Evaluation of Morphometric Parameters of Native Blood Cells by Atomic Force Microscopy

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We propose and tested a method for studies of native blood cells by atomic-force microscopy in a humid chamber preserving viability, size, and shape of biological objects. The method has some advantages over scanning in a liquid cell: it allows studying non-fixed blood samples in the form of suspension of live cells and excludes mechanical and chemical influences on the cells.

Key Words: *native cell; morphometric parameters; humid chamber; semicontact atomic force microscopy*

In modern probe microscopy, various methods of scanning of blood cells using atomic force microscope are used. Two methods of examination of blood cells based on routine preparing of blood smears are known [5]: application of the suspension of fixed blood cells onto slides followed by their scanning in air medium [3] and scanning in liquid cells by atomic force microscopy (AFM) in contact and tapping modes. Drawbacks of these methods are examination of dead fixed cells treated with chemicals distorting their morphometric parameters. Long-term process of sample processing and modification of cell surface with Hanks saline containing Ca^{2+} ions are associated with the risk of artificial changes of cell shape.

Liquid cells used in probe microscopy do not yield objective results, because sample preparation requires firm adhesion of the cells to the surface. Treatment of cell surfaces with chemical agents (polylysine, Ca^{2+} ions, *etc*.) induces destruction or activation of cell structures and modification of their shape. Moreover, blood cells can be pushed off the substrate with probe tip or can stick to the cantilever. AFM images obtained during scanning in liquid cell have low spatial resolution [6] and hence, do not allow evaluation of the character of cell membrane damage. This hampers objective evaluation of cell shape and size. The use of the proposed method for examination of native cells provides the possibility of studying intravital geometric parameters of the object without its exposure to modifying agents.

Morphometric parameters of anucleate cells (human erythrocytes) were evaluated using AFM. For preparing cell suspension, the blood was collected without heparin. The cells were suspended in physiological saline, a drop of the suspension was placed on a clean degreased slide. For preserving cell nativity, the preparations were placed into a humid chamber saturated with water vapors and closed with a membrane with a hole for the probe. Scanning in an atmosphere saturated with water vapors makes it possible to create optimal microenvironment for the cells and to maintain their viability. Morphometric parameters of cells were studied using an Iintegra Vita NT-MDT scanning probe microscope (configuration on the basis of Olympus IX-71 inverted light microscope). AFM images were obtained in a tapped mode. For minimization of the biological object drag effect caused by the presence of a lateral component, we used scan frequency of 0.6-0.8 Hz. Scanning was performed with NSG03 (NT-MDT) silicon probes with tip radius of 10 nm and spring constant of 1.1 N/m. AFM measurements were performed on at least 30 cells from each blood sample. The number of scanned cells was limited by the lifetime of native state of erythrocytes.

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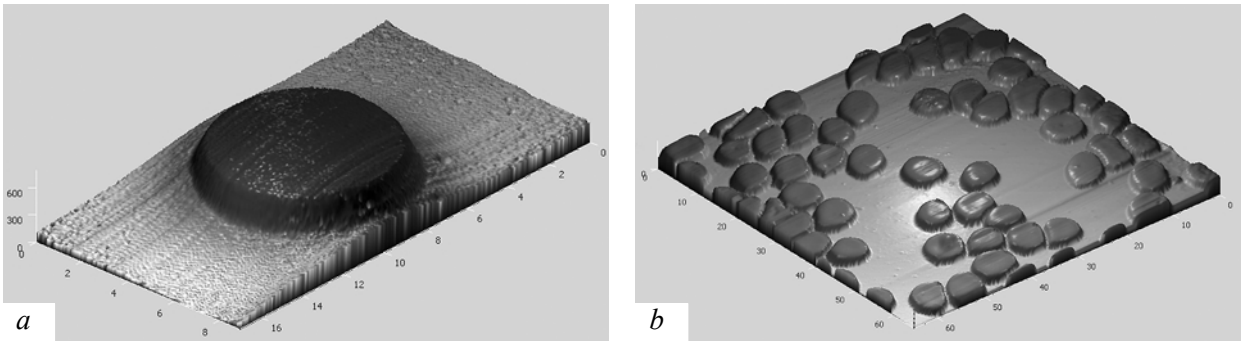

Fig. 1. AFM images of human erythrocytes. *a*) native cells; *b*) cells fixed with methanol and scanned in the liquid cell.

The duration of scanning for each cell was about 30 sec, the total time for analysis of one suspension drop did not exceed 2-3 min.

For obtaining objective parameters, we compared geometrical parameters of blood cells scanned in a humid chamber and cells fixed in methanol and scanned in a liquid cell. The preparations of samples for liquid cell included routine blood smear processing. For adhesion of cells to the substrate, the smear was fixed for 2-3 min in methanol and placed into the liquid cells of atomic-force microscope filled with physiological saline. Scanning was performed on a water bath using a NSG03 cantilever with beam length of 100 μ and spring constant of 1.1 N/m. Scans of 30 cells were obtained and used for measuring geometrical parameters of cells.

The data were processed statistically using Student *t* test.

Scanning yielded images of single native human erythrocytes in the humid chamber (Fig. 1, *a*) and erythrocytes fixed with methanol and scanned in the liquid cells (Fig. 1, *b*). The cell structure was different: native cells had rough surface, while fixed erythrocytes had smoothened surface relief. Moreover, the scans obtained in the liquid medium were characterized by low spatial resolution, so that the character of changes on the cell surface cannot be observed.

Analysis of morphometric parameters of human erythrocytes revealed an increase in the volume and height of methanol-fixed cells by 49 and 45.8% ($p<0.05$) compared to native cells (Table 1).

The observed differences in the height and volume of cells scanned in the liquid cell compared to native cells are related to modifying effect of fixatives enhancing cell adhesion to the substrate and low resolution of scans obtained in the liquid cell. Fixation of cells with methanol induces conformation changes in solid elastic protein backbone of the cell membrane [2,4,7].

Reproducibility of the results in different experiments with the same blood sample was evaluated by mean square deviations of the compared rows of variables. For evaluation of reliability of the obtained results, confidence probability was calculated by common methods [1]. Calculations showed that for confidence intervals not exceeding one mean square deviation ($\pm\sigma$) of the measured physical parameter the confidence probability for all N objects varied within the interval of 0.85-0.95. This confidence probability attests to significant reliability and accuracy of measurements of morphometric parameters. For verification of the conformity of the experimental data to normal distribution, asymmetry and excess of the distribution were analyzed. They were equal to zero, which confirmed normal distribution of experimental data.

The method of evaluation of morphometric parameters of native cells by tapping-mode AFM developed by us provides objective data on their size and shape due to the use of the humid chamber creating optimal conditions for the maintenance of native shape of cells. Moreover, this method excludes modifying effects of anticoagulants and substances enhancing cell

TABLE 1. Morphometric Parameters of Human Erythrocytes Obtained by AFM (*M±m*)

Scanning conditions	Morphometric parameters of cells		
	diameter, μ	cell volume, μ^3	cell height, μ
Native cells	7.396±0.140	31.96±1.78	0.483±0.020
Cells fixed with methanol and scanned in the liquid cell	7.139±0.070	62.750±2.010*	0.892±0.020*

Note. *$p<0.05$ compared to native cells.

adhesion to the substrate and mechanical influences on the cell surface during sample processing. The method can be used in general and clinical physiology for all living organisms and biological objects.

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