Investigation of $CdCl_2$ Influence on Red Blood Cell Morphology

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ABSTRACT

The authors studied the main methods of qualitative morphology evaluation and abnormal shapes visualization of red blood cells (RBC). We made the comparison of known sample preparation methods and selected the optimal one, which had no significant impact on the change in morphological features of cells. The purpose of the article is the formation of physiologically and morphologically abnormal RBC and its investigation by atomic force microscopy (AFM). It is established that AFM is an important tool to visualize the abnormal forms of RBC, especially with the usage of Nova PX software, which allows obtaining a more complete picture of the dimensional characteristics and parameters of RBC. In conclusion, the possibility and prospects of using AFM in studies of morphology and visualization of abnormal forms of RBC are describe.

Key Words: *AFM, RBC, morphology, heavy metals.*

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INTRODUCTION

Today atomic force microscopy (AFM) is one of the most promising methods of studying the structural features of macromolecules, because it allows to obtain images of objects with high resolution, comparable to the level of X-ray analysis, in conditions in which macromolecules are not subjected to rigid processing and show their natural activity [1-3]. It is also a powerful tool for determination of morphology [4], examination of the particle size [5, 6] and surface organization of the synthesized materials [7]. AFM also allows studying the properties of individual macromolecules including the distribution of surface charges, the mobility of individual sites, conformational changes depending on the conditions, and the strength of the specific interaction between molecules [8-10].

Recently, the most interesting and complete experimental data on the structure of the erythrocyte and the cytoskeleton were obtained by AFM [11, 12]. In 2013, the calculation of erythrocyte volume was carried out according to the measurement of the obtained membrane

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surface scan, and then a diagonal section of the erythrocyte scan was made to measure its height geometric characteristics [13].

AFM visualizes microobjects with high spatial resolution and can be used for local micromechanical tests [14-16]. Skorkina et al. [14] and Ebner et al. [15] showed that AFM, being a modern nanotechnology tool for measuring the local elasticity of cell membranes, can be used to determine the young people's modulus and surface potential of RBC, RBC-containing solutions and other blood components. A statistically significant correlation was found between the increase of young's modulus and surface potential of RBC, accompanied by shape-changing to echinocytes and spheroechinocytes after long-term storage of RBC-containing solutions for 35 days at standard temperature conditions of +4 °C [17]. Lobov et al. [16] considered three ways of preparing RBC for the AFM including without fixation of cells and fixation in glutaraldehyde solutions with concentrations of 0.5 and 2.5 %, with the aim of identifying the most suitable method to obtain high-quality AFM images of blood cells.

MATERIAL AND METHODS

Wistar rat's RBC were used for the experiment. Without any influence on RBC's shape (method - [18]) was produced deposition from suspension on cover glasses, which were pretreated with 1% glutaraldehyde. Initial investigations helped to determine the optimal condition of glasses' covering that was 2.5% glutaraldehyde prepared in 20 mm Hepes buffer and a Ringer-Locke solution. Glutaraldehyde reduces pH and has a significant effect on RBC with the formation of bonds between the molecules of cell membranes and organelle membranes. It produces a strong single network due to the "crosslinking" of mainly cellular proteins [19, 20].

Preparation of objects for AFM scanning.

Dry preparations were prepared from 50 µl of RBC with 1950 µl of 10 µg/l $CdCl_2$ solution. Prepared samples were placed in a shaker-thermostat (1 hour, 300 rpm, and 37 °C). After thermoshaking, the experimental samples were centrifuged, the incubation solution was drained and 1 ml of fixing solution was added. The resulting composition was applied to the cover glass and evenly distributed over the surface. The preparation was dried on air for 20 minutes at +22 °C before AFM scanning.

Atomic force microscopy

Scanning was carried out by AFM "NT-MDT" (Zelenograd, Russia), model "Ntegra Life", in the laboratory of Nanobiotechnology and Biophysics, the North-Caucasus Federal University (Stavropol, Russia). The cantilever model was HA_NC (Etalon) side B, with a radius of curvature < 10 nm, and the length of the beam of 124 µm. Data analyzing was performed with specialized software "Nova". A semi-contact method with a generated frequency of 153 kHz was used. The scanned area of the sample was 9.2 × 9.2 microns.

RESULTS AND DISCUSSION

AFM scanning of the cover glass surface with fixed erythrocytes showed images of RBC with physiologically abnormal, and different shapes, morphological features, and geometrical sizes. Imaged RBC differed from the discoid biconcave shape, which is most likely due to the interaction of heavy metal salts with the cell membrane.

Optical microscopy image of RBC is shown in Fig. 1, which also had morphologically abnormal cells. The surface of RBC clearly revealed contrast spots (marked with arrows), which are "columnar" outgrowths. It was not possible to reliably visualize the dimensional parameters of these formations using optical microscopy methods, that is why we decided to use the AFM method. Fig. 2 shows the same sample that was subsequently scanned by AFM.

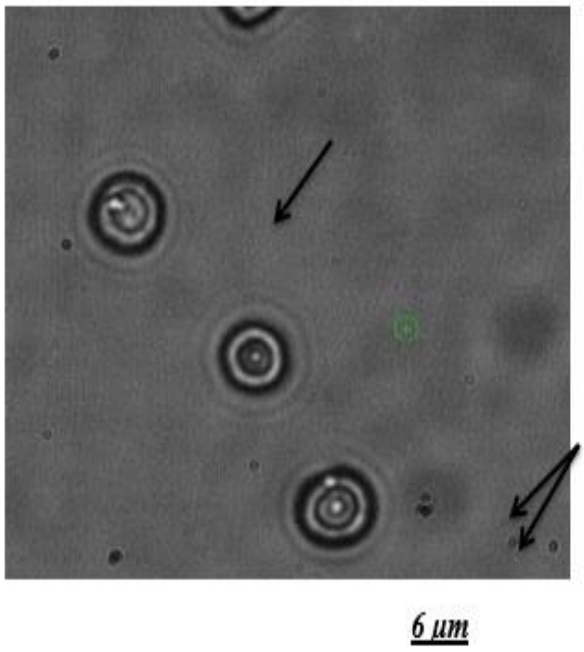

Fig. 1. Red blood cells under a microscope.

AFM scanning of the experimental sample obtained a 3-dimensional image of a single RBC. According to Fig. 2, the area of the depression clearly revealed the presence of hilly shape "fused outgrowths", contrasting sharply with respect to the surface of the sample. The ability to build a 3-dimensional picture of the image allowed us to clearly observe the shape and parameters of unusual RBC. Characterization of the size and other geometrical parameters was done by the usage of Nova PX software.

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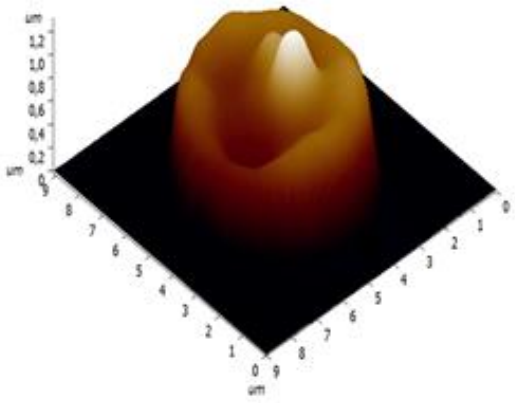
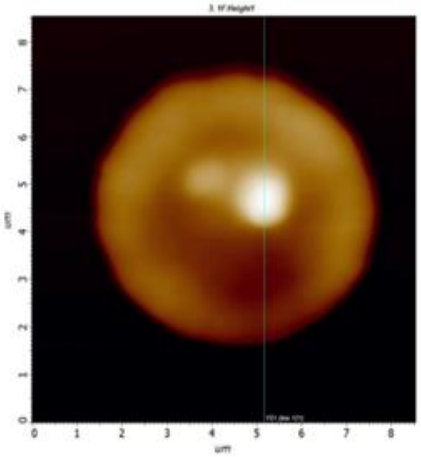
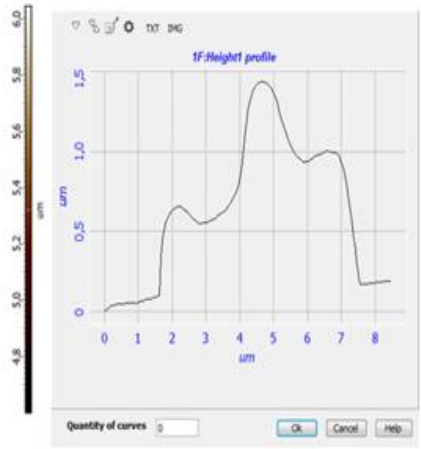


Fig. 2. 3D image of abnormal erythrocyte cells obtained by AFM (scanned in the air).

Using the possibility of constructing a cross-section profile of the studied object, such morphological characteristics as horizontal and vertical cross-section profiles were studied, which clearly demonstrated the shape, size, and height of the abnormal blood cells.



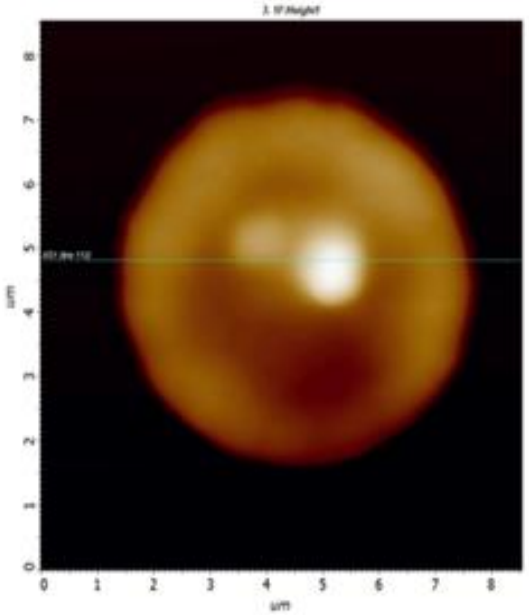
(A)



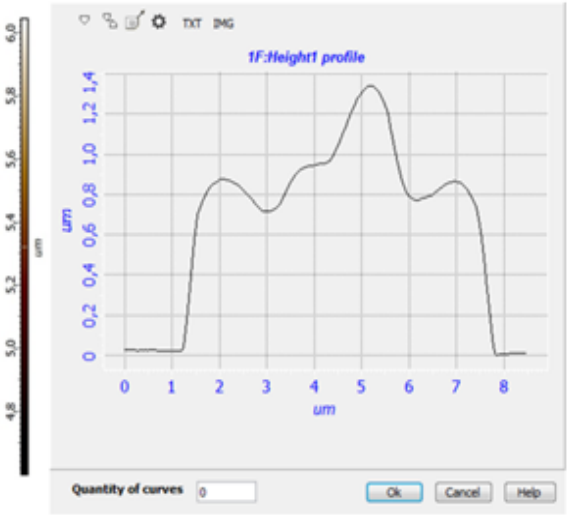
(B)

Fig. 3. AFM image of a red blood cell: a) 2D image with the projection of the vertical profile section; b) A graphic representation of the vertical profile section.

According to the AFM (Fig. 3-4), it is possible to determine not only the size of the object but also the size of the components that are part of or in interaction with the test sample. Thus, the width (diameter) of the studied RBC sample was within 6-6.5 microns, and the height of this abnormal form of blood cells was about 1.5 microns. The outlines of the volume of the cell itself, as well as the shape and location of the "outgrowths" in the central part of the erythrocyte cavity were clearly visible.



(A)



(B)

Fig. 4. AFM image of the erythrocyte: a) 2D image with the projection of the horizontal profile section; b) A graphic representation of the profile of the horizontal section.

A comparison of AFM images of all samples (figure 5), showed how the native biconcave form of RBC (control sample) changed to an uncharacteristic biconvex form reaching a peak of 1.5 µm (prototype E).

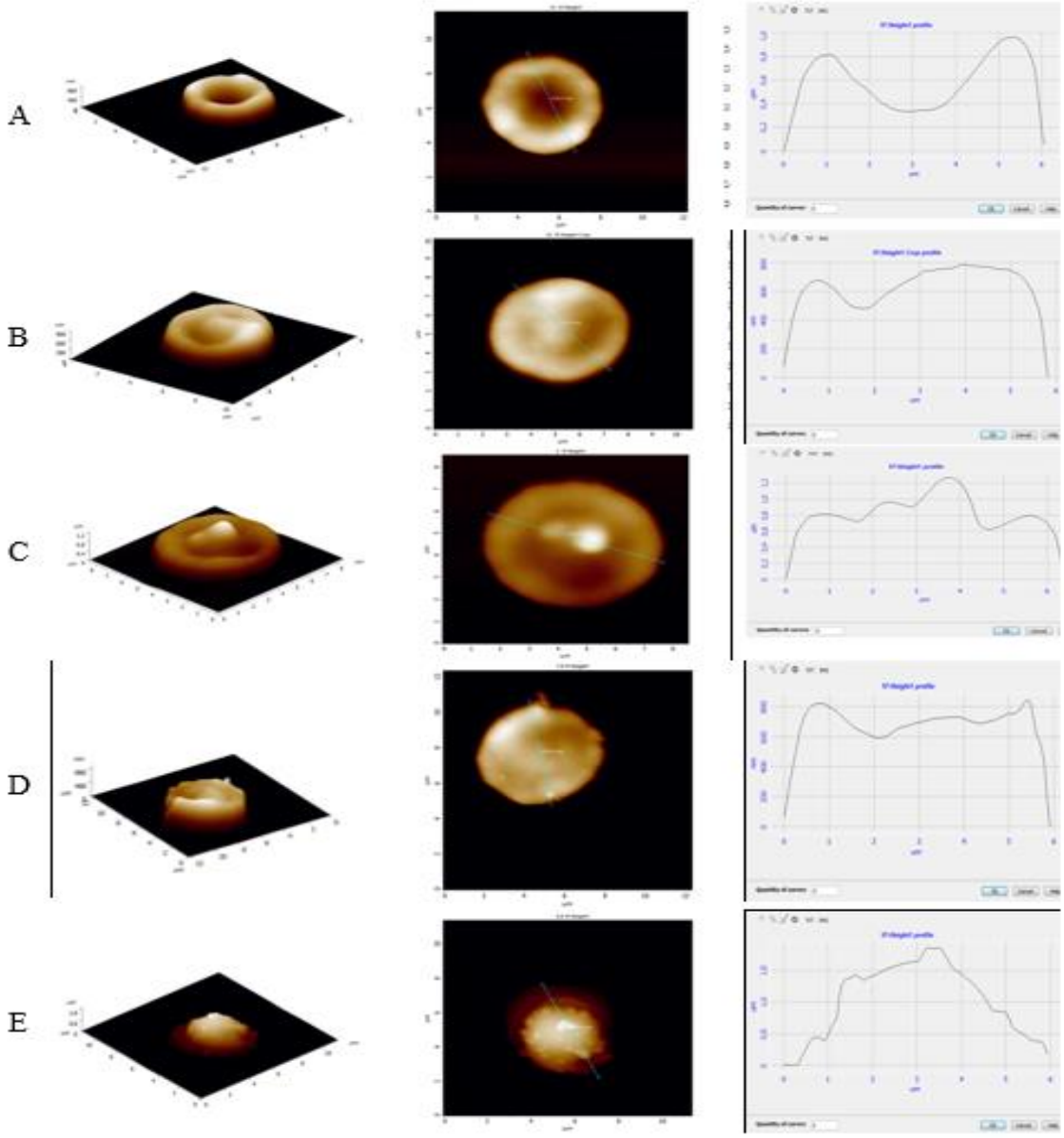


Fig. 5. AFM of control and experimental samples of RBC

Table 1 shows the results of the statistical calculation of the detected abnormal RBC shapes. According to the data, the increase of $CdCl_2$ content affects the number of deformed shapes of RBC. However, analysis of the effect of $CdCl_2$ content on the number of complete destructed cells did not show a direct relationship. Thus, at a concentration of 100 µg/l, the number of destroyed cells was 37, and at 1000 µg/l it was 14, even 2 times less than 10 µg/l $CdCl_2$-treated samples. This dynamics is not described by standard equations of probabilistic expectation and requires more studies to determine a certain trend.

Table 1. Analysis of RBC morphology deformation in control and experimental groups.

Sample	$CdCl_2$ content (µg/l)	Damaged (over 50%) or destroyed	Deformed but intact	% of all pathological forms of normal cells
A	0	2	6	8%
B	1	19	48	67%
C	10	28	52	80%
D	100	37	58	95%
E	1000	14	84	98%

Thus, generally, the experiment showed that traditionally used methods of visualization of objects in the micrometer range (mainly optical microscopy) can not accurately characterize their morphological and parametric characteristics. We can assume the prospects of using AFM methods in the study of biological objects of small size.

CONCLUSION

The results showed that simple methods of sample preparation allow us to carry out AFM scanning with producing images of individual objects with detailed morphological parameters and use it for qualitative assessment of the morphofunctional activity of cells. AFM images of RBC have significantly higher resolution than images of cells obtained by light microscopy.

It is necessary to take into account that some dimensional characteristics may differ from the reference values, both in a larger and in a smaller direction. Because, the test sample was exposed to heavy metal salts and has an abnormal shape, and also due to possible errors associated with sample preparation. But, the fact that it is possible to study the object and visualize unusual changes in shape and volume, compared with the methods of light microscopy, can not cause any doubt.

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