Evaluation of the local elasticity modulus of biological cells on the basis of the shells theory

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Abstract
The development of theoretical models and experimental methods of cell mechanics are actual task. On the bases of the shell theory we consider the elastic properties of the cells and compare them with the experimental results which was received using atomic force microscopy (AFM) date. The experimental calculations were made on the bases of the Herz model. It was shown that the shell theory allows to evaluate the modulus of elasticity of the cell membrane and Herz model is a complex characteristic of the membrane and cell’s internal components.

Keywords: Modulus of elasticity, cell, shells theory, atomic force microscopy

1. Introduction
Mechanical properties are fundamental properties of cells and tissues. They characterize a number of cytophysiological and cytopathological processes. The cell mechanical parameters are possible to use as certain markers of the pathology [1, 2]. The study of elastic properties allows to obtain new knowledge about biological cells and also is of clinical interest. That’s why the development of theoretical models and experimental methods of cell mechanics are actual task.

There are several methods to evaluate the elastic properties of the cells. In our experimental investigation we use atomic force microscopy (AFM). One of advantages of AFM is an opportunity to investigate the local mechanical properties of single cells including the properties of separate cellular structures as cytoskeleton and plasma membrane. Studying with AFM methods the surface layer of a single cell (thickness from some nanometers to some hundred nanometers), we deal with a composite material, the mechanical properties of which are mainly conditioned by the properties of cortical actin cytoskeleton, two- or three-dimensional network from natural biopolymers (proteins) [3]. The cytoskeleton structure reorganization leads to the mechanical properties of cells change. Early we proposed the method of the local elastic modulus estimation at nanoscale using a spherical indenter [4]. And now the goal is to create a theoretical model of this process using a shells theory. In experiments the AFM function of force spectroscopy was used. It is possible to obtain force curve by recording the cantilever deflection while the tip is in contact with the body. The force curve contains information about long- and short-range interactions and represents a basis for estimation of sample elastic (Young’s) modulus. Also it is possible to evaluate the adhesion between the AFM tip and the cell. We noticed that membrane interacted with the tip and stretched for some time and decided to solve the task about spherical shell under the action of normal force directed outwards.

2. Methods

2.1. Force spectroscopy
The procedure of force spectroscopy constitutes a standard mode of AFM. The method consists in the
realization of the contact deformation of a specimen using the probe and in quantifying the relationship between probe interaction force and distance [5]. We record the cantilever deflection as the sample moves up, reaches the tip and is retracted. The force curve is obtained by monitoring the movement of the reflected laser beam from the rear of the cantilever. The force curves are the relation between the bend of the cantilever and the position of the probe. Knowing the displacement of the sample in the vertical direction and the amplitude of cantilever bending, it is possible to calculate the total external force that has been applied to it and the resulting deformation of the sample. The force curve is applied to the calculation of Young’s modulus (Fig. 1.).

![Fig. 1. The regime of static force spectroscopy: typical force curve](image)

The figure shows the different stages of the indentation process. At the beginning of the force curve recording, the tip is distant from the sample and approaches, but does not contact it (a). Since there is no contact the cantilever deflection is constant. As soon as the tip actually touches the sample, the cantilever moves upward (b). Further, as the tip indents the sample, the cantilever arm moves downward (c). The sample is then retracted displaying a reverse behavior to action (c), as indicated by the curve (d) which shows the deflection during retraction. However, when adhesion between sample and tip occurs, the tip will adhere to the sample beyond the point of contact (e), until it finally breaks free again and the deflection returns to zero (f). The approach curve exactly retraces the pathway to point (e) provided there is no piezohysteresis. We have been able to quantify the local elastic properties of the erythrocytes using the force spectroscopy mode. The force curve is obtained by recording the cantilever deflection as the tip is brought into contact at the fixed point and then retracted. The Young’s modulus was calculated using the Hertz model describing the elastic deformation of two bodies in contact under load [6]. We consider that the indented sample is assumed to be extremely thick in comparison to the indentation depth. In this case the elastic modulus can be calculated as described earlier [7, 8].

2.2. Shells theory

The problem of cell deformation as a result of the contact stretch can be considered from the point of the shells theory [9]. To describe the interaction between the AFM tip with the cell under stretching deformation (Figure 2), we use the known solution for a spherical shell under the action of normal force to the shell surface of (Figure 3).
Fig. 2. Scheme of interaction between the indenter and the erythrocytes membrane when the AFM tip with draw from the surface.

It is assumed that the stresses and displacements are very small at some distance from the force application point, the tangent moving along the middle surface is considerably smaller than the movement in the normal direction, the shell is infinite in all directions (to eliminate the influence of the boundary effects), the shell material is elastic and conforms to the Hooke's law [9-12].

Fig. 3. Spherical shell loaded with a normal concentrated force: 
- thickness of the shell, 
- radius of the shell

Then the relation for determining the deflection of the shell is:

\[
    h = \frac{P l^2}{2 \pi D} \left\{ -kei(x) - k_R \left[ (1 + \nu) \left( \frac{\pi}{2} Y_0 (c \sqrt{2k_R}) + \text{ker}(x) \right) + \frac{1}{2} c \text{ker}'(x) \right] + (\eta - \varepsilon) \text{ker}(x) + \frac{t}{4} c \text{ker}'(x) \right\}
\]

where

\[
    D = \frac{E l^3}{12 (1 - \nu^2)}; \quad l = \frac{\sqrt{rt}}{\sqrt{12(l - v^2)}}
\]

\[
    k_R = \frac{t^2}{r^2}; \quad \varepsilon = \frac{v t^2}{10(l - v^2) l^2}
\]

\[
    \eta = \frac{t^2}{3(l - v)^2}
\]

\( E \) - Young's modulus of the sample, Pa; \( \nu \) - Poisson's ratio of the sample; \( R \) - radius of the AFM tip, m; \( h \) - the difference between the coordinate tip contact point and the coordinate of separation when the AFM tip withdraw from the surface (moving at material stretching), m; \( P \) - applied load, N; \( r \) - radius of the shell, m; \( t \) - thickness of the shell, \( \varnothing \) - the contact area, m\(^2\); \( l \) - characteristic size; \( c \) - dimensionless quantity, equals to \( c = \varnothing / l \); \( kei (c) \) - Thompson function; \( k_R = l^2 / R^2 \) - coefficient characterizing the thickness of the shell.
Y₀ - Bessel function of the second type and of the zero-order, \( \text{kei}(x) \) and \( \text{ker}(x) \) - Thompson (Kelvin) functions. Asymptotic representation of these functions has the form:

\begin{align}
\text{ker}(x) &= \sqrt{\frac{\pi}{2x}} e^{\alpha(-x)} \cos \beta(-x) \\
\text{kei}(x) &= \sqrt{\frac{\pi}{2x}} e^{\alpha(-x)} \sin \beta(-x) \\
\text{kei}(x) &= \sqrt{\frac{\pi}{2x}} e^{\alpha(-x)} \sin \beta(-x)
\end{align}

\begin{align}
\alpha(x) &\approx \frac{x}{\sqrt{2}} + \frac{1}{8x\sqrt{2}} - \frac{25}{384x^3\sqrt{2}} - \frac{13}{128x^4} \ldots \\
\beta(x) &\approx \frac{x}{\sqrt{2}} - \frac{\pi}{8} - \frac{1}{8x\sqrt{2}} - \frac{1}{16x^2} - \frac{25}{384x^3\sqrt{2}} \ldots
\end{align}

If the force is distributed over the small circle surface with radius is equal to \( c \), then at point \( x = 0 \) the movement can be calculated by the following expression:

\begin{align}
\hat{h} &= \sqrt{\frac{12(I-v^2)}{\pi}} \frac{Pr}{E1^2} \left[ \frac{1}{c^2} + \frac{1}{c} \text{ker}'(c) - \frac{1}{2} (I+\nu)kR \ln \sqrt{2kR} - \frac{1}{4} kR \right] + \\
&+ \frac{3}{5\pi} (I-v)(2-v) \frac{Pr}{E1} \left[ \text{ker}(c) + \frac{c\text{ker}'(c)}{2(2-v)} \right],
\end{align}

where \( c \) - dimensionless quantity and equals (9):

\begin{equation}
\hat{c} = \frac{\nu}{l}
\end{equation}

3. Results and discussion

From the point of view of the indentation, the radius of a circle corresponds to the contact area. Contact is provided by the forces of adhesion (we consider the withdrawal of the tip out of the contact). Thus, in order to present the equation (8) in the form, which could be used to calculate the modulus of elasticity, we use the theory of Johnson - Kendall – Roberts. This theory is taking into account the effect of cohesion. Contact area according Hertz theory is defined as follows:

\begin{equation}
a = \left( \frac{PR}{K} \right)^{1/3}
\end{equation}

The reduced modulus of elasticity:

\begin{equation}
K = \frac{3}{4} \left( \frac{(I-v_1^2)}{E_1} + \frac{(I-v_2^2)}{E_2} \right)
\end{equation}

If the modulus of elasticity of the indenter is in several orders higher than the modulus of elasticity of the material (as we consider), then in formula (11) the second term can be neglected and the penetration into material of the absolutely rigid sphere (AFM tip) can be described as function of \( v_1, E_1 \).

As a result of the surface forces action the contact area increases and describes by the following equation [10]:
If the surface forces are neglected ($\Delta \gamma = 0$), then (12) is transformed into Hertz formula. In the case when zero load applied the contact area (radius) can be calculated as:

\[
a = \left(6 \pi R^2 \Delta \gamma / K \right)^{1/3}
\]

When the material is stretched, that the load is negative, the contact radius will be reduced. Since the radicand must be positive, the maximum stretching force is:

\[
P = -\frac{3}{2} \pi R \Delta \gamma
\]

Then the surface energy of the body:

\[
\Delta \gamma = -\frac{2P}{3\pi R}
\]

To determine the contact area at maximum stretching force, we substitute (4.18) and (4.14) (4.15):

\[
a^3 = \frac{3PR(1-\nu^2)}{4E}
\]

Returning to the shells theory, we adopt and substitute in (4.12). Expressing (4.11), the modulus of elasticity, we obtain the system of equations:

\[
E = \frac{\sqrt{12(1-\nu^2)}}{\pi} \frac{Pr}{ht^2} \left[ \frac{1}{c^2} + \frac{1}{c} \text{ker}^2(c) - \frac{1}{2} (1+\nu)kR \ln \left(2kR - \frac{1}{4} \right) \right] +
\frac{3}{5\pi (1-\nu^2) (2-\nu)} \frac{P}{ht} \left[ \text{ker}(c) + \frac{c\text{ker}^2(c)}{2(2-\nu)} \right],
\]

\[
c^2 = \frac{3Pr(1-\nu^2)}{4El^3}
\]

As a result, the values of the elastic modulus solution and contact area were obtained from the expression (4.20). The values of load and displacement were chosen on the basis of experimental data. Shell radius ($r$) was assumed to be 2 µm (average thickness of the red blood cell membrane) [13]. The shell thickness ($t$) initially wondered equal to 10 nm, which corresponds to the average thickness of the cell membrane. However, the calculated value of the modulus of elasticity significantly different from the known values of these parameters for real membranes. So in the case of the force is equal to 1.5 nN and displacement - 50 nm, the value of the modulus was 15 times greater than in the calculation of Hertz. The values of the modulus of elasticity coincide, if the shell thickness was 60 nm. This difference can be explained by the influence of the cell internal components in Herzt (calculation module for compressive strain). Since it is impossible to determine accurately the thickness of the cell membrane with the cytoskeleton (shell), the calculation of the modulus of elasticity using the theory of shells is rather complicated.

Thus, we conclude that consideration of the local adhesion stretching of the erythrocyte by AFM tip using a solution about spherical shell deformation under the action of normal force (17) is difficult. Because, in practice, it is necessary to use the constant, determination of which is a difficult research task.
4. Conclusions

The shell theory, namely, the decision of the spherical shell under the action of the normal applied force was used to determine the modulus of elasticity of cells. We obtained the system of equations that can be used to calculate the elastic modulus on the basis of the force spectroscopy. However, in practice this approach may be useful if it is necessary to evaluate the elastic properties of the cell membrane. In other cases Herzt model is more convenient.

References