Estimation of Triboresistance of Erythrocytes during Surface Scanning with the Use of Atomic Force Microscopy

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This paper deals with the processes taking place on the surface of a sample during its scanning with the use of an atomic force microscope in the tapping mode. In this research, erythrocytes fixed on the surface of polycarbonate have been selected as the samples. Erythrocyte surface topography determines the ability of red blood cells to interact with medications. Alterations of the cell surface topography cause changes of its potential and ionic conductivity, which affects the lifetime of the red blood cell, as well as the ability of the cell to respond to environmental changes. During the process of scanning of the erythrocyte surface in the tapping mode, interaction between the probe and the surface, including friction, causes the destruction of the surface. Friction not only makes errors in the resulting image, but also can change the surface properties of the erythrocyte. Estimation of triboresistance based on so-called action parameters allows us to choose the correct scanning mode and other parameters, which helps us to avoid the destruction of the surface. In this paper, triboresistance of the surface of erythrocytes has been estimated. Based on the results of the research, scanning parameters have been chosen.

Keywords: *triboresistance, surface nanoscanning, atomic force microscopy, scanning probe microscopy, action parameters.*

1. INTRODUCTION

Nowadays, researching of processes, taking place during scanning with the use of atomic force microscopy, when the probe is touching the surface of the sample, is relevant. It is interesting from the point of surface research on micro- and nano-level, as well as from the tribological point of view.

This paper deals with erythrocytes and processes taking place during scanning their surface. The surface of erythrocytes determines their properties, including tribological ones, which make it important to research the surface. Taking into consideration the size of erythrocytes, this research can be performed only with the use of scanning probe microscopy. Erythrocytes have several different functions that are defined by properties of their surface.

2. TRIBORESISTANCE OF ERYTHROCYTES

The properties of erythrocytes membranes define the stability of their structure and their behavior during deformation. Triboresistance characterizes interaction at micro- and nano-scale between the probe and the surface of the object being scanned.

Received: April 2015, Accepted: June 2015
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doi:10.5937/fmet1503254M

2.1 Scanning probe microscopy

Scanning probe microscopy allows surface properties research at micro- and nano-scale [1].

There is scanning force microscopy (atomic force microscopy) as well as scanning tunnel microscopy.

In a scanning tunnel microscope the probe interacts with the surface with the help of constant tunnel current in a span between the probe and the surface.

In an atomic force microscope there is a force interaction between the probe and the sample.

All scanning probe microscopes have a sensor – a cantilever with a probe (Fig. 1).

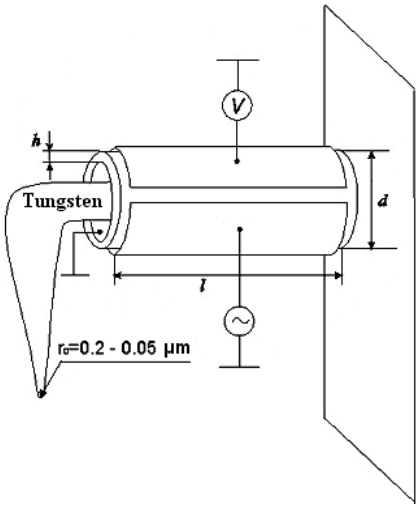

Figure 1. A sensor of a scanning probe microscope

A piezoresonance element of a tunneling current probe sensor and a force interaction sensor of scanning probe microscope represent a consolidated piezoceramic tube. A solid electrode is attached to the inner side of the tube, while two isolated electrodes are attached to the outer side. Tungsten probe is fixed in the opening of the tube.

The piezotube is fluctuated with the help of sinusoidal electric voltage applied between the solid inner electrode and one of the outer electrodes. Voltage, which is proportional to the fluctuation amplitude, is measured between the inner electrode and another outer one. A tungsten wire with diameter 100 micrometers is attached to the loose end of the tube.

The loose end of the wire is used as a probe and is chemically sharpened. The fillet radius is between 0.2 – 0.05 micrometers. The probe is electrically attached to the inner electrode, which is attached to the grounded housing. During measuring the tunneling current piezotube is used as a hard passive beam. Electrical shift is applied to the sample relatively to the grounded probe.

An atomic force microscope gets all the surface information via mechanical fluctuations of the cantilever beam or changes in piezotube fluctuation amplitude during physical contact between the probe and the sample surface.

The probe is moving consequently, line by line, along the surface (changing X and Y coordinates). However, during surface scanning it is not the surface obtained, but some potential function, describing interatomic interaction between the surface and the probe [1].

2.2 Erythrocyte

Erythrocytes or red blood cells are the most numerous blood cells. They have regular disc-shaped form. Red blood cells are thicker at edges and thinner in the center. They have biconcave disk form on the cut. Such structure helps erythrocytes to saturate with maximum amounts of oxygen and carbon dioxide while moving through the blood system. The disc surface area of a red blood cell is 1.7 times bigger than the area of the sphere with the same volume. The disc can experience moderate changes without cell membrane getting stretched. There is no doubt that the form of biconcave disc given the surface of erythrocyte gives it ability to transfer the bigger amount of substances. Moreover, this shape makes it possible for the red cells to attach themselves in fibrin network in case of formation of a blood clot. The main advantage of the shape is that it allows red blood cells to pass through the capillary. Erythrocytes can be twisted in their narrow middle part, while their contents flow from the wider end to the center. This allows red blood cells to enter a narrow capillary [2].

The main functions of red blood cells are the following: oxygen transfer from lungs to tissues, carbon dioxide transfer from tissues to lungs, nourishing and protective functions, acid-base blood balance management. Transport function is transporting of the oxygen, carbon dioxide, amino acids and so on. Red blood cells also have serious influence on specific and non-specific immunity and blood coagulability. Regulatory function is performed by red blood cells with the use of hemoglobin, which they consist of. The external boundary of erythrocytes is a plasma membrane. O_2, CO_2, Cl^- and HCO_3^- can be diffused through this membrane. Red blood cells also regulate acid-base blood balance and water metabolism [2].

This makes research of mechanical properties of erythrocytes membrane a relevant task. The main goal of this work is estimation of triboresistance during erythrocyte surface scanning.

2.3 Triboresistance

During nanoscanning, there is interaction between the probe and the sample. This interaction includes different forces: van der Waals forces, dipole-dipole interactions, electrostatic forces and friction forces [4]. The probe has its influence on the surface layers of the sample. The sample resists this influence. To estimate this resistance, in our previous paper, we have introduced triboresistance that is based on so-called action parameters [3,5,6].

The result of nanoscanning of a sample surface is a matrix of numbers. Each element of the matrix represents the height Z in the corresponding point of the surface. According to our method, the cumulative summations of all elements of the matrix should be performed in two directions: in the rows and in the columns of the matrix. When we perform the cumulative summation in the rows, we get the result in the last column of the obtained matrix. Performing the cumulative summation in the columns, we obtain the result in the last row.

After that, wavelet transform and fast Fourier transform should be performed for the obtained cumulative sums. As a result, we get the spectral energy density of the cumulative sums, which allows us to define some important parameters, such as maximum value of the energy $E_{\max}$ and the band $[f_1 : f_2]$ where the spectral energy density $E(f)$ is defined:

$$E(f) = \left|X(f)\right|^2 = \left|\int x(t)\, e^{-2\pi i f t}\, \mathrm{d}t\right|^2 . \tag{1}$$

The cumulative spectral energy density can be defined as follows:

$$\varepsilon = \int_{f_1}^{f_2} E(f)\, \mathrm{d}f . \tag{2}$$

Action parameters are defined as follows:

$$p_1 = \lg\left(\frac{\varepsilon^*}{(f_2 - f_1) f_{\max} \hbar}\right), \tag{3}$$

$$p_2 = \lg\left(\frac{E_{\max}}{f_{\max} \hbar}\right). \tag{4}$$

where $E_{\max}$ represents the energy maximum value of the spectral density of the energy, ε^* the maximum cumulative value of the spectral density of the energy, $f_{\max}$ the frequency corresponding to $E_{\max}$, $\hbar$ the Planck's constant ($\hbar = 6.62 \cdot 10^{-34}$ Js).

According to the authors' terminology, triboresistance is defined as follows:

$$t_1 = p_1 \cdot 10^{-2}, \quad (5)$$

$$t_2 = p_2 \cdot 10^{-2}. \quad (6)$$

2.4 The results of experiments

The red blood cell surface scanning has been performed with the use of the scanning probe microscope "NanoEducator" in tapping mode with initial amplitude of the oscillations of the probe.

The results of scanning are shown in Figure 2.

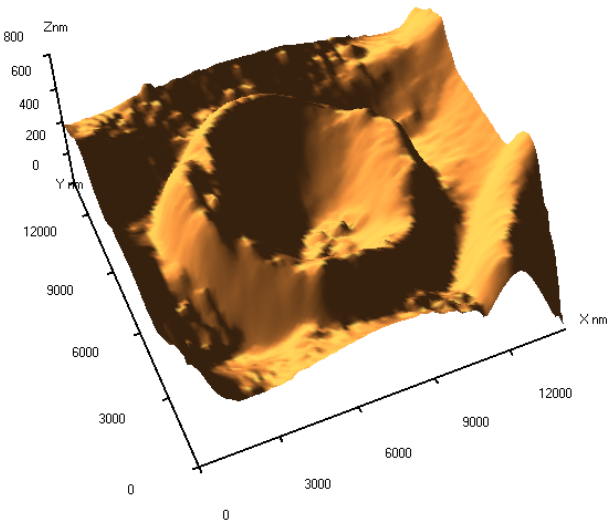

Figure 2. The surface of the erythrocyte scanned with initial amplitude of the oscillations of the probe

Calculations described in section 2.3 have been performed. The results are shown in Table 1.

Table 1. Triboresistance of the erythrocyte scanned with initial amplitude of the oscillations of the probe

Triboresistance	
In the rows	In the columns
$t_1 = 0.212$ $t_2 = 0.220$	$t_1 = 0.165$ $t_2 = 0.173$

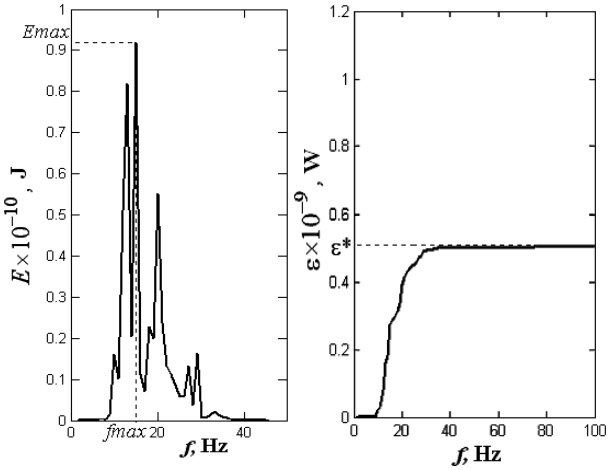

Figure 3. Spectral energy density of the cumulative sum in the rows (left) and cumulative spectral energy density in the rows (right)

Spectral energy density of the cumulative sum in the rows and cumulative spectral energy density in the rows are shown in Figure 3.

Spectral energy density of the cumulative sum in the columns and cumulative spectral energy density in the columns are shown in Figure 4.

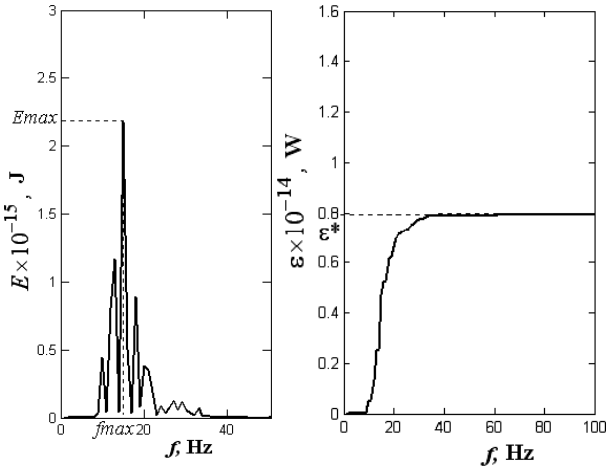

Figure 4. Spectral energy density of the cumulative sum in the columns (left) and cumulative spectral energy density in the columns (right)

Based on the results of triboresistance estimation, amplitude of the oscillations of the probe has been decreased in order to decrease deformation of the surface. The result of scanning is shown in Figure 5.

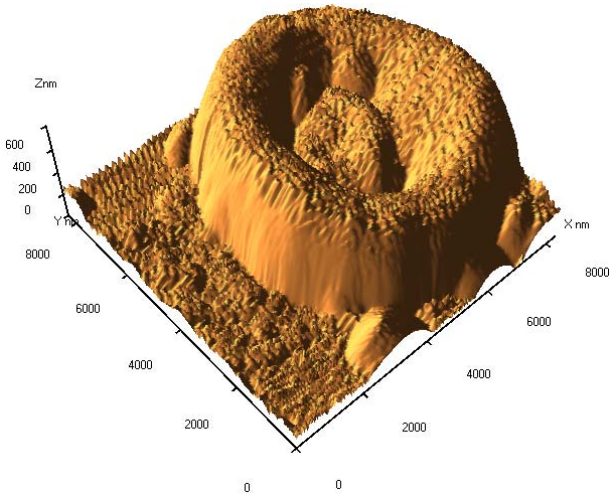

Figure 5. The surface of the erythrocyte scanned with corrected amplitude of the oscillations of the probe

The same calculations from section 2.3 have been performed for the obtained data. The results are shown in Figures 6 and 7 and Table 2.

Table 2. Triboresistance of the erythrocyte scanned with corrected amplitude of the oscillations of the probe

Triboresistance	
In the rows	In the columns
$t_1 = 0.206$ $t_2 = 0.211$	$t_1 = 0.172$ $t_2 = 0.180$

Comparison of results of triboresistance estimation for both cases is shown in Table 3.

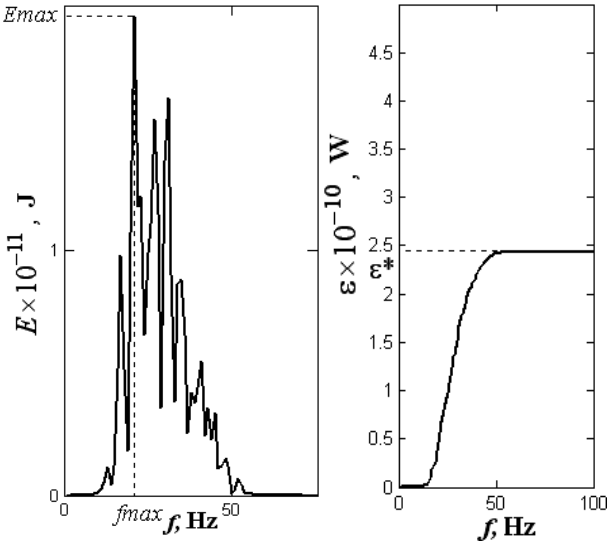

Figure 6. Spectral energy density of the cumulative sum in the rows (left) and cumulative spectral energy density in the rows (right)

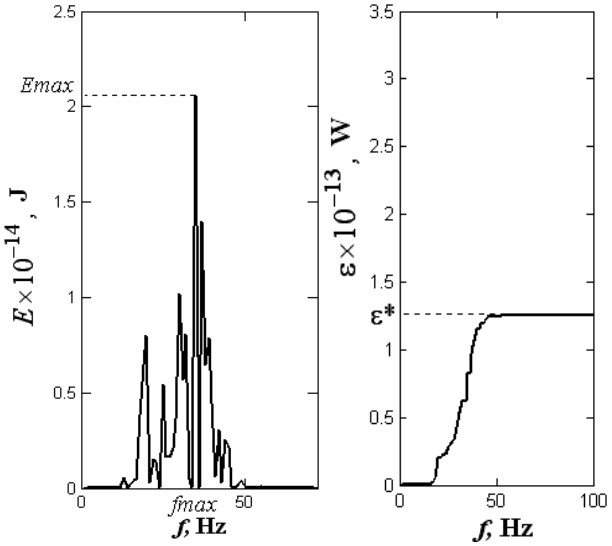

Figure 7. Spectral energy density of the cumulative sum in the columns (left) and cumulative spectral energy density in the columns (right)

Table 3. Comparison of triboresistance

The conditions of experiment	Triboresistance	
	In the rows	In the columns
Initial amplitude of the oscillations of the probe	$t_1 = 0.212$ $t_2 = 0.220$	$t_1 = 0.165$ $t_2 = 0.173$
Corrected amplitude of the oscillations of the probe	$t_1 = 0.206$ $t_2 = 0.211$	$t_1 = 0.172$ $t_2 = 0.180$

3. CONCLUSION

Experiments have shown that triboresistance during scanning in the rows has increased, which can be explained by decreased deformation of the red blood cell surface during the probe and the surface contact. This leads to decreased probability of artifact occurrence on the resulting image.

The developed technique of triboresistance estimation leads to simplification of scanning mode selection. Later, when the database of scans of biological objects in vivo and in vitro is created, this technique will allow us to determine mechanical properties of cells, which will make it possible to research elastic properties of red blood cells as well as their ability to recover their shape after passage through a narrow capillary.

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ПРОЦЕНА ТРИБООТПОРНОСТИ ЕРИТРОЦИТА ПРИ СКЕНИРАЊУ ПОВРШИНЕ ПОМОЋУ МИКРОСКОПА АТОМСКИХ СИЛА

Виктор М. Мусалимов, Павел П. Коваленко, Светлана Ј. Перепелкина

У раду су разматрани процеси који се одвијају на површини узорка при његовом скенирању, помоћу микроскопа атомских сила, у полуконтактном режиму. Као узорци за испитивање су одабрани еритроцити (црвена крвна зрнаца) који су били причвршћени за површину поликарбонатне основе. Топографија површине црвених крвних зрнаца одређује њихову способност да реагују на лекове. Промена топографије површине зрнца проузрокује промену његовог потенцијала и јонске

проводљивости, што утиче на век трајања црвених крвних зрнаца, као и на њихову способност да одговоре на промене у окружењу. При скенирању еритроцита у полуконтактном режиму долази до интеракције између сонде и површине еритроцита, укључујући и појаву трења, што проузрокује разарање површине. Појава трења не само да ствара грешке у добијеној слици, већ може да промени и површинске карактеристике еритроцита. Процена трибоотпорности на основу такозваних акционих параметара омогућава избор одговарајућег режима и осталих параметара скенирања, што помаже да се избегне разарање површине при скенирању. У раду је процењена трибоотпорност површине еритроцита, а на основу резултата испитивања су изабрани одговарајући параметри скенирања.