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## Method for calculation of intercellular pressure in erythrocyte according to atomic force microscopy

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### ABSTRACT

The article aim is, within a simple mechanical model of the erythrocyte morphology, to carry out the calculation and comparison of the calculated data to the experimental data obtained using atomic force microscopy of blood cells after femtosecond laser irradiation. The paper proposes a model of the erythrocyte representing the erythrocyte as a homogeneous elastic body with the elasticity depending on the distance to the center of the erythrocyte. The model is based on data from atomic force microscopy obtained by various authors, in particular the data on rigidity of the membrane, which depends on the position of the measuring point on the surface. In the developed model the elasticity of the membrane changes depending on the distance to the center within 1-1.6 kPa. The calculation of the elastic properties is made by the finite element method, which allowed us to determine the dependence of the erythrocyte morphology on pressure on the membrane, which varied within the range of 0.5-2 kPa. The good agreement between calculated and experimental data confirms the consistency of the model and allows us to conclude that the morphology of the erythrocyte is largely determined by the elastic properties of the membrane and intercellular pressure.

### KEYWORDS

Model of the erythrocyte; Atomic force microscopy; The geometric characteristics of the erythrocyte; Modeling of the elastic properties; Intercellular pressure.



## INTRODUCTION

Erythrocytes are blood cells performing gas-transport function of transporting oxygen from lungs to organs and tissues, and carbon dioxide in the reverse direction. Mature erythrocytes have no nucleus and organelles and have a form of a biconcave disk with the maximum area to volume ratio providing the optimum gas exchange. Specific features of the biconcave erythrocyte's cytoskeleton and construction of its the cellular membrane allows it to sustain significant deformation while passing through narrow capillary with consequential restoration of the form.

It is known that plasticity of erythrocytes reduces as the form changes, for instance, in case of cellular senescence. Spherocytes, drepanocytes, stomatocytes have significantly reduced plasticity, while plasticity of echinocytes is altered too. Oxygen transport is provided by hemoglobin that accounts for about 98% of protein mass of erythrocyte's cytoplasm. This speaks of almost homogenous contents of erythrocytes.

Besides, the form of red blood cells depends on osmotic pressure in erythrocytes and in blood plasma as well as on condition of the cytoskeleton of the cellular membrane of erythrocytes, which in turn impacts elastic properties of red corpuscle membranes. It is known that the protein content in erythrocytes is higher, while the content of low molecular agents is lower than in plasma. Osmotic pressure produced by high intracellular concentration of proteins in erythrocytes is compensated by low concentration of low molecular agents to a considerable degree<sup>[1]</sup>.

In recent years, the most interesting and complete experimental data about the structure of the erythrocyte and the construction of the cytoskeleton were obtained with atomic force microscopy methods (AFM)<sup>[2-4]</sup>. The most inspiring atomic force microscopy data were obtained in<sup>[5-7]</sup> claiming the elastic coefficient (rigidity coefficient or Young modulus) of the erythrocyte membrane is normally equal to 1,4-1,7 kPa<sup>[5]</sup>, while the other source's<sup>[6]</sup> data indicates rigidity in the center and on the edge differs by 25-40%. Direct observation of the fine structure of the filament network of the erythrocyte membrane using AFM methods allowed to determine the size of the mesh as a 50-70 nm range<sup>[7]</sup>. In spite of the swab test leads the erythrocyte to drying on the air and losing over the half of its volume, the proportions of the main geometrical characteristics remain the same<sup>[3,4,8-13]</sup>.

The present paper presents a model that takes into account gathered experimental data obtained through methods of force atomic microscopy and lend mathematical support for interrelation between elasticity properties of the membrane and the form of the erythrocyte. The model is based on the following assumptions: 1) the erythrocyte is a homogenous elastic body; 2) elastic properties of the erythrocyte are dictated by rigidity of its membrane; 3) rigidity of the membrane is a differentiable function of the distance from the center of the erythrocyte. The model is chosen quite simple in order to be able to apply the finite elements method usually applied to mechanical system. In the long run, the given model will allow to create atomic force microscopy computational methods to determine elastic stress in living objects. In this paper we propose to evaluate the intracellular pressure of erythrocytes based on the calculations of the elastic properties of the erythrocyte membrane and change its morphology under intercellular pressure.

## MECHANICAL MODEL OF THE ERYTHROCYTE

For a first approximation the model takes into account the influence of rigidity of the membrane to the form and state of the erythrocyte<sup>[12]</sup>. The pivot point of the developed model is that it considers the impact of elastic properties of the erythrocyte membrane as the main factor of its form change. This impact in turn depends on membrane's cytoskeleton condition and osmotic pressure both in the erythrocyte itself and plasma. Let's switch to cylindrical coordinates and introduce a symmetry axis to the geometrical model of the erythrocytes. We denote the horizontal axis as  $\mathbf{r}$ , and the vertical axis as  $\mathbf{z}$ . In order to solve the problem with the finite elements method, let's introduce its domain of definition with axial symmetry about  $\mathbf{z}$ . The Figure 1a shows a three-dimensional model of the erythrocyte, its complete form is obtained by turning the section by 360° around  $\mathbf{z}$ . The initial data are maximum values: the initial radius of the erythrocyte  $r_0$ , and the thickness  $h = 2.0 \mu\text{m}$ . According to<sup>[5]</sup> the elastic coefficient (rigidity coefficient or Young modulus) of the erythrocyte membrane is normally

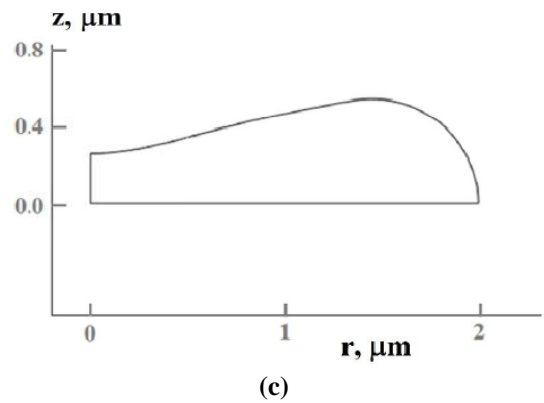
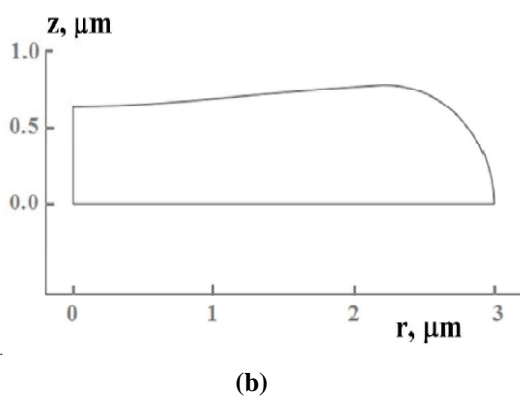
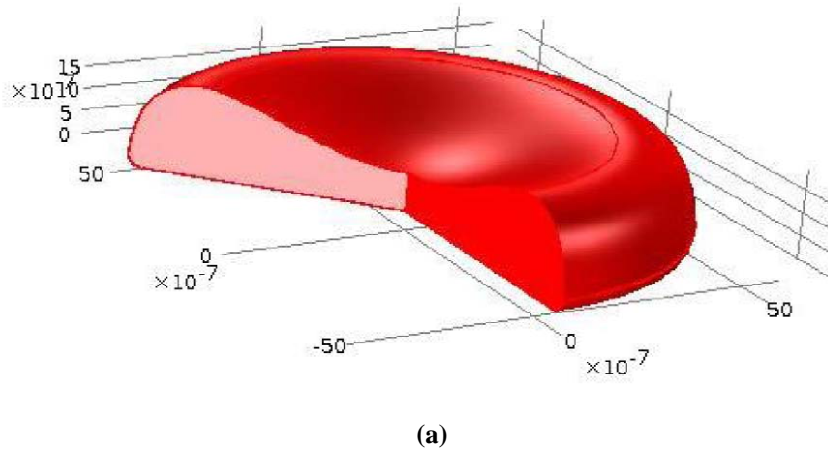
equal to 1.4-1.7 kPa, and<sup>[2,6]</sup> specifies that rigidity in the center and on the edge of the erythrocyte differs by 25-40%. To take this experimental fact into the account, we introduce a dependency of the Young modulus as follows:

$$E = E_0(2 - \exp[-(r/R)^2]),$$

where the scale factor is  $E_0 = 1000Pa$ .

Young modulus steady built up from 1000 Pa to 1632 Pa. Such change in Young modulus provided a smoothness of the erythrocyte contour in the distorted state for the linear modulus change with the sharp angle with respect to the axis. Poisson's ratio was  $\nu = 0.33$ , and the pressure to erythrocyte sides varied from 0,1 to 2 kPa.

A simulation of erythrocyte morphology was conducted using the finite elements method under different pressures to the membrane. The three-dimensional model of the erythrocyte with external pressure is presented at the Figure 1a. The initial size of the erythrocyte form was equal to 2 and 8  $\mu m$  along the z and r axes respectively. Then the model of the erythrocyte was stressed by external pressure from 500 to 2000 Pa. The computations assumed the pressure  $P$  is homogenously applied along the entire surface of the erythrocyte, so the morphology change is only caused by the internal characteristics of the erythrocyte that are factored into the model.



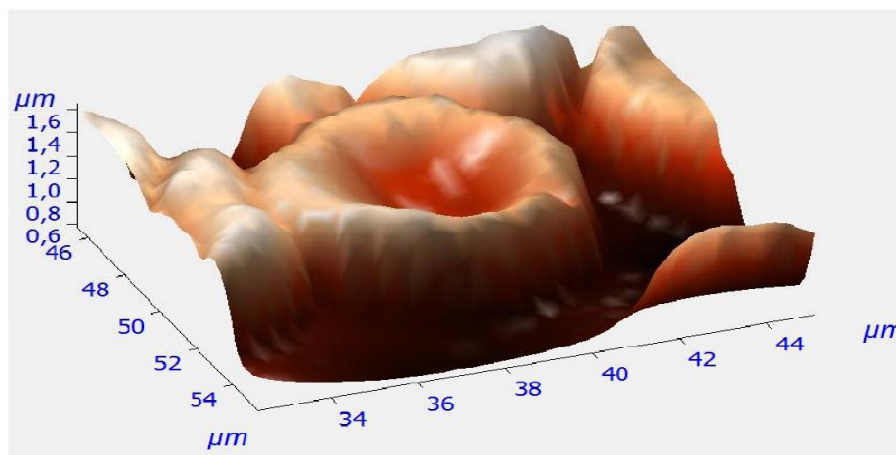
**Figure 1: Geometrical model of the erythrocyte: a) three-dimensional model of the erythrocyte under a pressure of 2000 Pa; b) cross section of the erythrocyte under external pressure of 1000 Pa; c) cross section of the erythrocyte under external pressure of 2000 Pa**

The Figure 1 (b and c) shows how the erythrocyte morphology changes depending on growth of pressure on to the membrane. We can see that if low values of pressure comparable to membrane's elasticity are applied (Figure 1b), the form of the erythrocyte almost doesn't change and is close to the initial form (Figure 1a). This form is peculiar to erythrocytes having their metabolic functions disturbed

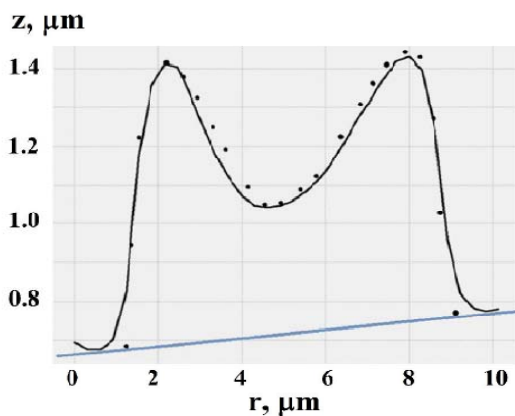
and, therefore, having low pressure difference inside and outside of the membrane. In the previous paper<sup>[13]</sup> the author solved an optimization problem for elastic energy minimum and area to volume ratio maximum. The results of computations of the optimization model were similar to results in the context of a simple mechanical model<sup>[12,13]</sup>.

## EXPERIMENTAL AND CALCULATED DATA COMPARISON

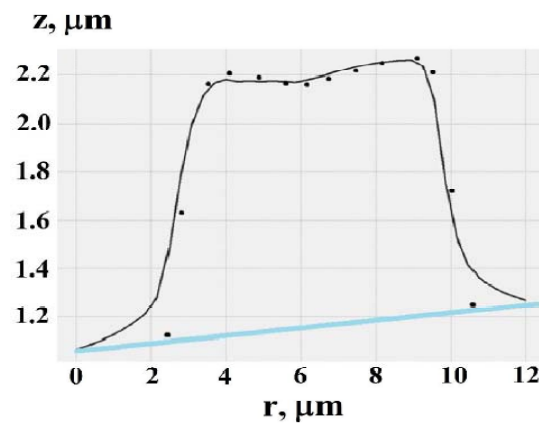
To compare experimental and calculated data we take the atomic force microscopy data for the blood film of a bronchial asthma female patient<sup>[10,12]</sup>. The scan displays both normal erythrocytes and the deformed ones, which allows comparing form and size of erythrocytes in health and disease. To determine morphological features of erythrocytes, a central cross section of the erythrocyte scan has been made along the symmetry axes (Figure 2). Sections of erythrocytes with deformed morphology are presented on Figures 2b and 2c. While determining the geometrical features of erythrocyte sections we noticed that the height and the width of normally formed biconcave erythrocytes are respectively 0.6-0.8  $\mu\text{m}$  and 8  $\mu\text{m}$ . The same parameters of morphologically deformed erythrocytes are respectively 0.8-1.1  $\mu\text{m}$  and 8-10  $\mu\text{m}$ . Therefore, these erythrocytes have changed their form and have increased the size.



(a)



(b)



(c)

**Figure 2: Erythrocytes in an atomic force microscope: a - three-dimensional image of an erythrocyte is normal, b and c - cross sections of erythrocytes with normal and the changed morphology. Lines signify experimental AFM data, dots signify the calculation results for different pressures: b –  $P=2000\text{Pa}$ , c –  $P=500\text{Pa}$ . The line below the plot indicates the slope angle of the measured sample**

These data well conform to results obtained with the Coulter method. The aforementioned patient had the average volume of erythrocytes measured by Coulter counter equal to 97 femtoliters or  $97 \mu\text{m}^3$ <sup>[10,12]</sup>. Using the atomic force microscopy the amount of erythrocytes in the air was calculated. The volume of the erythrocyte shown on the Figure 2a was  $34 \mu\text{m}^3$ , and the ones shown on Figures 2b and 2c – 38 and  $46 \mu\text{m}^3$  respectively. The volume of morphologically deformed erythrocytes was

approximately 12-35% higher than the volume of biconcave erythrocytes of the normal form, because the latter have a cavity. Therefore, drying has reduced the volume of erythrocytes 2-3 times but retained the original geometrical characteristics ratios, which according to some authors renders use of atomic force microscopy to estimate red blood cell morphology possible<sup>[9]</sup>.

Comparison of the morphology calculation results with atomic force microscopy data for суды in various states is of our main interest. Comparison of various forms of erythrocytes using atomic force microscopy data and models presented on Figures 1 and 2 should be done taking into account the change of the form caused by sedimentation of the erythrocyte. Indeed, the erythrocyte in the model and in physical solution is a symmetrical object. However, sedimentation affects the form of the erythrocyte. At first, the erythrocyte membrane sticks to the base and therefore spreads causing elongation of the form. Second, due to sticking of the membrane to the base, the upper and the lower cavities of the erythrocyte turn to a single cavity with an overall depth equal to the sum of both initial cavities' depths. Third, the swab test causes drying of the erythrocyte, which leads to reduction of the volume of the erythrocyte and its thickness 2-3 times.

Taking into account all said above, comparison of erythrocyte morphology was performed using the AFM methods and calculations. The Figure 2b displays a normal erythrocyte having cavity depth of 0.3-0.4  $\mu\text{m}$ , diameter of 7-8  $\mu\text{m}$  and width of 0.7  $\mu\text{m}$ . This form corresponds to the erythrocyte model presented on the Figure 1c having cavity depth of 0.52  $\mu\text{m}$ , diameter of 4  $\mu\text{m}$ , width of 1.05  $\mu\text{m}$ . Since the model doesn't take adhesion and sticking processes accompanying AFM investigation into account, the calculation data are different from the AFM data. Presuming that if the erythrocyte sticks and retains its volume constant, it stretches, therefore the depth and the thickness of its cavity should proportionally decrease.

The model cannot factor in all process of volume changes caused by drying, therefore we perform data normalization and only then we compare them. We reduce the width and the thickness of erythrocytes to the same units and then we recalculate the data with regard to the change in the height caused by summing up of two cavities (multiplication by 2). Then we apply these data to the experimental values obtained from AFM (Figures 2b and 2c). The calculations took into account the slope angle of the sample during the measurement as an addition of the slope line values to theoretical values. The normal form of the erythrocyte in the model implies the difference between inner and outer pressure equal to 2000 Pa (Figure 1a), while the deformed erythrocyte has this value equal to 500 Pa (Figure 2c). We can see that the normalized calculation well fits the experimental data.

We have to make few remarks regarding the model and the process of comparison of the obtained data. The erythrocyte model assumes only the elasticity of the membrane depends on the distance from the center of the cell. The erythrocyte membrane functions as a regulator for ion channels and sodium ion and other agent contents in the erythrocyte, therefore affecting the osmotic pressure inside. Hemoglobin contents in the erythrocyte also impacts the oncotic pressure and the conditions of the membrane of the erythrocyte and its morphology. Therefore, the pressure difference in the erythrocyte model reflects these values as a whole. However, erythrocytes in the air should have their osmotic pressure significantly decreased when measured by AFM methods, while the oncotic pressure should remain the same. That is why the value of the pressure difference inside and outside of the membrane in the erythrocyte model is equal to 2kPa, which is close to the oncotic pressure (0.03-0.04 atm or 3-4kPa) of blood.

## CONCLUSION

The present paper offers a model of the erythrocyte taking into account its elastic characteristics and estimating its morphology. The model represents the erythrocyte as a homogenous elastic body with the elasticity depending on the distance to the symmetry center of the erythrocyte. The computation of the elastic properties is made by the finite element method. Within a simple mechanical model a dependence of the erythrocyte morphology on pressure difference on the membrane, which varied within the range of 500-2000 Pa was built. This allowed to estimate the factors impacting the form of erythrocytes. A comparison of the calculated data with the data obtained with atomic force microscopy allowed concluding the consistency of the model. The model also allows to perform indirect estimation of the pressure difference inside and outside of the erythrocyte membrane by linking it to the oncotic pressure.

The assumption about dependence of rigidity of the membrane on the distance from the center that's took as a basis of the model on one hand is experimentally confirmed in certain papers on atomic



force microscopy<sup>[2,6]</sup>, and on the other hand has an important conclusion. Since the model indirectly confirms changes in elasticity of the membrane surface, an apparent question arises: what does cause this elasticity dependence? Taking into account that the cytoskeleton of the erythrocyte membrane consists of the filament network produced by the spectrin protein and also the fact that elastic properties of the membranes are mostly produced by binding protein band 3, we can conclude that the simulation data argue for the following assumption.

In the center of the erythrocyte membrane elastic characteristics of the erythrocyte are 1.5 times less than on the edge, which could be caused by lower concentration of the protein band 3 and by sparser mesh of the cytoskeleton in comparison with that of the edge. Atomic force microscopy allows determining the fine structure of the membrane cytoskeleton<sup>[7]</sup>. And according to these data the structure consists of mesh cells of 50-70 nm. Characterizing landscape on such a scale is a task of high complexity that still prevents to determine the degree of changes in the fine structure on the membrane surface. Therefore, within further development of the model, a problem of determining changes in the fine structure of the membrane across the erythrocyte surface as the distance from the center increases. On the other hand, there is a problem of determining changes of the protein band 3 concentration.

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