relationship between the persistence length *(l_p)* and contour length *(L_C)* is shown. For persistence length, correlations in the tangent direction are lost. The contour length of the chain is the distance between two nodes, measured as the mean length of the chain at the maximum physical extension.

Semiflexible networks typical in rubber-like materials have $l_p << L_C$; therefore, the elasticity is determined mainly by the entropic factors. The number of possible configurations for the fibers is reduced by stretching, and entropy is a log function for the number of these configurations. Nevertheless, analysis of biopolymer networks is a much more complex issue that solved in the most prestigious journals. According to Storm et al. (2005), the energy of a biological chain can be modeled as follows:

$$U = \int_0^{L_c} \left\{ \frac{\kappa}{2} |u''|^2 + \frac{f}{2} |u'|^2 \right\} dz \,, \tag{2}$$

where z is the projected coordinate along the end-to-end vector, κ is the bending stiffness related to persistence length via $\kappa = k_B T\, l_p$ (k_B is the Boltzmann constant), f is the applied force, and $u(z)$ describes deviation of the filament from its straight conformation. In small deformations, the entropic factor dominates, as the polymer chains can still embody a number of potential conformations. At higher deformations, the energetic factor prevails, which results in *strain-stiffening* (Onck et al., 2005), and the stress grows rapidly with increasing strain. However, other issues arise at higher deformations, such as the loss of fiber stability, and, therefore, more corrections could be necessary (Chadhuri et al., 2007). Strain-stiffening is typical for biological materials (Fung, 1993; De Santis et al., 2004; Kochova et al., 2008) and has several interesting consequences.

The validation of these theoretical models seems to be successful, especially with experimental data characterizing artificial biopolymer networks created *in vitro* and separate from other biopolymers (e.g., collagen network, vimentin filaments, and fibrillar actin microfilaments). However, the *in vivo* experiments performed in living cells remain a

problem, even for simple cell cultures, where cells are separate and free from the context of the entire tissue. The main issue is that the cells, which are supposed to be the object of the experiment, are remodeling during the experiment, thus altering its conditions. This raises a fundamental paradox: how can we measure the mechanical behavior of living cells if they react to our measurement tools (Bao & Suresh, 2003)?

Another issue is that living structures, unlike artificial biopolymers, behave as open systems, far from thermodynamic equilibrium. The cells require a constant influx of energy and behave as dissipative systems. For the application of non-equilibrium thermodynamics as devised by Onsager and Prigogine for cell membrane transport, see e.g., Dickel (1984). Biopolymer networks *in vivo* are in a non-equilibrium state, which results in the presence of a permanent mechanical *prestress*, not caused by any external force. The elastic behavior of these networks is non-linear (Fig. 2). Due to prestress, the material is more stiff, which has been demonstrated in experiments on actin cytoskeletal microfilaments (Gardel et al., 2006). While the elastic module for *in vitro* actin networks reaches 0. 1 Pa, the moduli in living cells were 10,000 times higher. Although this difference is also caused by the presence of additional networks, it can be concluded that prestress increases stiffness in living cells by at least 10 times. Prestress is based in part on active forces generated by molecular motors (such as within the contractile apparatus of muscle cells, see Fig. 3). Other chemical energy-driven phenomena might take part in prestress, such as ion pumps regulating cell volume and intracellular osmotic pressure.

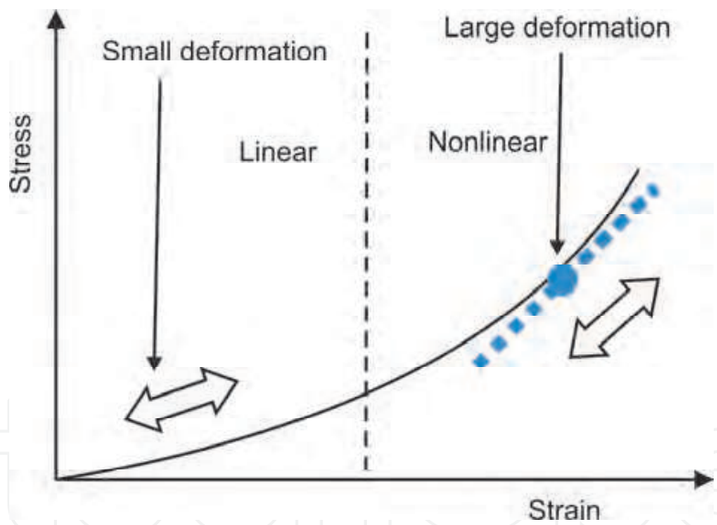

Fig. 2. Graphical interpretation of the stress-strain relationship describing the nonlinear strain-stiffening effect, which is characteristic of higher deformations in biological materials. For an explanation, see the text in this section related to modeling the energy, stiffness, and forces acting on a biopolymer chain (Storm et al., 2005; Chadhuri et al., 2007).

3. The living cell as a physical system

The cell is the basic functional and morphological unit in living organisms (Alberts et al., 2002). The typical cell size is 10-20 μm, and the shape can be spheroidal, ellipsoidal, fusiform, polyhedric, or squamous. Cells are either free units or are surrounded by other cells and an extracellular matrix within tissue. There are two types of cells: prokaryotic

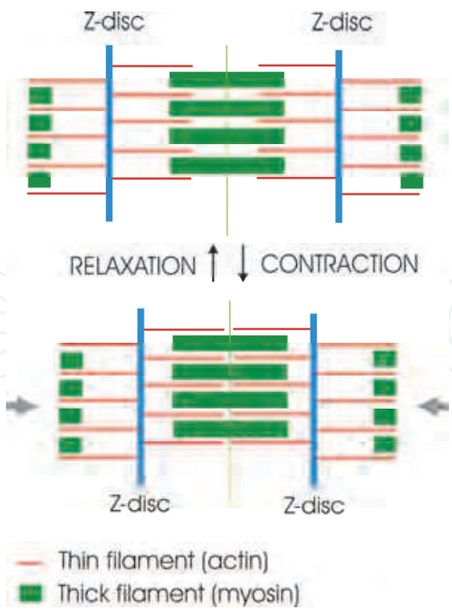

Fig. 3. Arrangement of myofibrils within contracted and relaxed sarcomeric muscle cells and fibers. The schematic illustrates the information in the paragraph on prestress generated by molecular motors and proteins associated with actin microfilaments (Gardel et al., 2006).

(bacteria) and eukaryotic (containing the cell nucleus and other membrane-bound compartments). Because of the plasma membrane (all cells) and even the cell wall (bacteria, plants, fungi, algae), the cell is an autonomous thermodynamic unit. The plasma membrane serves as an interface between intracellular and extracellular space. However, it does not insulate the cell from the extracellular environment. Rather, it is a selectively permeable barrier that hosts membrane transport systems. It is also involved in cell adhesion, cell signaling, ion conductivity, anchoring the cytoskeleton, attaching the extracellular matrix, maintaining cell volume, and the electric membrane potential. Maintaining stable and constant conditions within the cell, i.e., cell homeostasis, entails multiple dynamic equilibrium adjustments and regulatory mechanisms, which require approximately 30-70% of the energy available to the cell.

The intracellular space is compartmentalized by a number of membrane structures, while the space among them is filled with a colloidal liquid, the cytoplasm. In addition to the organelles and various inclusions, the intracellular space is reinforced by a scaffold, the cytoskeleton. This provides the cell with structure and shape. Cytoskeletal elements interact with cellular organelles and membranes. The cytoskeleton is also involved in cell motility, intracellular transport, cell division, and other processes.

From the physics point of view, the cell can be regarded as a structure comprised of heterogeneous materials, which correspond to the cytoskeleton, nucleus and other compartments. These constituents are considered continuous bodies with various material characteristics. Each of these materials is a viscoelastic continuum with a high degree of anisotropy and non-linear behavior. This approach is used to perform computational simulations of experiments performed with real cells using variety of techniques (e.g., atomic force microscopy as well as twisting, compressing, and pulling the cells, see Fig. 4-5). Describing the cellular structures as a sum of mechanical continua is useful only in tasks with dimensions exceeding the level of intracellular changes (Vaziri & Gopinath, 2008). Models

based on the interaction of multiple physical phases can simulate interactions between the cytosol and solid objects using constitutive laws that describe the flow of fluid through a porous medium, such as Darcy's law (Rohan & Cimrman, 2010). Such multiphase models are applicable when analyzing experiments based on the interaction of liquid and solid phases, such as micropipette aspiration experiments (Fig. 4C, see also Mofrad & Kamm, 2006).

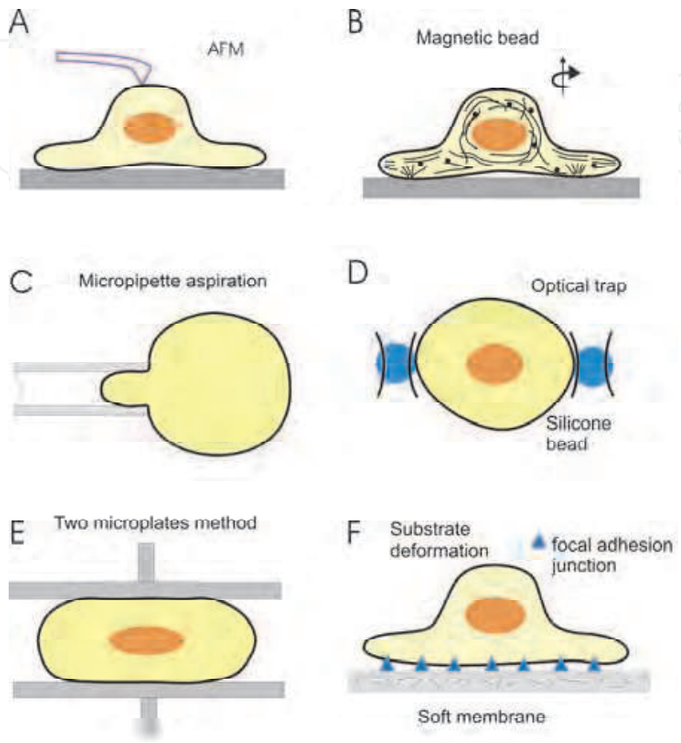

Fig. 4. Schematics of experimental methods currently used for measuring mechanical properties at the cellular level. These include atomic force microscopy (A), magnetic twisting and pulling cytometry (B), micropipette aspiration (C), optical particle trapping and optical tweezers (D), the two microplates method (E), and traction force microscopy (F).

However, for a better understanding of the cell as a physical system, more structures must be employed in our models. Probably the most important is the cytoskeleton (Fig. 6, see also Boal, 2002). The three main types of cytoskeletal filaments present in eukaryotic cells are microfilaments, intermediate filaments, and microtubules (Alberts et al., 2002). In prokaryotic cells, several types of structural and functional homologs of these cytoskeletal filaments were found (Carballido-López & Errington, 2003).

The *actin filaments* are the thinnest in the cytoskeleton at only 7 nm in diameter and are referred to as microfilaments. They are composed of linear actin polymers and are capable of generating force by elongation and shrinking at the ends. They also act as tracks for the movement of myosin molecules that attach to the microfilament, sliding along them. Actin microfilaments can be found both in the cortical and the deep cytoplasm. They are involved in cell motility, phagocytosis, cell division, and the transmission of force between the extracellular and intracellular structures. Several actin-binding proteins (e.g., α-actinin) contribute to actin cross-linking, thus modulating its *in vivo* mechanical properties. Unlike artificial microfilaments, *in vivo* actin is stiffer from prestress.

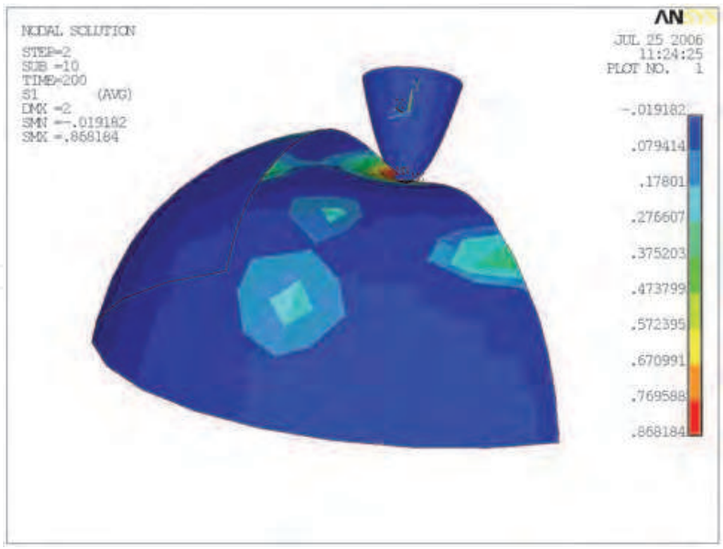

Fig. 5. Distribution of principal stress in the plasma membrane of a cell during a simulation of the indentation test. The figure demonstrates simulations by considering cellular compartments as viscoelastic mechanical continua with variable material properties (with kind permission of Dr. J. Burša, according to Burša & Fuis, 2009).

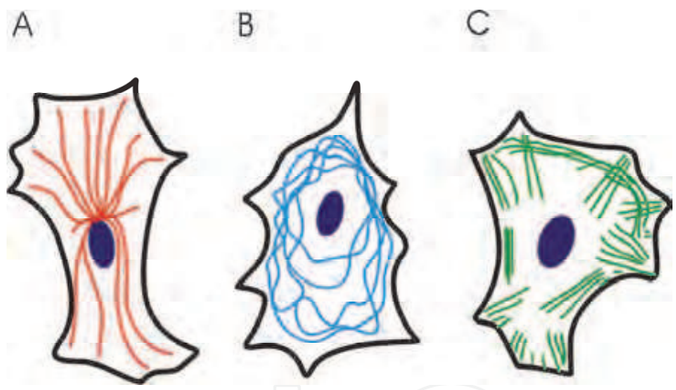

Fig. 6. Schematics for three different distribution patterns of cytoskeletal filaments. (A) Microtubules *(red)* are hollow cylindrical filaments with a diameter of 25 nm, radiating from the cell nucleus. Microtubules are organized by the microtubule organizing centers (centrioles and basal bodies). They are capable of growing and shrinking to generate force. They can be used by motor proteins that support the movement of organelles and other cellular factors along the microtubules. (B) The intermediate filaments *(blue)* have an average diameter of 10 nm, and they are mostly cytoplasmic (except the nuclear filaments known as lamins). These filaments are deformable proteins that can be stretched to several times their initial length. Such a large deformation is possible due to their hierarchical structure (Qin et al., 2009). (C) The actin microfilaments *(green)* are the thinnest cytoskeletal filaments (7 nm in diameter). They are assembled in highly versatile bundles and networks, they can be cross-linked and they resist both tensile and compressive forces. They are distributed both in the cortical cytoplasm and throughout the entire cell. They are able to generate force by either elongating or depolymerizing, and they work as part of the actomyosin molecular motor (Alberts et al., 2002).

The *intermediate filaments*, approximately 10 nm in diameter, are more stable than actin filaments. Like actin filaments, they help maintain the cell-shape by resisting tensional forces. Therefore, the cells are able to resist a tension of 10^2-10^5 Pa (Bao & Suresh, 2003). Intermediate filaments organize the internal structure of the cell and anchor the organelles.
Microtubules are rigid, hollow cylinders approximately 25 nm in diameter, and are polymers of the protein tubulin. They exhibit a highly dynamic behavior as they polymerize and depolymerize. They are commonly organized by the centrosome, play role in the intracellular transport of organelles and vesicles, and construct the cilia, flagella, and mitotic spindles. They resist compression, and thus, in *tensegrity models* (tensional integrity, Stamenovic, 2006), they are considered rigid bars (rods). These rigid microtubules are interconnected by prestressed, extensible microfilaments (cables) (Fig. 7). These models are used to explain cellular tension and complex interactions between stiffness and prestress, thus describing even viscoelastic properties (Canadas et al., 2002). Tensegrity models may be combined with continuum models. However, they are highly sensitive to the spatial arrangement of the cables and rods.

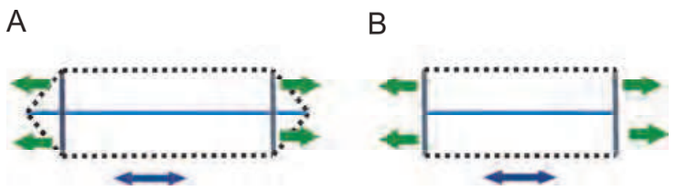

Fig. 7. A combination of rigid bars (rods, full lines) and viscoelastic fibers (cables, dotted lines) illustrate the tensegrities (Stamenovic, 2006) in models based on the balance between tension and compression in linear cytoskeleton components. In a steady state (A), the oblique cables are prestressed so that they can carry the tensile force, balanced by compression of the rigid rods (B). This approach may be used to demonstrate the non-trivial explanation of prestressed behavior in intracellular biopolymer networks.

As demonstrated by the tensegrity models, whenever physics aims to describe cell mechanics, it tends to choose a remarkable phenomenon, which becomes the basis for physical analysis. The cell has been considered a either mixture of polymer networks, a prestressed construction created from rods and fibers, or a multiphase continuum. Each of these strategies can explain certain features of cell behavior and may be used for planning and describing various experiments. Still, there is a demand for more general models, often inspired by non-trivial physical approaches. An interesting model is based on the similarity between cell behavior and the rheology of colloidal materials.

4. Biological cells and soft glassy materials

Despite the obvious presence of cytoskeletal semiflexible networks within the cells, physical description of these networks does not explain their complex behavior. The *in vivo* networks are not passive. On the contrary, the cell is a living structure; the networks undergo constant remodeling, and the activity of the molecular motors change over time. Despite the molecular level of these adaptive processes, their outcome is global and even macroscopic, i.e., at the level of the entire cell or tissue. Therefore, the next task is to describe the physical global effects from these molecular processes.

Recent advances in the rheology of soft glassy materials are at least a partial response to this problem (Fabry et al., 2001; Bursac et al., 2005; Deng et al., 2006). An experimental description of the frequency dependence for the complex modulus of the cell *G(f)* via magnetic cytometry (Fig. 4B) showed that the complex modulus of the cell consists of an elastic modulus *G´* and a loss of this modulus *iG´´*, i.e., $G = G´ + iG´´$. These measurements were transformed into traditional elastic and loss moduli using a geometric factor, α, that depends on cell shape and thickness. For frequency dependence of the complex modulus for a semiflexible polymer network (e.g., fibrillar *in vitro* actin), *G* is either independent from the frequency (at lower frequencies) or is related (for higher frequencies) as follows:

$$G \sim f^{3/4}. \tag{3}$$

Equation (3) complies with the theory of entropic elasticity. At higher frequencies, the heat-driven bending fluctuations of the semiflexible fibers contribute to the complex modulus. This relationship (3) has also been measured for cell cytoskeletons *in vivo*. However, there should be a considerable contribution from prestress *in vivo*, generating higher stiffness in the cytoskeletal network. Because of prestress, the energetic component should prevail over the entropic contribution (represented by the ¾ index in Equation (3)). Another discrepancy was found at lower frequencies. Unlike *in vitro* networks, the real cytoskeleton has a frequency dependence even at lower frequencies:

$$G \sim f^{\alpha}, \tag{4}$$

where α varies between 0.1-0.3. Such behavior no longer complies with semiflexible polymer networks. Instead, it corresponds to special materials referred to as *soft glassy materials* (Sollich, 1998). These material represent various foams, emulsions, slurries and colloidal systems. These "glassy materials" share features of structural disorder and metastability. Local elements in this material are either too large, or they are trapped by their neighbors. Therefore, they cannot rapidly reach heat-induced equilibrium, and their rearrangement via thermal activation, referred to as *aging,* is rather slow. However, this rearrangement can be facilitated by external energy (Fig. 8). The behavior depends on mesoscopic elements, which are regions small enough for a macroscopic piece of material to contain them in a large number, thus providing a description of its behavior as an average over elements. They are also large enough such that deformations at the scale of a single element can be described using an elastic strain variable (Sollich, 1998).

The application of these experiments and conceptual tools to the cytoskeleton provides evidence for a non-equilibrium cytoskeletal state. Prestress corresponds to the "densities" of colloidal particles trapped within the glassy system, causing the metastability. The ATP (adenosine triphosphate)-driven molecular motors (e.g., myosin, dynein, kinesin) and cytoskeleton-associated proteins rearrange the network *in vivo*, thus modifying the local non-equilibria within the cytoskeletal configuration. As a result, the macroscopic behavior of the cytoskeleton is observed (Fabry et al., 2001). The value of α is the central characteristic for the entire process. When α=1, the material is a fluid, and when α=0, the material is solid. As soft glassy materials, the cells may be near the *glass transition*. Upon activating the contractile apparatus, α (Equation 4) decreases to zero, which corresponds to the glassy transition. In contrast, the loosening and relaxing the cytoskeleton leads to an increase in α. The "glassy dynamics" appear at frequencies of 10^2 Hz, which is the transition frequency

between the characteristics described by Equation (3) and (4). In other words, describing the cell in terms of soft glassy rheology is useful for processes at a scale of approximately 0.01 s. However, this is exactly the time scale for many cellular events, such as cell motility, movement of kinocilia, and phagocytosis, among others. (Deng et al., 2006).

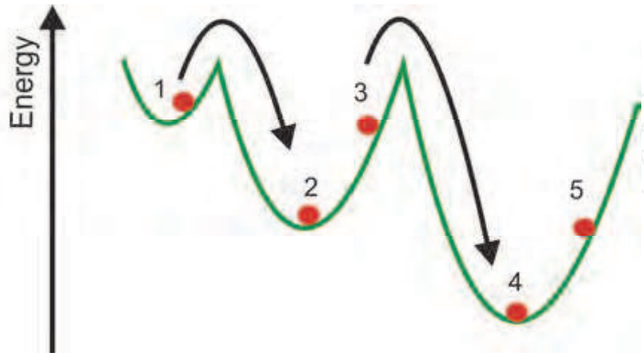

Fig. 8. Schematic illustrating the soft glassy rheology model of the cytoskeleton. The natural reorganization and dynamics for intracellular biopolymers can be modeled as a series of transitions (red positions labeled 1-5) between a fluid and solid state.

It is noteworthy that when applying soft glassy rheology to cell behavior, the physical approach is far from a "naive reductionism". There are no simulations at the molecular level. Conversely, the molecular processes remain in the background, varying only as a typical response to the stimulating frequencies. However, the "global" parameter, α, appears to be more important, as it characterizes the condition of the cytoskeleton and its typical response. The individual molecular mechanisms likely contribute to changes in α, thus affecting the global cellular response. Even the definition and experimental identification of such a parameter must be appreciated.

5. Cells and "intelligent materials"

In multicellular organisms, the cells are arranged in tissues, which usually compose organs. The cells are interconnected with cell-to-cell junctions that involve both plasma membranes and the intercellular matrix. The extracellular matrix consists of fibers and a ground "amorphous" substance. The prevailing matrix fibers in vertebrates are the collagen, elastin, and reticular fibers. The ground substance is constructed mainly of glycoproteins and glycosaminoglycans. In some tissues, the cells prevail, while other types of tissue are formed mainly by the matrix. Quantitative analysis of the tissue requires either a combination of microscopy and image processing or, preferably, unbiased *stereological methods* (Howard & Reed, 2005). The tissue can be characterized using volume fractions of the constituents (Fig. 9A), a description of the degree of anisotropy of the cells, the spatial orientation of the matrix fibers, cell clustering, the fiber length density, the microstructure surface area (Fig. 9B), and the numerical density of the objects in three dimensions, among others. Volumes, surfaces, lengths, numbers, and other microstructural characteristics can be estimated using stereological methods (Howard & Reed, 2005), which apply various geometrical principles for fair sampling of histological sections (Gundersen & Jensen, 1987). Stereology is based on methods related to the three-dimensional measurements available on the sections. A typical example includes the point-interception method, which estimates the area by counting the

intersections between a randomly placed test grid and the objects of interest. The total number of points intersecting with the objects of interest is then multiplied by the reference area corresponding to each point. This is the reason stereology is also known as "stochastic geometry".

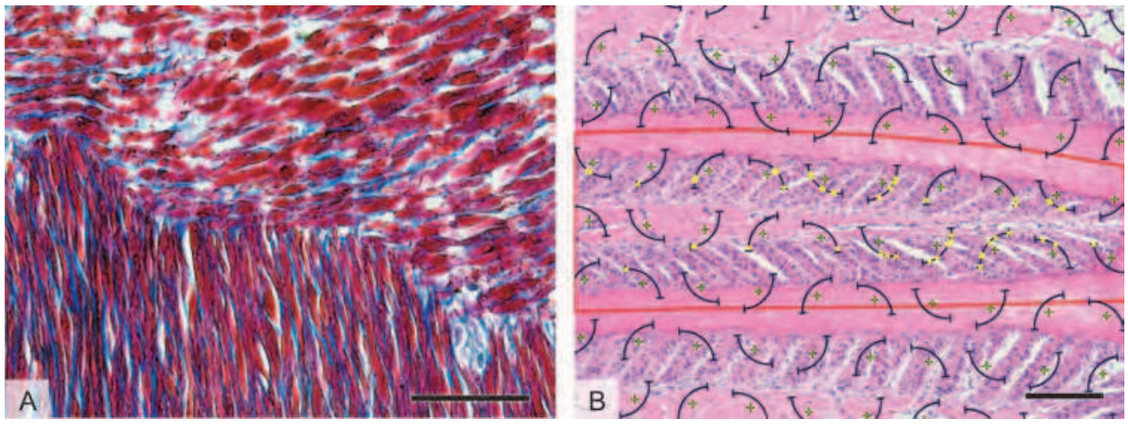

Fig. 9. Micrographs of composite biological tissues undergoing quantitative histological analysis. To elucidate how the principal constituents in biological tissues contribute to the mechanical behavior of macroscopic biological samples, either the volume fractions or surface densities of the tissue compartments must be assessed, preferably using unbiased point-interception stereological methods. (A) Smooth muscle *(red)* within the amphibian intestine is arranged in two perpendicular systems. The volume fractions from the active component (smooth muscle, stained *red*) and passive matrix (collagen fibers, *blue*) can be quantified. (B) A section through a junction for two types of tissues at the border between the dermis and epidermis of an equine hoof. Repeating epithelial lamellae interdigitate with the collagenous connective tissue of the dermis. The lamellar surface area can be estimated by counting the intersections *(yellow)* between the lamellae and a linear stereological system (circular arcs, *black*). The *red* borders delimit the reference space that corresponds to a single primary lamella, thus, the lamellar surface can be related to a volume unit of the tissue via the surface density of the junction. Mallory trichrome stain (A) and hematoxylin-eosin stain (B). Scale bars 50 μm (A), 100 μm (B).

In biological tissues, the cells are interconnected with the matrix fibers. The most abundant are collagen fibers. In fact, the hierarchical organization of collagen is complicated. Bundles of collagen fibers are constructed from fibrils, which consist of microfibrils, made from protofibrils that are overlapping trihelices of tropocollagen. In small deformations, the collagen fibers have a wavy appearance, and, unlike elastin fibers, they almost never contribute to the stress-strain behavior (Fig. 10, region I).

With increasing deformation (Fig. 10, region II), the collagen fibers become stretched and contribute to the total elastic modulus of the tissue. Further deformation (Fig. 10, region III) is characterized by fully stretched collagen fibers, which lead to sample stiffening. When increased stress reaches the ultimate strength of the tissue (region IV), microcracks are initiated and propagate through the tissue (Fig. 11). According to the arrangement of the collagen fibers, different tissues have different extensibility under uniaxial loading. For example, the mesentery of the peritoneal cavity can be distented by 100-200%, while ureters, arteries and veins can extend by approximately 60%; in addition, the cardiac muscle and

tendons can extend to 15% and 2-5% of their initial length, respectively (Viidik, 1979; Ahlfors et Billiar, 2007). Therefore, we must consider that biological tissues have a complex microstructure and a non-linear, non-Hookean stress-strain response. Even under physiological conditions, they are subjected to large deformations.

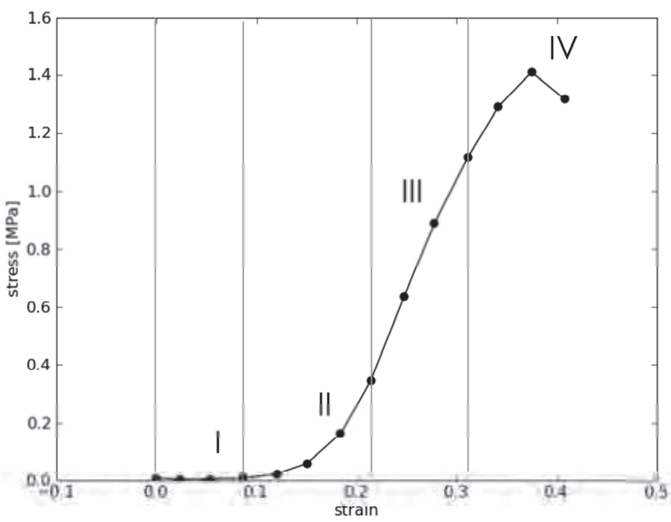

Fig. 10. An illustration of the non-linear stress-strain relationship for connective tissue exposed to a uniaxial tensile test. Region I represents the linear region for small deformations, region II is characterized by tissue stiffening, region III is the linear region for large deformations, while, at the end of region IV, the rupture is initialized.

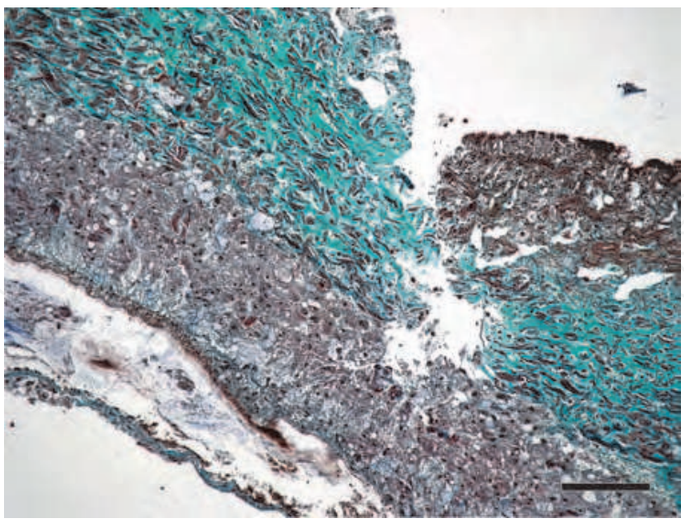

Fig. 11. A micrograph of a tissue rupture induced by uniaxial tensile loading that exceeds the ultimate strength of the connective tissue. The tissue sample shows the pedal integument of a pulmonate gastropod *Arion* sp. and consists of smooth muscle *(reddish to brown)* and collagen fibers *(green)*. Green trichrome stain, scale bar 200 µm.

From the perspective of rheology, the tissues can be considered viscoelastic materials, and therefore, they have properties such as stress relaxation, creep, and hysteresis. These parameters are identified using mechanical experiments, while preserving experimental conditions close to the *in vivo* state. The viscous properties result from biopolymers and the presence of intracellular and extracellular fluid. The intracellular fluid corresponds to approximately 40% of the tissue weight, while the extracellular fluid composes approximately 20% of the tissue weight. Deformation of the tissue is accompanied by redistribution of the fluid; however, the fluid renders the tissues incompressible.

Materials that can adapt to their environment and those that, more modestly, can alter their properties as required by their users are referred to as "intelligent". This is observed, for example, in the muscle tissue, which can change the microscopic contractility of the cytoskeletal filaments, causing either macroscopic peristalsis of the intestine, blood vessel vasoconstriction or blood vessel vasodilatation.

Let us consider a possible physical description for these mechanisms, enabling the number of N cells to modulate the mechanical behavior of the entire tissue. The free energy of a cell $F_{cell}(\mathbf{A}_C,\alpha)$ is determined by a set of working parameters $\mathbf{A}_C$ (describing the cell geometry) and a set of intrinsic parameters α (governed by the cell itself, e.g., the activity of molecular motors and degree of polymerization of the actin network, etc.). Now let us define the free energy of the extracellular matrix F_{ECM}, which is determined by the working parameters $\mathbf{A}$ of the sample and the values for all cells present within the sample $\mathbf{A}_c^i$, $i = 1,\ldots,N$. These values define the geometry of the cells and, therefore, the geometry of the matrix. At the level of the entire tissue sample, the working parameters for individual cells are considered free variables, as they are not controlled from "outside" of the cells; rather, they are set by the cells to minimize the free energy of the entire system:

$$\Delta F = \min_{\mathbf{A}_c^i}\left\{\Delta F_{\mathrm{ECM}}(\ldots,\mathbf{A}_c^j,\ldots) + \sum \Delta F_{\mathrm{cell}}(\mathbf{A}_c^i,\alpha_i)\right\}. \tag{5}$$

As the cells are much softer than the matrix, the consequence of Equation (5) is that the geometry of a cell is adapted to minimize deformation of the matrix, i.e., the cells will be deformed rather than the matrix. The contribution of the free energy is small; therefore, should the deformation mainly involve the cells, the intracellular processes would not be able to change the free energy of the entire tissue, i.e., they would not be able to alter the mechanical properties of the entire tissue.

In addition, the shape of the cells should not be deformed without limits, i.e., the values for parameters $\mathbf{A}_c^i$ are limited. It is well-known that most animal cells tend to maintain their shape and volume using a variety of compensating mechanisms related to homeostasis and constant intracellular osmotic pressure (Pollard & Earnshaw, 2004). Therefore, constant volume is another factor limiting the shape of the cell. Maintaining constant cell volume is an efficient way to enable additional intrinsic mechanisms to alter the tissue stiffness. This can be demonstrated using a model structure constructed of elastic balloons (or balls) interconnected with linear springs (Fig. 12); the balls represent the cells, and the springs are analogous to the tissue matrix. Similar to living tissues, this material is incompressible as a whole (the space inside and among the balls is filled with fluid). Let us study orthogonal deformations of this material, which can be described as changes in individual dimensions $\Delta x_{i0} \rightarrow \Delta x_i$, i = 1,2,3, while $\beta_i \equiv \Delta x_i / \Delta x_{i0}$ is the relative deformation in the i-th direction (the incompressibility of the material can be expressed as $\beta_1\beta_2\beta_3 = 1$). Change in the

internal energy during deformation is determined by deformations of the springs and balls. For compressible balls (i.e., their wall is permeable to the fluid), the ratio between deformations of the balls and springs could be optimized in all three directions independently from each other. If the balls are much softer than the springs, an external mechanical loading in the system causes a deformation of the balls only such that the overall stiffness of the material is close to the balls' stiffness. Should the balls' wall become impermeable, such optimization would be impossible. Changes in the free energy during deformation within a system made of incompressible balls ΔF would be much higher than the change in free energy for a system containing compressible balls ΔF_0:

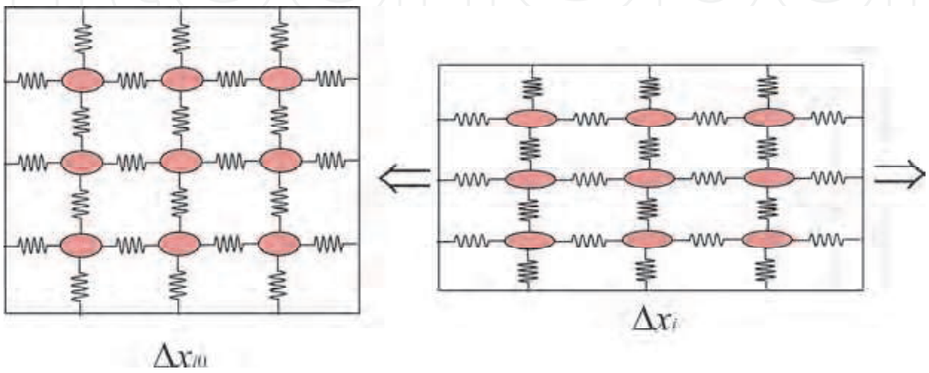

Fig. 12. An illustrative explanation of a two-dimensional model formed by flexible balls connected with linear springs (according to Holeček & Moravcová, 2006). Due to the incompressibility of the system as a whole, the total volume for the tissue model is constant. However, an external force may deform both the balls and springs, while the maximum deformation occurs in the balls, as this deformation requires less energy. For compressible balls, the material becomes extremely soft and deformation occurs almost entirely in the balls. This approach appears to be useful for modeling non-trivial modulation of macroscopic mechanical behavior of tissues (or even entire organs) by setting the properties of various cell types and intercellular fibers or junctions.

$$\begin{aligned} \Delta F &= \Delta F_0 + \Delta F', \\ \Delta F' &>> \Delta F_0. \end{aligned} \tag{6}$$

While the free energy ΔF_0 corresponds to linear elastic material, the free energy ΔF corresponds to non-linear material. This is caused by the optimization process described by Equation (5), which describes a system with non-linear relationships (Holeček & Moravcová, 2006). The involvement of the balls is determined by physical and geometric parameters describing the inner structure of the material. The more behavior of the material that is controlled by the balls entirely, the nearer the product of $\beta_1^{eff}\beta^{eff}{}_2\beta^{eff}{}_3$ to 1, where β_i^{eff} describes an efficient relative deformation in the i-th direction:

$$\beta_i^{\text{eff}} = \frac{1+\delta_i}{1+k_i}\left(\frac{\Delta x_i}{\Delta x_{i0}} - 1\right) + 1 \cdot \tag{7}$$

δ_i is the ratio of the distance between the balls to the diameter of the ball in the i-th direction (in a non-deformed condition, thus corresponding to the ratio of initial spring lengths and

the initial ball diameters), and k_i is a ratio between the ball stiffness and the stiffness of the spring in the *i*-th direction. In any part of the material, provided that

$$\beta_1^{\text{eff}} \beta_2^{\text{eff}} \beta_3^{\text{eff}} = 1, \tag{8}$$

the geometric and mechanical parameters are optimized such that even with incompressible balls, the material behaves as if the balls were compressible, i.e., deformation of the material relies on deformation of the balls only. Under the condition described by Equation (8), the local inner energy $\Delta F'=0$ (Fig. 13). However, even a small change in either δ_i or k_i will impair Equation (8). Further deformation of the entire material also depends extensively on deformation of the springs, which are much stiffer than the balls. This leads to an increase in inner energy ΔF, necessary for local deformation. Equation (8), therefore, indicates the presence of a local minimum for deformation energy among the intrinsic material parameters at a given external deformation.

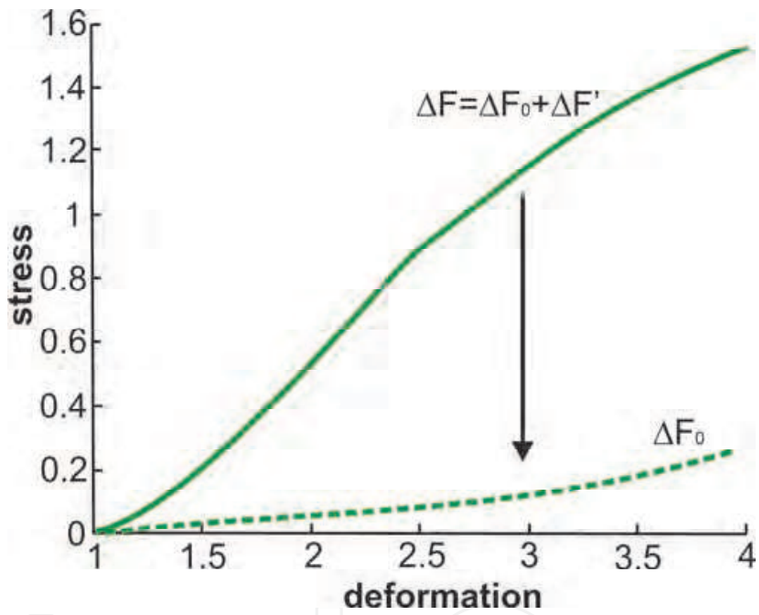

Fig. 13. Numerical simulation of free energy for a model constructed from balls and reinforced with springs *(full line)*. For high deformability of the balls, the energy of the material drops to a minimum and the stress-strain characteristics of the material are illustrated with a *dashed* line. Along this minimum, it is possible to change the stiffness of the material in an efficient manner. This simulation demonstrates the considerable number of macroscopic mechanical characteristics in biological tissues that result from the properties of its microscopic constituents.

Nevertheless, do the living cells really use this principle? Taking the interaction between the actin and myosin myofilaments as an example, it is obvious that the sliding of the myosin heads along the actin microfilaments corresponds to changes in the initial lengths of the springs. Using these intrinsic processes driven by chemical energy, the cell changes the geometric parameters δ_i, thereby setting $\beta_1^{eff} \beta^{eff}{}_2 \beta^{eff}{}_3$ close to 1. Indirect evidence for such a structural optimization is demonstrated by comparing the efficiencies of the smooth and skeletal muscle. Both muscle cell types share the same intrinsic mechanisms for the sliding

myofilaments, i.e., altering the overlap between the actin and myosin filaments. However, the ultrastructural organization of the contractile apparatus (i.e., the geometric properties of the system) varies. In skeletal muscle, the contractile apparatus is arranged as periodical sarcomeres. In smooth muscle cells, the actin and myosin chains stretch across the cytoplasm, and spatial reorganization of the contractile machinery optimizes force development during contraction. The smooth muscle cell can be shortened to 2/3 its initial length, while contraction in a skeletal muscle fiber is limited to approximately 1/4. According to the experiments performed, the skeletal muscle requires 10-300 times the chemical energy (ATP) to achieve the same isometric contractile force compared with smooth muscle (Mofrad & Kamm, 2006). In smooth muscle cells, each isometric contraction is accompanied by a lower number of interactions between actin and myosin (each of these interactions is compensated with the ATP), which more efficiently maintains muscular tone. The drawback of this adaptation is that the onset of contraction is much slower in smooth than skeletal muscle.

6. Conclusion

Modeling the mechanical behavior of living cells and tissues is one of the biggest challenges in biomechanics. The models should comprise the properties of the cytoskeleton, plasma membrane, cell-to-cell junctions, as well as fibrillar and amorphous extracellular matrix. The macroscopic mechanical parameters of tissues can be identified much easier than microscopic parameters; therefore, the models serve as translators between macroscopic and microscopic phenomena. We reviewed several current approaches used in various branches of biophysics to devise non-trivial models of biological tissues.

Beginning with thermodynamics, which led us to study the free-energy dependence of microscopic parameters for living tissues, we continued with special mechanical properties of living cells (Bao & Suresh, 2003) and the important role of prestress in the cytoskeleton. We mentioned the tensegrity models (Stamenovic, 2006) and the approach in which the biological cell is regarded as a soft glassy material (Sollich, 1998). Active adaptations of intracellular structures require a physical description of such cytoskeletal remodeling (Bursac et al., 2005). We then discussed the problems with description and experimental identification of properties in living tissues and used an example to outline a purely mechanical explanation for the way that smooth muscle cells efficiently control the mechanical behavior of the entire tissue. We discussed the current methods for quantitative microscopic analysis of biological samples using stereological methods (Howard & Reed, 2005). We also outlined the importance of simple physical models in understanding the important features of biological structure functions (e.g., Holeček & Moravcová, 2006). To enhance this review's availability to a broad audience, a number of illustrations accompanied the text.

We conclude that the mechanical properties of biological cells and tissues depend on their underlying complex microstructure. At the tissue level of organization, computer simulations based on a stochastic interpretation of real morphology allow us to pursue the effects from stiffness and elasticity of both the cytoskeleton and extracellular matrix on the mechanical behavior of entire organs. Despite the limitations in our knowledge and methods, physical models of cell mechanics offer powerful tools for researching the structural principles governing living matter.

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8. References

Ahlfors, J. E. W. ; Billiar, K L. (2007). Biomechanical and biochemical characteristics of a human fibroblast-produced and remodeled matrix. *Biomaterials*, Vol. 28, pp. 2183-2191, ISSN 0142-9612.

Alberts, B. ; Johnson, A. ; Lewis, J. ; Raff, M. ; Roberts, K. ; Walter, P. (2002). *Molecular Biology of The Cell,* (4th edition), Garland Science, ISBN 978-0815332183, New York.

Bao, G. ; Suresh, S. (2003). Cell and molecular mechanics of biological materials. *Nature Materials*, Vol. 2, pp. 715-725, ISSN 1476-1122.

Boal, D. (2002). *Mechanics of the Cell*, Cambridge University Press, ISBN 978-0521796811, Cambridge.

Burša, J. ; Fuis, V. (2009). Finite element simulation of mechanical tests of individual cells. *IFMBE Proceedings,* Vol. 25, pp. 16-19, ISSN 1680-0737.

Bursac, P. ; Lenormand, G. ; Fabry, B. ; Oliver, M. ; Weitz, D.A. ; Viasnoff, V. ; Butler, J.P. ; Fredberg, J. J. (2005). Cytoskeletal remodelling and slow dynamics in the living cell. *Nature Materials*, Vol. 4, pp. 557-561, ISSN 1476-1122.

Canadas, P. ; Laurent, V.M. ; Oddou, C. ; Isabey, D. ; Wendling, S. (2002). A cellular tensegrity model to analyze the structural viscoelasticity of the cytoskeleton. *Journal of Theoretical Biology,* Vol. 218, pp. 155-173, ISSN 0040-5809.

Carballido-López, R. ; Errington, J. (2003). A dynamic bacterial cytoskeleton. *Trends in Cell Biology,* Vol. 13, pp. 577-583, ISSN 0962-8924.

Carnot, S. (1824). Réflexions sur la puissance motrice du feu et sur les machines propres à développer cette puissance. Bachelier, Paris.

Chadhuri, O.; Parekh, S.H.; Fletcher, D.A. (2007): Reversible stress softening of actin networks. *Nature,* Vol. 445, pp. 295-298, ISSN 0028-0836.

De Santis, R. ; Sarracino, F. ; Mollica, F. ; Netti, P.A. ; Ambrosio, L. ; Nicolais, L. (2004). Continuous fibre reinforced polymers as connective tissue replacement. *Composites Science and Technology,* Vol 64, pp. 861-871, ISSN 0266-3538.

Deng, L. ; Trepat, X. ; Butler, J.P. ; Millet, E. ; Morgan, K.G. ; Weitz, D.A. ; Fredberg, J. J. (2006). Fast and slow dynamics of the cytoskeleton. *Nature Materials,* Vol. 5, pp. 636-640, ISSN 1476-1122.

Dickel, G. (1984). The variational principles of Onsager and Prigogine in membrane transport. *Faraday Discussions of the Chemical Society,* Vol. 77, pp. 157-168, ISSN 0301-7249.

Fabry, B. ; Maksym, G.N., Butler, J.P; Glogauer, M.; Navajas, D.; Fredberg, J.J. (2001). Scaling the microrheology of living cells. *Physical Review Letters,* Vol. 87, pp. 148102-1-148102-4, ISSN 0031-9007.

Fung, Y.C. (1993): *Biomechanics. Mechanical Properties of Living Tissues*. Springer-Verlag, ISBN 03-879-7947-6, New York.

Gardel, M.L. ; Nakamura, F. ; Hartwig, J.H. ; Crocker, J.C. ; Stossel, T.P. ; Weitz, D.A. (2006). Prestressed F-actin networks cross-linked by hinged filamins replicate mechanical

properties of cells. *Proceedings of the National Academy of Sciences,* Vol. 103, pp. 1762-1767. ISSN 1091-6490.

Gundersen, H.J.G. ; Jensen, E.B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy,* Vol. 147, pp. 229-263, ISSN 0022-2720.

Holeček, M.; Moravcová F. (2006). Hyperelastic model of a material which microstructure is formed by "balls and springs". *International Journal of Solid and Structures,* Vol. 43, pp. 7393-7406, ISSN 0020-7683.

Howard, C.V.; Reed, M.G. (2005). *Unbiased Stereology: Three Dimensional Measurement in Microscopy,* (2nd edition), Garland Science/BIOS Scientific, ISBN 978-1859960899, New York.

Kochova P.; Tonar, Z.; Matejka, V.M.; Sviglerova, J.; Stengl, M.; Kuncova, J. (2008). Morphology and mechanical properties of the subrenal aorta in normotensive and hypertensive rats. *Biomedical papers of the Faculty of Medicine of Palacký University, Olomouc, Czech Republic*, Vol. 152, pp. 239-245, ISSN 1213-8118.

Mofrad, M.R.K.; Kamm, R.D. (2006). *Cytoskeletal Mechanics. Models and Measurements in Cell Mechanics,* Cambridge University Press, ISBN 978-0521846370, New York.

Onck, P.R.; Koeman, T.; van Dillen, T.; van der Giessen, E. (2005). Alternative explanation of stiffening in cross-linked semiflexible networks. *Physical Review Letters,* Vol. 95, pp. 178102-1-178102-4, ISSN 0031-9007.

Pollard, T.D.; Earnshaw, W.C. (2004). *Cell Biology*. W.B. Saunders Company, ISBN 0-7216-3360-9, Philadelphia.

Qin, Z.; Kreplak, L.; Buehler, M.J.; Schnur, J.M. (2009). Hierarchical Structure Controls Nanomechanical Properties of Vimentin Intermediate Filaments. *PLoS ONE,* Vol. 4, p. e7294, ISSN 1932-6203.

Rohan, E.; Cimrman, R. (2010). Two-scale modeling of tissue perfusion problem using homogenization of dual porous media. *International Journal for Multiscale Computational Engineering*, Vol. 8, pp. 81–102, ISSN 1543-1649.

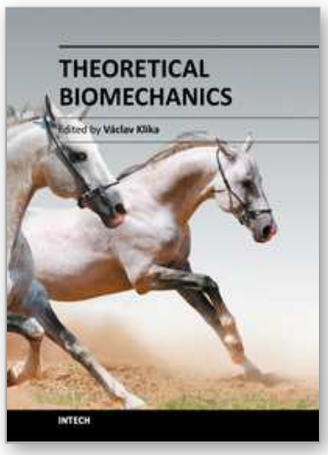
Sollich, P. (1998). Rheological constitutive equation for model of soft glassy materials. *Physical Review E*, Vol. 58, pp. 738-759, ISSN 1539-3755.

Stamenovič, D. (2006). Models of cytoskeletal mechanics based on tensegrity. In: *Cytoskeletal Mechanics*, Mofrad, M.R.K.; Kamm, R.D. (Eds), pp. 103-128, Cambridge University Press, ISBN 978-0521846370, New York.

Storm, C.; Pastore, J.J.; MacKintosh, F. C.; Lubensky, T.C.; Janmey, P.A. (2005). Nonlinear elasticity in biological gels. *Nature,* Vol. 435, pp. 191-194, ISSN 0028-0836.

Vaziri, A.; Gopinath, A. (2008). Cell and biomolecular mechanics in silico. *Nature Materials*, Vol. 7, pp. 15-23, ISSN 1476-1122.

Viidik, A. (1979). Biomechanical behavior of soft connective tissues, In: Akkas N. (Ed) *Progress in Biomechanics,* pp. 75-113, Springer and Sijthoff & Nordhoff´, ISBN 978-9028604797, New York.



Theoretical Biomechanics

Edited by Dr Vaclav Klika

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During last couple of years there has been an increasing recognition that problems arising in biology or related to medicine really need a multidisciplinary approach. For this reason some special branches of both applied theoretical physics and mathematics have recently emerged such as biomechanics, mechanobiology, mathematical biology, biothermodynamics. This first section of the book, General notes on biomechanics and mechanobiology, comprises from theoretical contributions to Biomechanics often providing hypothesis or rationale for a given phenomenon that experiment or clinical study cannot provide. It deals with mechanical properties of living cells and tissues, mechanobiology of fracture healing or evolution of locomotor trends in extinct terrestrial giants. The second section, Biomechanical modelling, is devoted to the rapidly growing field of biomechanical models and modelling approaches to improve our understanding about processes in human body. The last section called Locomotion and joint biomechanics is a collection of works on description and analysis of human locomotion, joint stability and acting forces.

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