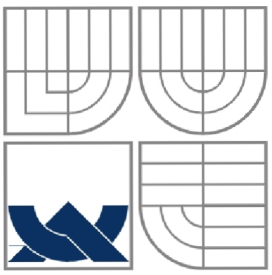

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VÝPOČTOVÉ MODELY MECHANICKÝCH ZKOUŠEK BUNĚK

COMPUTATIONAL MODELS OF MECHANICAL TESTS OF CELLS

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Abstract

This work introduces the problem of the biomechanics of cell and its computational modelling and testing. Further the work briefly describes the basic notions of biology and mechanical engineering. The aim is the research of available information sources and classification of the level of computational modelling and cell testing.

Key words: Computational modelling, cellular architecture, cell model, biomechanics of cell, tensegrity structure, viscoelastic model, tests of cell.

Abstrakt

Práce uvádí do problému biomechaniky buňky, jejího výpočtového modelování a experimentálních zkoušek. Stručně popisuje základní pojmy biologie a mechaniky. Cílem práce je provést rešeršní studii dostupných informačních zdrojů a zhodnotit úroveň výpočtového modelování buňky.

Klíčová slova: Výpočtové modelování, složení buňky, model buňky, biomechanika buňky, tensegritní struktura, visloelastický model, zkoušky buněk.

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Čestné prohlášení

Čestně prohlašuji, že bakalářskou práci na téma: *Výpočtové modely mechanických zkoušek buněk* jsem vypracoval samostatně pod vedením svého vedoucího bakalářské práce s použitím odborné literatury, kterou jsem všechnu citoval v seznamu literatury.

V Brně 23. 5. 2008 ……

Zdeněk Ančík

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Contents

1. Introduction

”The biomechanics engineering tries to find ways how to improve human life, but the most difficult is that the human live.”

The computational models of mechanical tests of cell are interdisciplinary issues. Therefore the access for successful solution of these issues involves biology and mechanical engineering. The biomechanics is defined as the application of engineering principles to biological systems. The biomechanics problems are generally applied on biomechanics objects and they are defined by these objects. In this case we specify the object as a eukaryotic cell.

The biomechanics of cell, applying mechanical engineering methods on eukaryotic cells, recently became an independent scientific discipline. The aim of the discipline is a research of mechanical loads effects on behaviour of the eukaryotic cell, especially conversion of mechanical signal to biochemical signal. This mechanism is called mechanotransduction.

The biomechanics of cell tries to answer many important questions about the cell, such as the way of transduction of the signal from surroundings into the cell, the processing of this signal in the cell, the invoking an appropriate response to the signal and identification corresponding structures which participate on this mechanism.

The attempt of scientists to answer these questions resulted in experiments on single cell, which are used for identification of the properties of the cell structures. These properties are utilized as an input data to computational modelling.

This work introduces the problem of the biomechanics of cell and its computational modelling. Further the work briefly describes the basic notions of biology and mechanical engineering. It concurs on the works published in Faculty of mechanical engineering, University of technology Brno. The aim is the research of available information sources and classification of the level of computational modelling and cell testing.

2. Cellular architecture

The cell theory was founded in 1838 and it is the base for cytology and modern biology. The cell is basic building and functional block of higher organism. Individual cell themselves are highly complex living entity. The structure of cell consists of individual elements so-called organelles, bindings between them and bindings with surroundings. There are more than 200 different types of cell in human body. The cells differ in size, shape, structure and function. There are two general cell types: eukaryotic cell, with nucleus, found in higher organism such as mammals, and prokaryotic cell, without nucleus, found in organism such as bacteria. This chapter is described the biomechanics of eukaryotic cell in short.

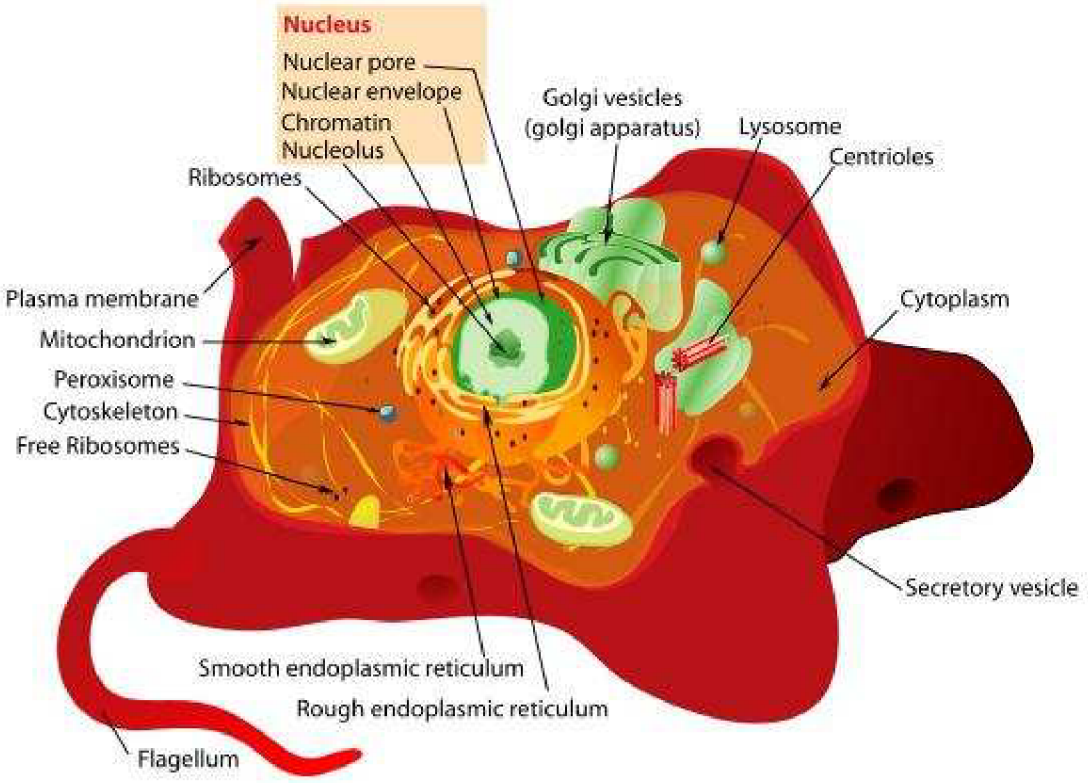

Figure 1. Structure and organelles found in eukaryotic cell.

2.1. Eukaryotic cell

This chapter will target the subsystems and organelles influencing mechanotransduction.

The main parts of eukaryotic cell are:

- plasma membrane (wall)
- cytoplasm (cellular plasma)
- nucleus (command centre)
- cytoskeleton (framework)

Plasma membrane

The plasma membrane is a thin and structured bilayer of phospholipid and protein molecules that envelopes the cell. This barrier separates a cell interior from its surroundings and controls what moves in and out. There are no elements for mechanical resistivity. It is ensured by the cytoskeleton.

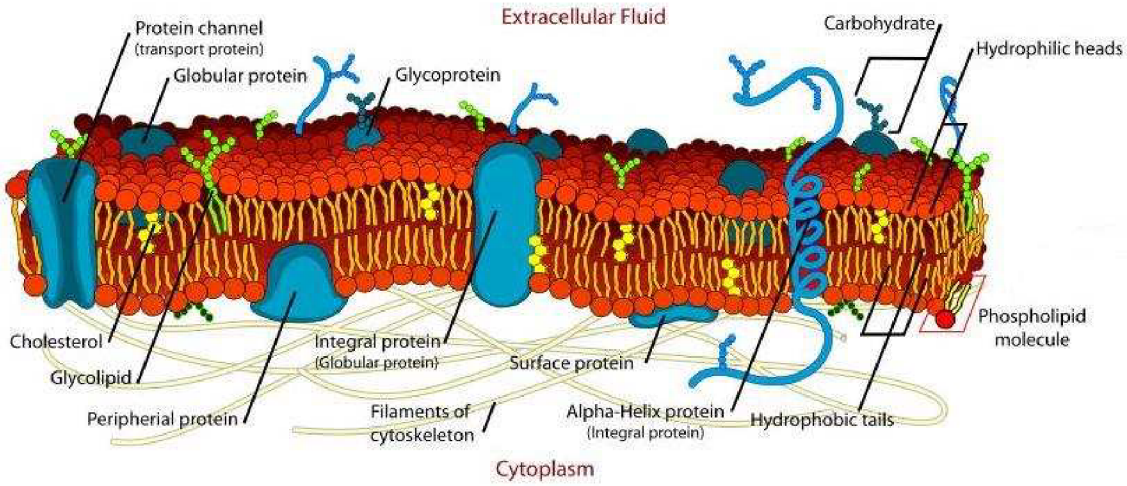

Figure 2. The cell plasma membrane.

Cytoplasm

The cytoplasm is liquid environment inside the cell. The organelles and other subsystems are placed there. It is inhomogeneous emulsion with different viscosity and density in other parts. (*Figure 1*)

Nucleus

The nucleus is a highly specialized organelle that serves as the information processing and administrative centre of the cell. This organelle has two major functions: it stores the cell hereditary material, or DNA, and it coordinates the cell activities including growth, intermediary metabolism, protein synthesis and reproduction. The main structures building up the nucleus are the nuclear envelope, nuclear cytoplasm, chromatin and nucleolus.

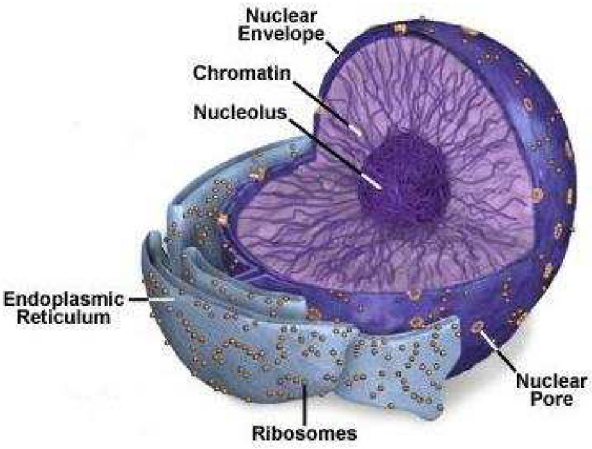

Figure 3. The structure of cell nucleus.

Cytoskeleton

The cytoskeleton is a dynamic structure that maintains cell shape, often protects the cell, enables cellular motion and plays important roles in both intracellular transport and cellular division. This organelle consists of long rod-shaped molecules attached to one another and to the other organelles by connecting molecules. It contains three main kinds of filaments: microfilaments, intermediate filaments and microtubules. The cytoskeleton is the main element which transfers mechanical loads and can be a key for understanding of machanotransduction.

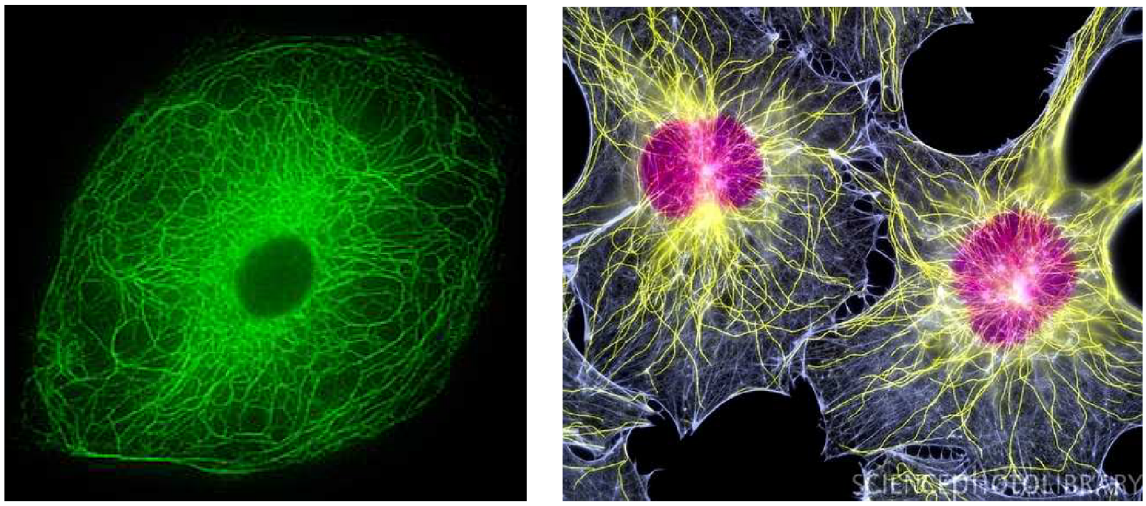

Figure 4. The cytoskeleton is imaged by fluorescence method. The nuclei are viewed as purple, microtubules are yellow and actin filaments are blue.

3. Methods of measuring the mechanical properties of cell

The external mechanical load is an essential part of cell life, which participates in many mechanical actions in human body. For better understanding to the biomechanics of cell it is necessary to know the mechanical properties of a single cell.

The experimental data are based on computational models and it is used as input parameters. This part describes measurement methods, which are used for measuring of the cell properties such as Yong's modulus, shear modulus, Poisson number etc. Due to special technologies the mechanical load can be applied on the particular parts of cell, even to individual organelles and cellular subsystems. Their participation in transduction of mechanical load can be proved on the basis of test results.

The response to the external mechanical load is different for every individual type of cell. It mainly depends on the function of the cell. Following factors of external mechanical load are essential:

- **Loading value of load**

Chondrocytes are the most loaded cells, whose stress intensity is around 20MPa. When the chondrocytes aren't loaded, they start atrophying. On the other side, when the load is higher, they are damaged.

- **Loading character**

The mechanical engineering distinguishes following main loading types: press, pull, shear, torsion, bend and their combinations. For example the one axis pulling is typical starting mechanism for function of a tenocytes.

- **Loading time variation**

Some types of cells are more sensitive to time variation of external mechanical load than to stable value of it.

The very special measurement methods are used to detect mechanical properties. They are listed bellow:

- **Mechanic methods**

  Cell poking

  Atomic force microscopy

  Tensile tests

  Microplate manipulation

  Micropipette aspiration

  Traction force microscopy

- **Magnetic methods**

  Magnetic twisting cytomery

  Magnetic tweezer

- **Optical methods**

  Optical tweezer

  Optical stretcher

  Defocusing microscopy

  Immunoflorescenece imaging

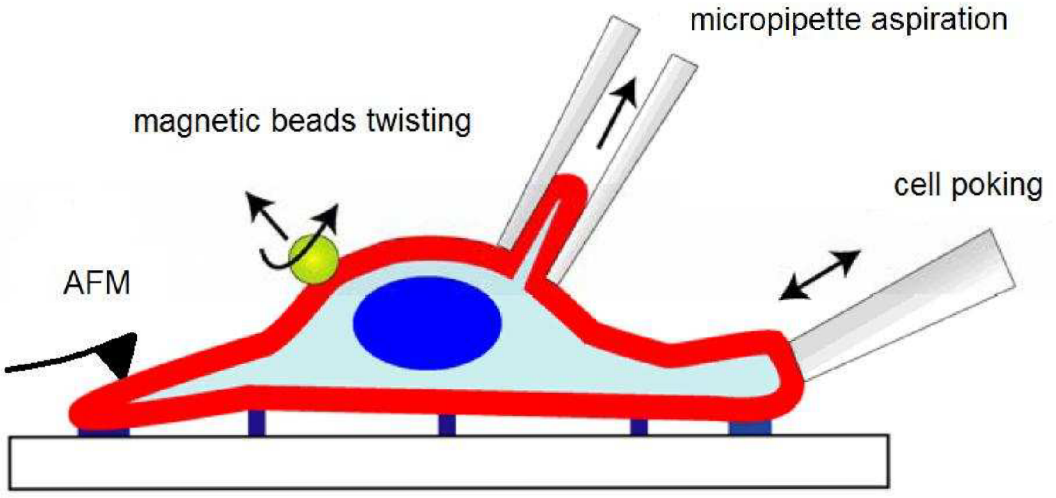

Figure 5. Schematic drawings of various methods for measuring mechanical properties of a living cell.

3.1. Defocusing microscopy

Defocusing microscopy is a recently developed technique that allows quantitative analysis of the membrane surface dynamics of living cells using a simple bright-field optical microscope. The base of this technique is proportional relation of the contrast of defocused images on the cell surface curvature, which causes light and dark contrast images that can be measured and quantitatively studied.

Figure 6. a) positive defocusing, b) in focus (object invisible,) c) negative defocusing

This technique is used mainly to determine size and amount of the membrane shape fluctuations. In *[19]* there is published the use of defocusing microscopy to measure optical and mechanical properties of the cell, such as cell refractive index, membrane bending modulus and effective cell viscosity.

Any deformation applied on the plasma membrane changes the local curvature. It can be detected and quantified through a simple analysis of the contrast patterns appearing in the defocused images, given by:

$$C = \Delta n * [\Delta f - h] * \kappa \qquad (1)$$

Where:

- C contrast generated by the curvature κ
- Δn difference between refractive indexes of the membrane and the surrounding
- Δf defocusing distance
- h vertical extension of the deformation

3.2. Immunoflorescenece imaging

Due to immunoflorescence imaging can by examined relation between cellular stiffness and higher order structure of cytoskeleton. In *[5]* there is investigated a distribution of F-aktin, myosin II and vinculin by indirect immunofluorescent. The base of this method is antigen, molecule, which is binding on antibody.

A primary antibody is bound to an antigen, secondary antibody coupled to fluorescent molecule is bound to the primary antibody.

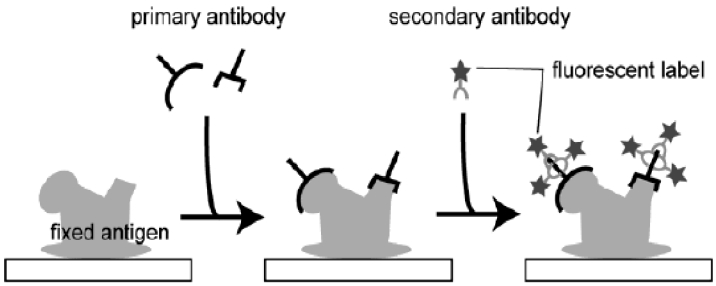

Figure 7. Schematic drawings of indirect immunofluorescent.

Stained proteins can be easily detected with fluorescence microscopes. The optical system of confocal laser scanning microscope is used in test.

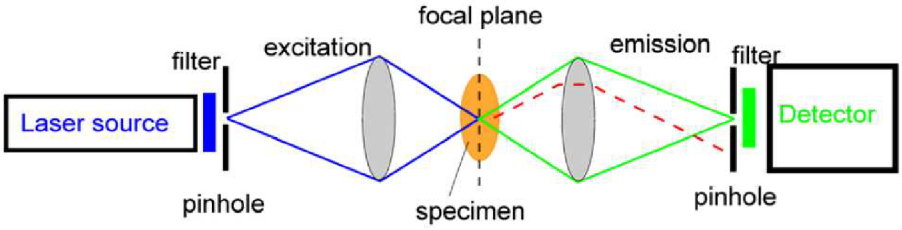

Figure 8. The optical system of confocal laser scanning microscope.

The excitation light is focused by pinhole 1 at a single point in the specimen. The pinhole 2 eliminates fluorescence light except that emitted from the focal point. The two-dimensional fluorescence image is showed by scanning excitation light across the specimen. Thereto, a three-dimensional image can by constructed by stacking each sectional image obtained at various levels.

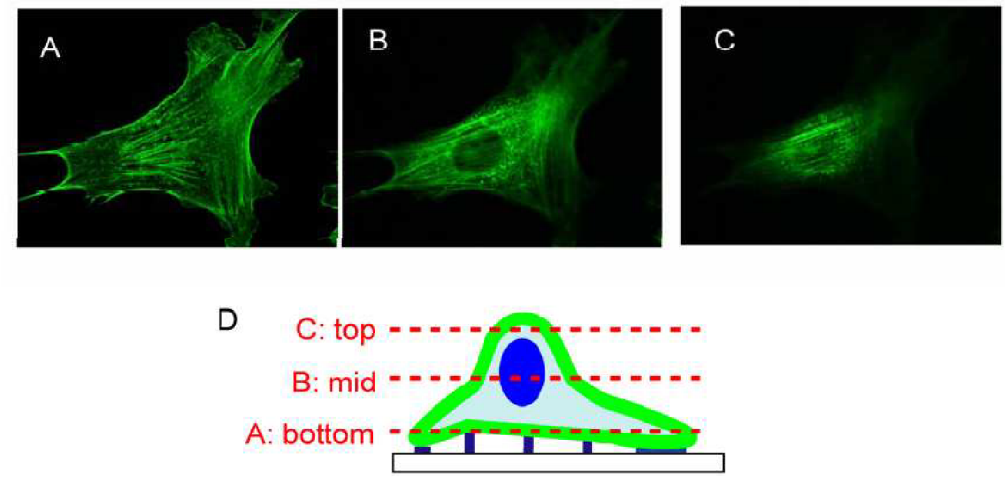

Figure 9. *Two-dimensional fluorescent images. A-C) these images are taken at different height levels, D) schematically shown.*

4. Computational models of cells

The computational model is constructed on the basis of obtained experimental data and accessible theoretical knowledge. The target of modelling is creating simplified object, whose characteristic properties and behaviour are the same as properties of real object. The complexity of model should be adapted with required accuracy and type of examine parameters. Then the different levels of models are constructed (two and three dimensional models, structural and unstructured models, static and dynamic model etc.).

This chapter is targeted to three dimensional viscoelastic and tensegrity model of the cell with regard to study of published knowledge and targets of this work.

4.1. Continual model of the cell

The measurement methods of the mechanical properties of cell, which resolving ability is lower than distance between each cellular elements (organelles and subsystems), examine mainly the macroscopic mechanical properties. The magnetic bear rheometry, micropipette aspiration and micropipette manipulation is possible to mention. For the computational modelling and identification of macroscopic mechanical properties of these tests are continual models sufficient.

The viscoelastic description of mechanical properties of continual model is mainly used in present. The viscoalestic response to application of the force impulse is indicated by experimental data from magnetic bead rheomery.

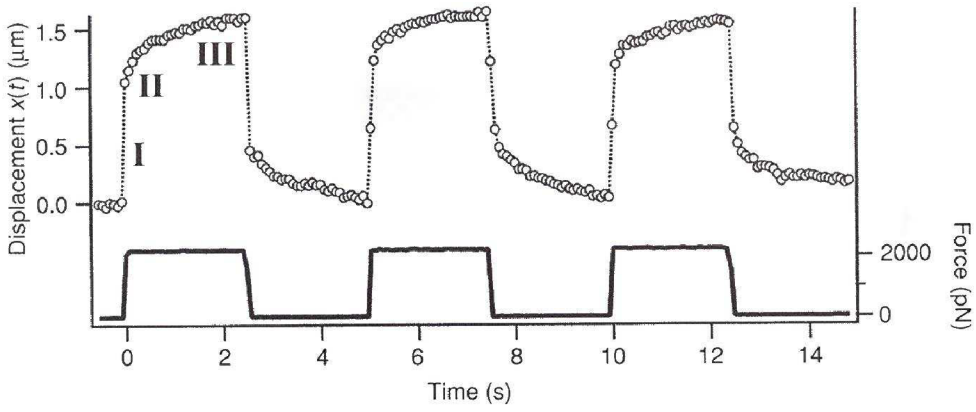

Figure 10. The creep response and relaxation curves.

The easier identification of constitutive parameters is the next advantage of viscoelatic description. The viscoelastic properties consist of elastic properties described by dashpot and elastic properties described by spring.

When the force is applied to dashpot and spring the rate of deformation are linearly related to the force:

- spring $F_s(t) = k_0 * x_s(t)$ (2)

- dashpot $F_d(t) = \eta_0 * \dot{x}_d(t)$ (3)

Where:
- k_0 spring constant
- x_s spring length (difference in unloaded and forced spring)
- η_0 dashpot constant
- $\dot{x}_d$ differentiation with respect to time

This combination of sprig and dashpot is known as the Maxwell body.

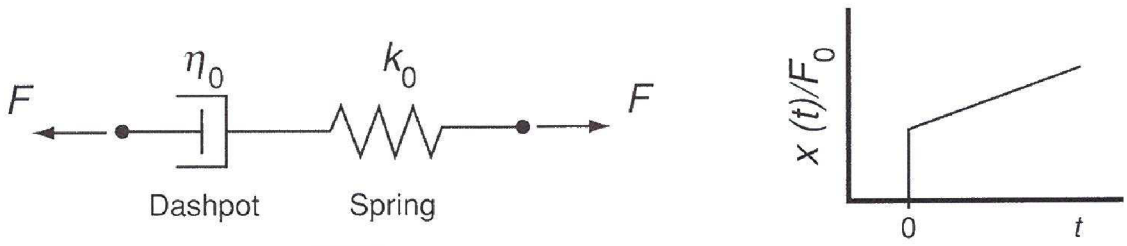

Figure 11. The parameter model of Maxwell body and the corresponding creep response to a step change in the applied force.

4.2. Published studies of continual models

4.2.1.

In *[6]* there the viscoelastic model is used for identification of mechanical properties such as Young elastic modulus E and viscous modulus η. The target of this work is attempt to estimate in living adherent epithelial alveolar cells the degree of structural and mechanical heterogeneity by considering two individualized cytoskeleton components. That is a cortical cytoskeleton and a deep cytoskeleton. The results from the magnetic twisting cytometry are used as an input data. These results show that the cortical cytoskeleton response is a faster, softer, moderately viscous, slightly tensed and easily damaged structure compared to the deep cytoskeleton structure which appears slower, stiffer, highly viscous, more tensed and fully elastic.

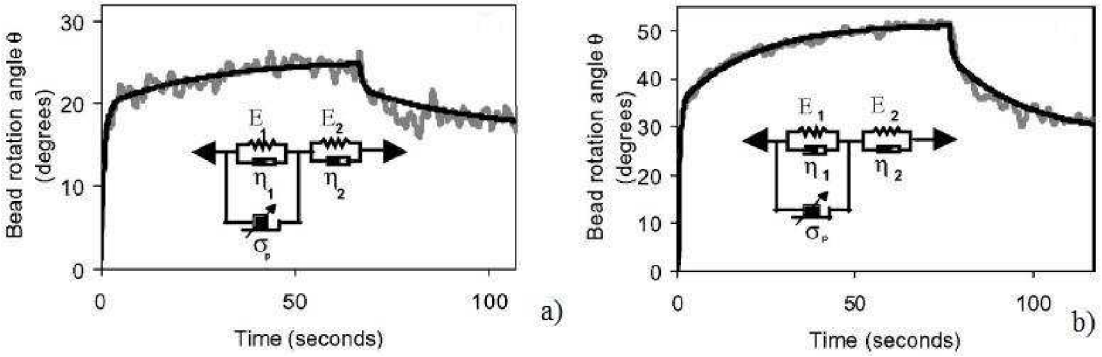

Figure 12. a) model of cortical cytoskeleton, b) model of deep cytoskeleton

4.2.2.

In *[7]* there is viscoelastic model used for computational model of isolated cell nucleus. The response on AFM and micropipette aspiration experiments is simulated in these cases. The model includes distinct components representing the nucleoplasm and the nuclear envelope. The nuclear envelope consists of three layers: inner and outer nuclear membranes and one thicker layer representing the nuclear lamina. The nucleoplasm is modelled as a viscoelastic Maxwell body with a single time constant. The nuclear envelope layer is taken as a linear elastic material.

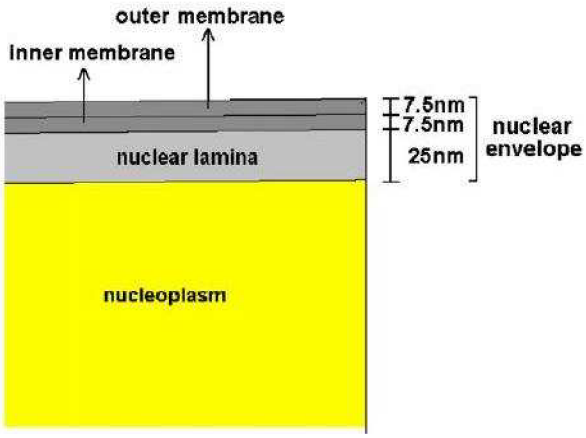

Figure 13. Nuclear elements considered in the computational model.

The model for an isolated nucleus in micropipette aspiration experiments is developed with the radius of 3 μm. The nuclear inner and outer membranes have thickness of 7.5nm and nuclear lamina is 25nm thick.

The computation is performed by Abaqus. It is modelling software using finite element method. The eight-node biquadric axisymmetric finite elements are used in calculations.

The input data to computational model are:

- nuclear envelope κ_{NL}=5mN/m; $\kappa_b(t=0)$=16mN/m; α_b=0.01; τ_b=5s
- nucleoplasm E=30Pa; τ=1s; ν=0.48
- load the pressure increased linearly to 300Pa in 10s then held constant

Where:

- κ_{NL} stretching stiffness of nuclear lamina
- κ_b stretching stiffness of lipid bilayer
- α_b ratio of the final apparent stiffness
- E Young elastic modulus
- τ characteristic time
- ν Poisson ratio

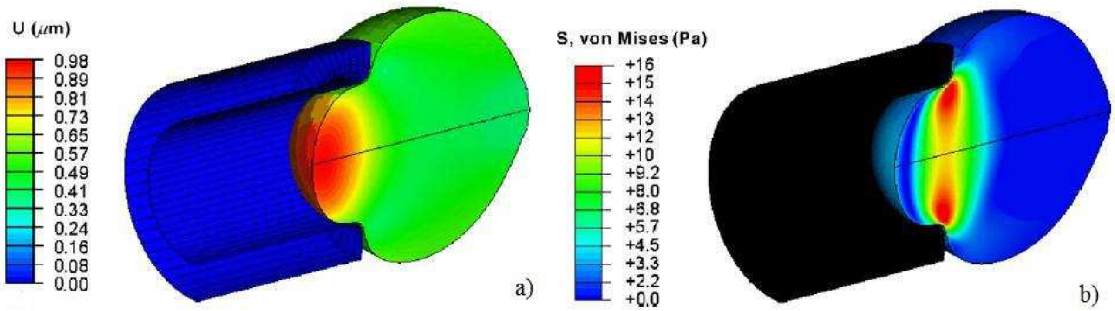

Figure 14. a) displacement field, b) effective stress field

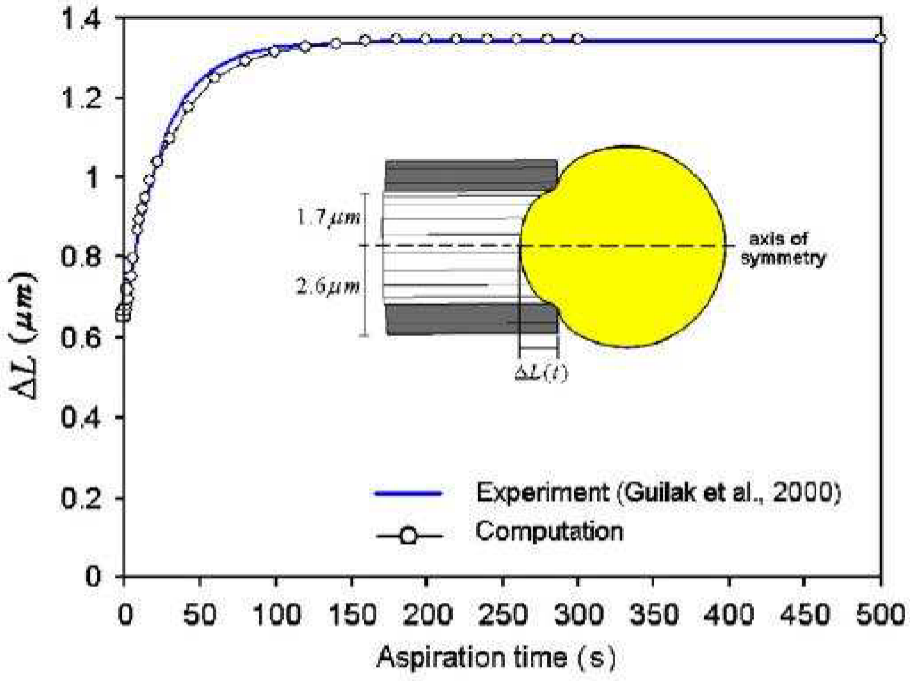

Figure 15. Comparison of the results of the computational model with the experiment.

4.2.3.

In *[8]* there is used same model for simulation of atomic force microscopy measure method.

The input data are:

- nuclear envelope κ_{NL}=5mN/m; κ_b=18mN/m
- nucleoplasm E=25Pa; ν=0.485
- load displacement of 1μm

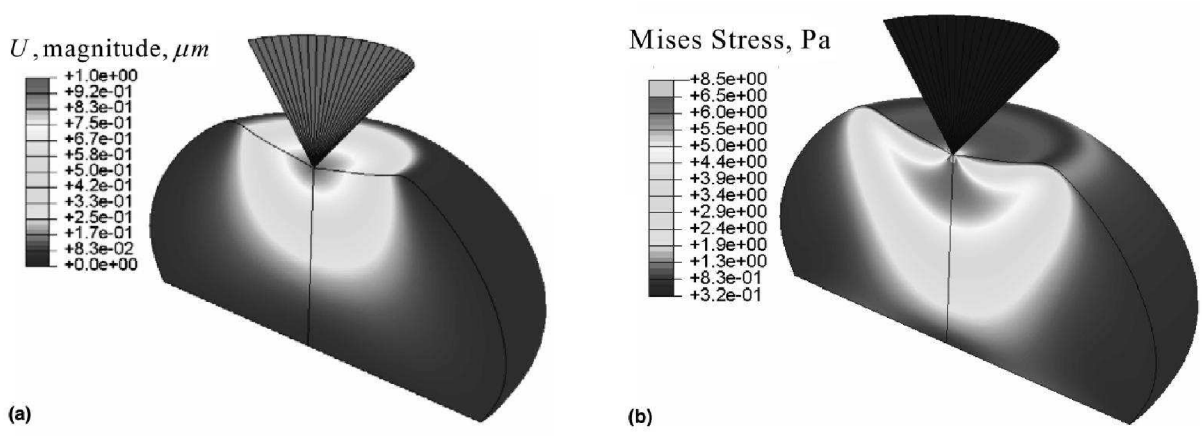

Figure 16. a) displacement field, b) effective stress field

4.3. Tensegrity model

The intercellular and extracellular elements, which are participant in mechanical behaviour of the cell, have the same properties as a tensegrity structure. This theory was described by Donald Ingber in 1980s. The term tensegrity is contraction of tensional integrity. This structure is composed of rigid bars compressed by continuous network of pre-stretched cables. The stability of structure is assured by pre-stretched cables. More formally:

"A tensegrity system is established when a set of discontinuous compressive components interacts with a set of continuous tensile components to define a stable volume in space."

The properties of tensegrity structure:

- proportional dependence on stiffness and pre-stress in cables
- no linear response to external load
- transfer of external load to the other elements of structure (global response)
- synergic action
- independence of stability of structure on interaction with surroundings
- statistic imbalance

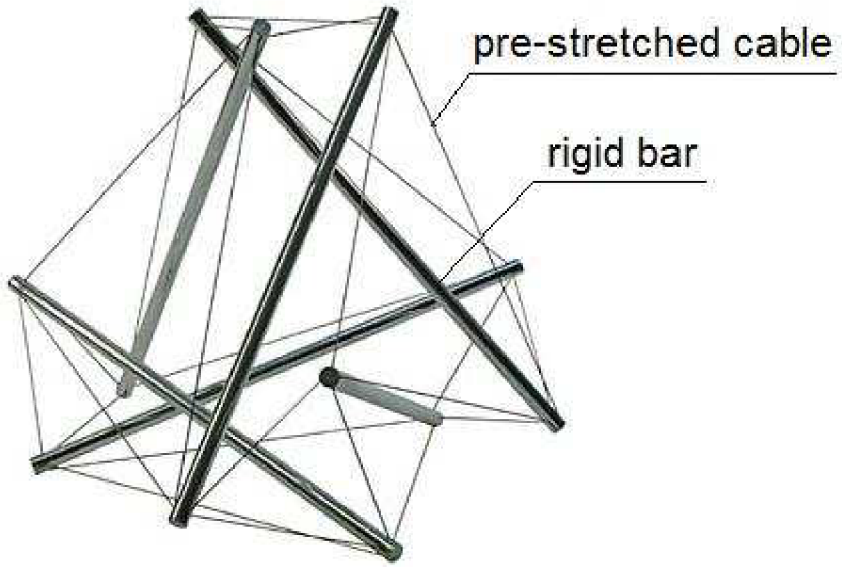

Figure 17. The tensegrity structure.

4.4. Published studies of tensegrity models

4.4.1.

In *[10]* there is used the tensegrity model composed of 6 rigid bars connected to a continuous network of 24 viscoelastic pre-stretched cables. The target is analysed the role of the cytoskeleton spatial rearrangement on the viscoelastic response of living cells.

These properties are considered in model:

- discrete nature of the cytoskeleton
- cell - cell and cell - extracellular matrix interactions
- cellular pre-stress

Relationships between the global viscoelastic properties of the tensegrity models, and the physical properties of the constitutive elements are examined in this study.

Global properties:

- viscosity modulus η
- elasticity modulus E

Properties of the constitutive elements:

- length of elements L
- initial internal tension T

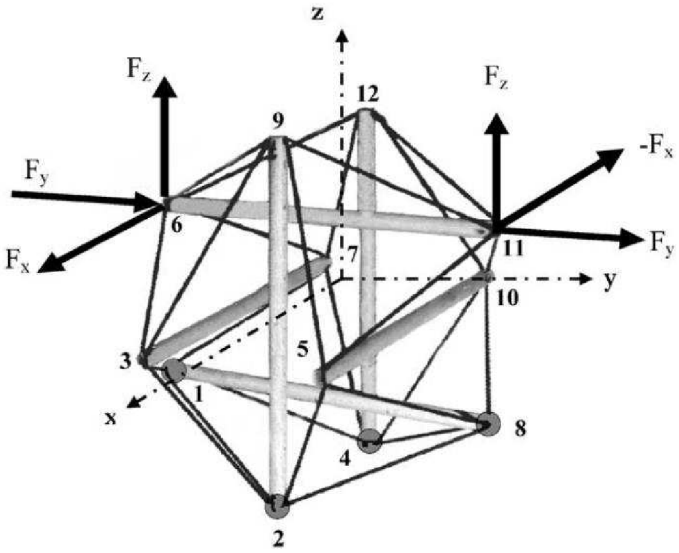

Figure 18. The tensegrity model.

The load of model is shown in *Figure 18*. External forces are applied at nodal points 6 and 11. Extension and compression forces F_z are applied along the z-axis. Shear forces F_y are applied along the y-axis and twisting torque is applied by opposite forces at node 6 F_x and at node 11 F_x.

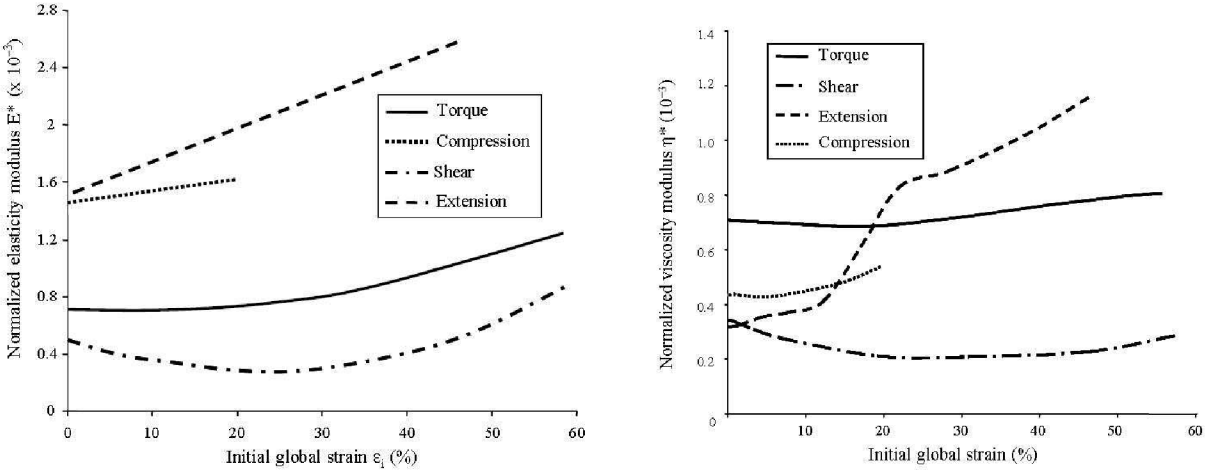

Figure 19. Normalized viscosity and elasticity modulus as a function of the global deformation.

4.4.2.

In *[9]* there are presented "non-regular" tensegrity models, which respect some basic properties of cytoskeleton.

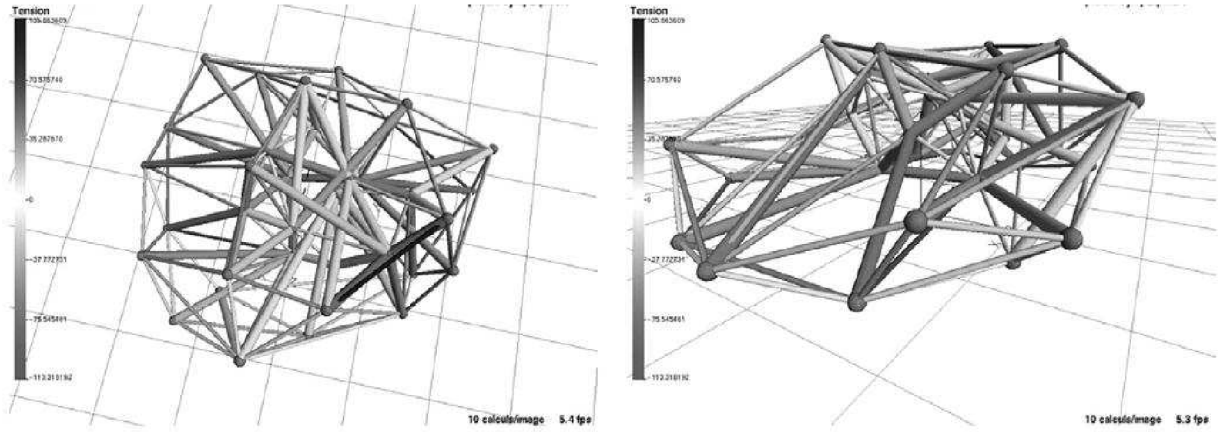

Figure 20. Models of an epithelial cell.

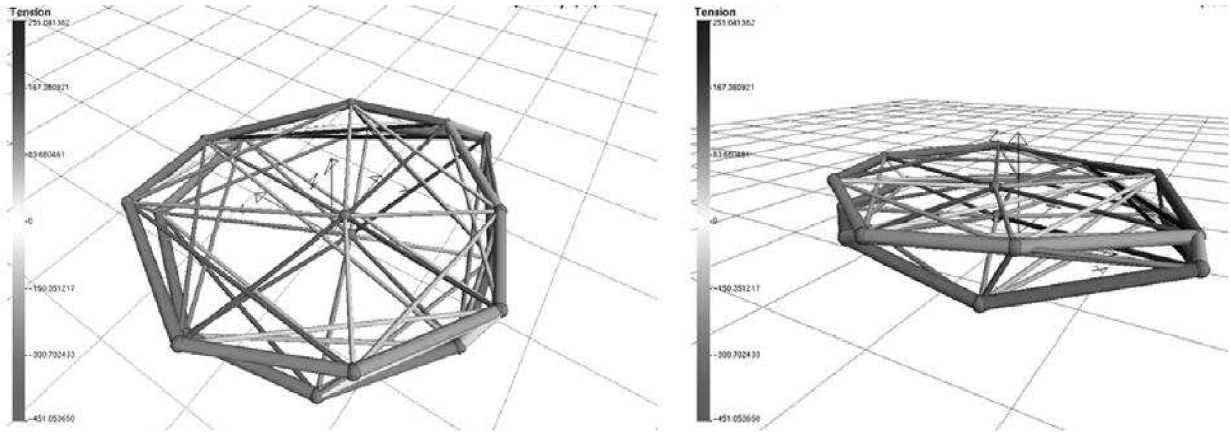

Figure 21. Models of a blood platelet.

4.4.3.

In *[1]* there is used high structure model, based on tensegrity structure. This model is considered with the cytoskeleton, membrane envelop, cytoplasm and nucleus. The cytoskeleton is composed of 30 rigid bars connected to a continuous network of 60 pre-stress cables. The rigid bars present the function of the microtubules, cables present actin filaments. The same miniaturized structure is used as a nuclear skeleton. These structures are connected together by 30 rigid bars, which present function of microtubules and intermediate filaments. This tensegrity structure is enveloped by shell element and felled up with an isotropous homogenous continuum. They presents membrane envelop and cytoplasm. A linearly elastic constitutive model is used for material properties.

The input data to computational model are:

- cytoskeleton rigid bars E=1,2e6kPa ; ν=0,3
- cytoskeleton cables E=2,6e6kPa ; ν=0,3
- connecting rigid bars E=2e6kPa ; ν=0,3
- membrane envelop E=10kPa ; ν=0,3
- cytoplasm E=0,001kPa ; ν=0,45
- nucleus E=0,005kPa ; ν=0,45

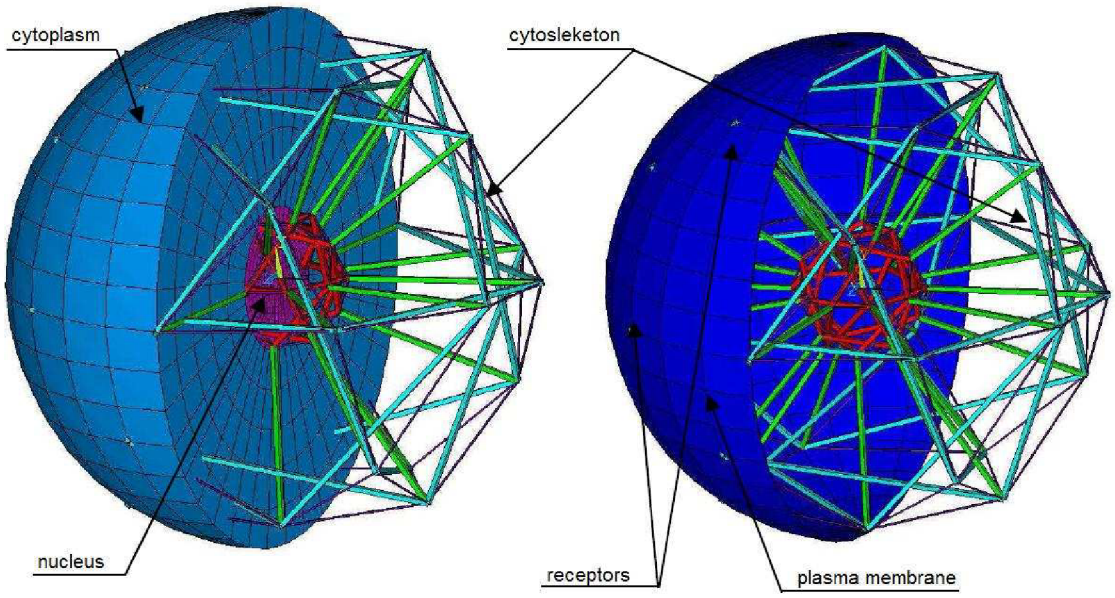

Figure 22. The structural model of cell – spherical shape.

This model is used for simulation of AFM measurement method. Above mentioned model is remodelled to adhesion shape by setting required displacement.

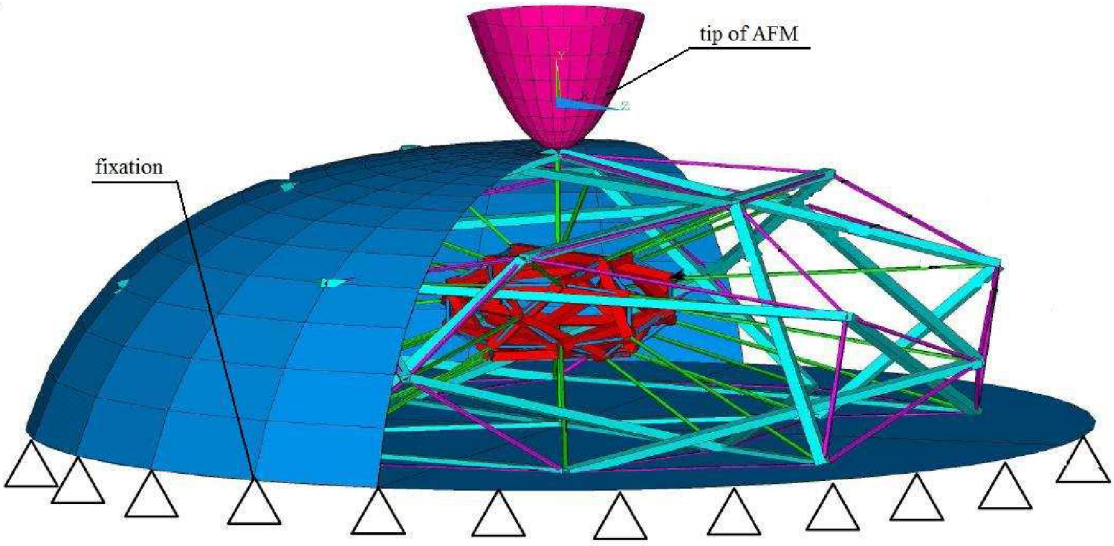

Figure 23. The computational simulation of AFM test.

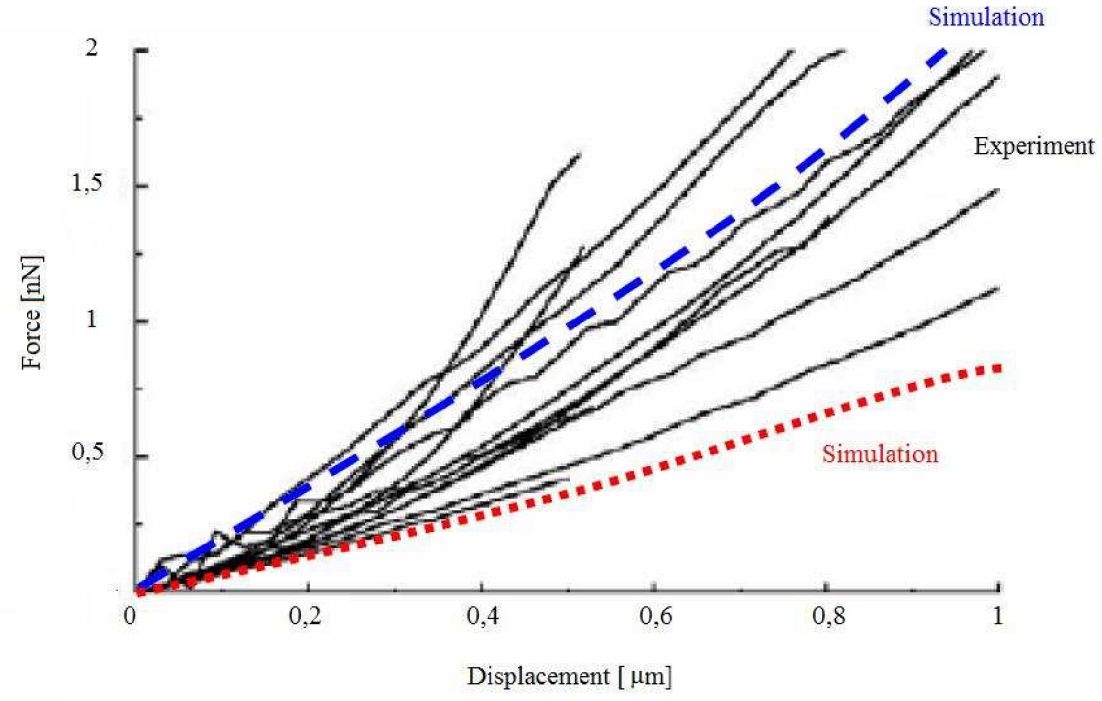

Figure 24. The comparison of computational model and experiment.

5. Conclusion

This bachelor thesis describes the biomechanics of cell on the basis of available information sources. Due to the technical intent of the study the basic knowledge of technical terminology is expected therefore more attention is dedicated to the biological term description.

The work follows the dissertation thesis published on the Faculty of mechanical engineering, University of technology Brno in 2007 and includes the sources which are not mentioned in that works. Due to high level of research and large information content of the earlier published works it was very difficult to find new information about this topic.

With respect to the high demands and difficulties related to cell testing the international cooperation is useful. Consequently for the information exchange and the presentation of the results the English language is generally used and it was considered appropriate to use English in this work as well.

6. References

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7. Appendix

There is the CD with electronic version of this work in PDF format.