RESEARCH OF ERYTHROCYTES MEMBRANES CHANGE BY LASER INTERFERENCE MICROSCOPY

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Abstract. The possibility of using laser interference microscopy to explore the morphological and functional state of erythrocytes was studied. The possibilities of this method based on the rapid determination of the structure and physiological state of erythrocytes were shown. The analysis of erythrocytes by laser interference microscopy showed that erythrocytes have a typical biconcave discocyte shape. On erythrocytes' surface, there is a slight heterogeneity due to the presence of membrane-bound proteins. The impact on erythrocytes of stress hormones caused changes in erythrocyte surface that were different from physiologically normal. Numerous loosening of the structure appeared on cell surface during erythrocyte incubation with cortisol. Incubation of cells with adrenaline caused a greater effect of erythrocyte membranes deformation which was expressed by the appearance of convex seals and spicules on the surface. The molecular mechanisms of membrane modifications arising under the action of adrenaline and cortisol were discussed. The results of the work may be interesting both for basic research of erythrocyte properties and for practical medicine.

Keywords: laser interference microscopy, erythrocytes, adrenaline, cortisol, morphology.

List of Abbreviations

μm – micrometer; nm – nanometer

Introduction

The properties and condition of erythrocytes are always in the focus of attention of scientists and clinicians so far as erythrocytes not only carry oxygen and carbon dioxide, but are able to bind and transport various substances non-specifically (Kuryanova, Tryasuchev, 2019). Erythrocytes provide suspension stability of blood, play an important role in the processes of adsorption and intercellular communication (Kozinets, 2002; Novitsky, 2004; Boyarinov et al., 2016). The properties of erythrocytes (deformability, osmotic resistance, ability to aggregate and agglutinate) change under the influence of regulatory factors (mediators and hormones) (Antipenko et al., 2017).

In this regard, there is a need for the development of adequate methods for assessing the functional activity of erythrocytes, the introduction of new methods of obtaining information about the physiological state of cells in general and under conditions of changes in homeostasis. Traditional erythrocytes analysis methods, such as electron microscopy and atomic force microscopy require cell fixation. This leads to a certain change in the true size of the cells and significantly complicates the study of erythrocytes in dynamics. One of the most effective modern highly informative methods for determining the state of biological objects is laser interference microscopy. This method allows you to measure the elasticity, roughness and rigidity of the cell surface and get a truly three-dimensional relief of the investigated surface. Interference microscopy has a high axial resolution as well as measurement accuracy and sensitivity, compared to conventional microscopy. The resolution limit of the laser interference microscope is 0.1 nm vertically and 10–100 nm in the plane of the object which allows us to solve the problems of monitoring the microstructure of reflective and transparent objects. Aim: the study by laser interference microscopy of erythrocyte membranes surface structure.

Materials and Methods

The object of the study was erythrocytes of human peripheral blood. The study was conducted with the permission of the local ethics committee at the Lobachevsky State University. Donor informed consent to participate in the study obtained.

Blood was taken in the morning on an empty stomach from the ulnar vein into vacuum tubes after which an erythrocyte suspension was obtained from venous blood.

Erythrocyte interference microscopy was performed by cell modification in vitro experiments. Several series of 20 experiments were carried out. The erythrocytes were preincubated with adrenaline (1×10^{-9} g/ml) for 15 min (1 series) and cortisol (5×10^{-7} g/ml) for 30 min (2 series).

Structural changes in red blood cell membranes were studied by laser interference microscopy (LIM) using a MII-340 laser interference microscope (Yekaterinburg, Russia) with a 30x objective (NA = 0.65), laser λ = 650 nm. To capture images, a VS-415U CCD video camera (NPK Videoscan, Russia) with a resolution of 782x582 pixels was used.

During the study, biological objects were placed on a mirror substrate, from which light passing through the cell is reflected. As a result, a double phase shift of the beam of a coherent light source is detected at each point of the object, and an interference image of the cell is formed using an additional wave from the same source. For the study, images of 10 sites with a monolayer arrangement of cells in the interference channel and reflected light in each sample were obtained.

The state of human red blood cells was assessed by recording the average value of the optical path difference (ORX) and the area of the phase image of the red blood cell. To obtain a reliable result, indicators were calculated using at least 100 cells from each sample.

Mathematical processing was carried out by the method of variation statistics with the calculation of the average value (M), the average error (m) and the determination of the significance of differences by the t-student criterion. Spreadsheet Microsoft Excel 2007 and program Statistica 6.0 were used for statistical processing of finding.

Results

The study showed that intact erythrocytes have the typical shape of biconcave discocytes, a slight heterogeneity due to the presence of membrane-bound proteins was distinguished on the surface and the distribution of hemoglobin is uniform (Fig. 1A).

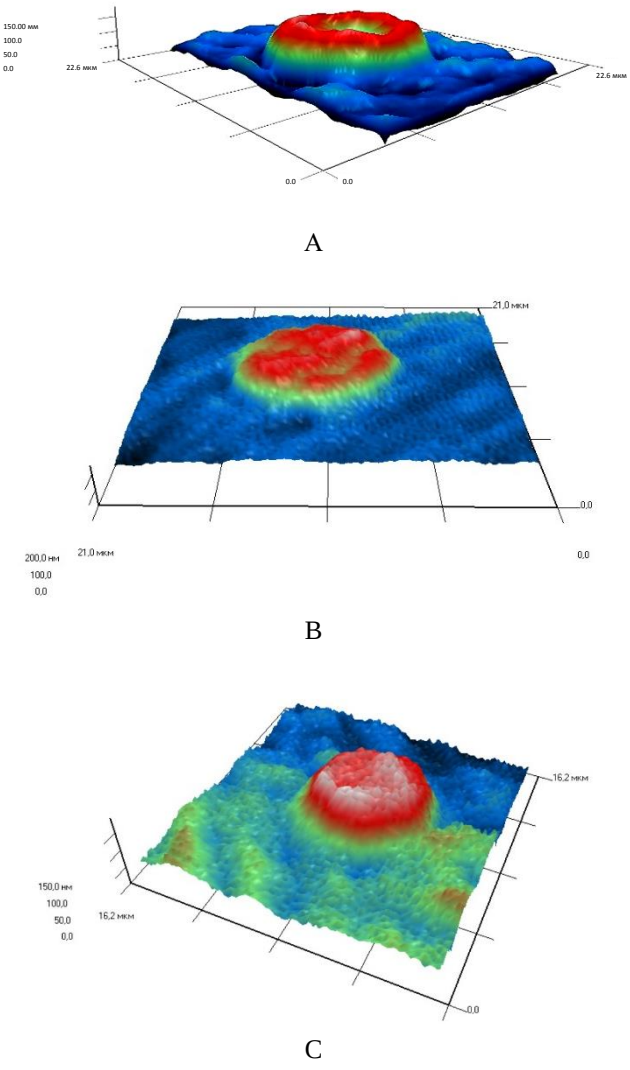


Fig. 1. 3Dphaseportrait of erythrocyte: A – intact cell, B – cell under the action of adrenaline, C – cell under the action of cortisol

The erythrocytes phase height was used to quantify the morphological and functional state of living erythrocytes. The phase height was determined as the maximum height of the profile

relative to the level of the substrate. The erythrocytes phase height was measured by the erythrocyte phase diameter.

The analysis of the erythrocytes phase height showed that after incubation of cells with adrenaline the phase height increased by 4% and after incubation with cortisol it decreased by 18% relative to intact values. The phase diameter after incubation of erythrocytes with adrenaline, cortisol significantly decreased by 2% and 3%, respectively, compared with the values of the intact group of cells (Table 1).

Table 1

The erythrocytes phase diameter (μm) and erythrocytes phase height (nm) of the studied groups in vitro

Groups	Phase diameter erythrocytes, (M ± m)	Phase height erythrocytes, (M ± m)
Intact	7,55±0,03	216,8±4,66
Adrenalin	7,39±0,06*	225,4±4,62*
Cortisol	7,37±0,03*	177,8±6,09*

Notice: * statistically different from intact group, $p < 0.05$.

The phase image of erythrocytes had a rough shape with bulges and outgrowths and sharply contrasted to the surface of the sample during incubation with adrenaline, cortisol. The cell geometry and the surface/volume ratio is changing changed, erythrocyte spherulation occurred (Fig. 1 B, C).

Upon incubation of erythrocytes with cortisol, numerous loosening of the cell surface structure appears on the cell surface (Fig. 2). Incubation of cells with adrenaline caused a greater effect of deformation of the erythrocyte membranes, which was manifested by the appearance of convex seals and spicules on the surface (Fig. 3).

Discussion

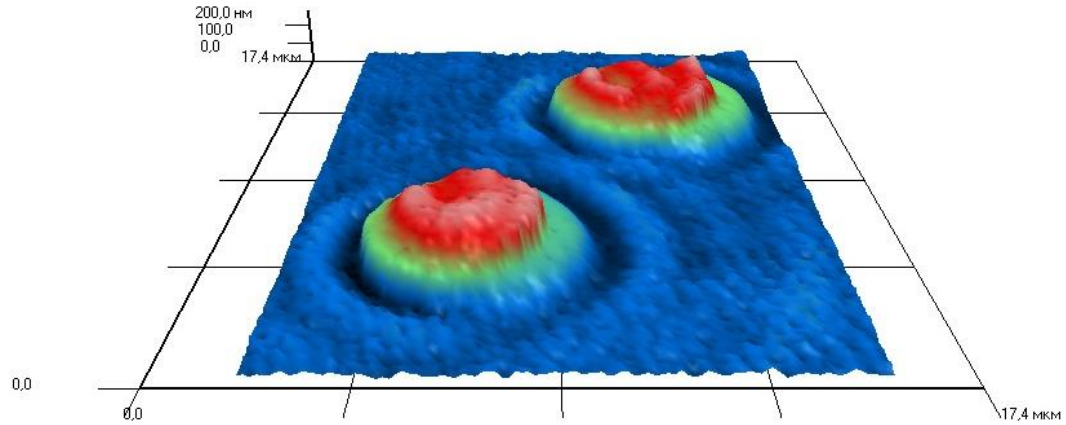
It was shown that the topography of the erythrocyte membrane nanostructure and surface roughness can be classified as independent morphological parameters of the membrane which describe changes in the structure and functional state of the membrane (Girasole et al., 2007; Girasole et al., 2010; Kozlova et al., 2012; Girasole et al., 2012). Investigation of cell surface by laser interference microscopy revealed macro- and microstructural changes. It was noted that macrostructural changes are mediated by the state of the inner surface of the membrane and microchanges indicate a surface structural change (Moroz et al., 2012; Kozlova et al., 2012). Apparently the appearance of spicules under the action of adrenaline is mediated by an increase in the concentration of hemin. It was shown that an increase in hemin during oxidative stress leads to the formation of spicules and the formation of echinocytes and spheroechinocytes (Sergunova et al., 2016).

The observed effects are probably caused by the adrenaline oversaturation of hormonal receptors with subsequent modification of the properties of the membranes and the functional state of the cells (Golenda et al., 1996). It is also likely that the detected changes during the incubation of cells with adrenaline are partially mediated through the activation of free radical oxidation, the intensification of lipid peroxidation processes entails a violation of the structure and properties of erythrocyte membranes (Deryugina et al., 2019b).

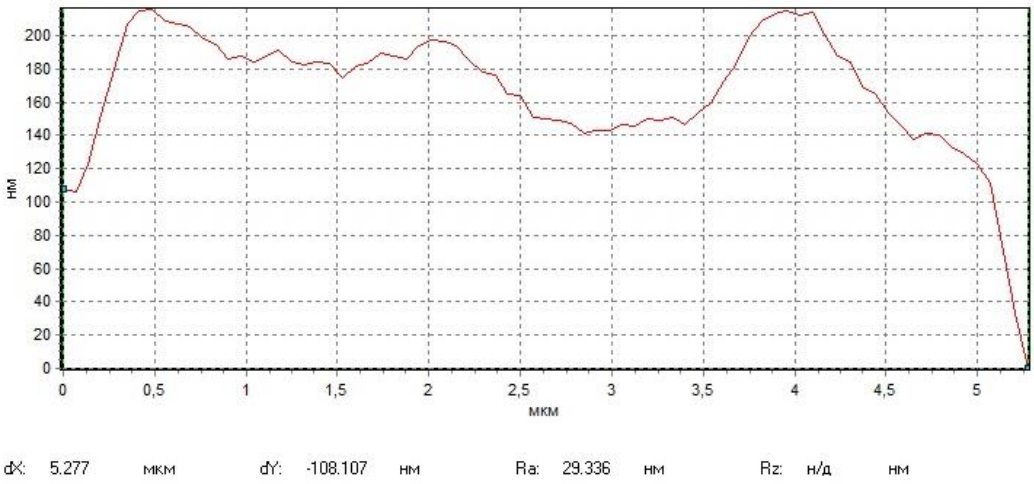
Under the action of steroid hormones on erythrocyte membranes, the mechanisms of their structural self-organization change, the active groups of the hormone interact with CO- and NH-groups of proteins, and membrane phospholipids. This leads to the formation of complex protein-lipid domains in the membrane, displacement of the water dipole from the domains and, as a result, loosening of the membrane (Panin et al., 2011).

It was shown that the change in roughness correlates well with the functional state of the cell (Girasole et al., 2007; Girasole et al., 2012).

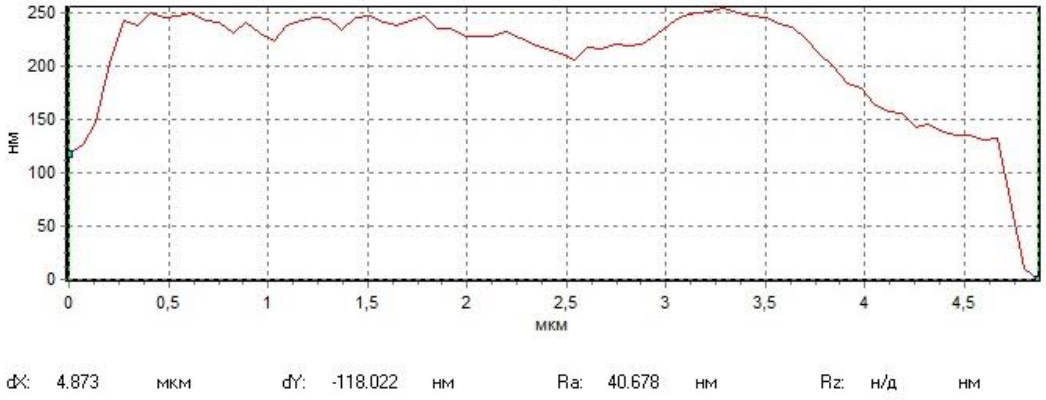
Thus adrenaline determines the urgent activation of the organism under stress and causes an increase in glucocorticoids which exhibit an adaptive role in stressful situations (Deryugina et al., 2019a). Adrenaline stimulates oxidative processes and leads to macroorganization of the erythrocyte membrane. On the contrary cortisol



A

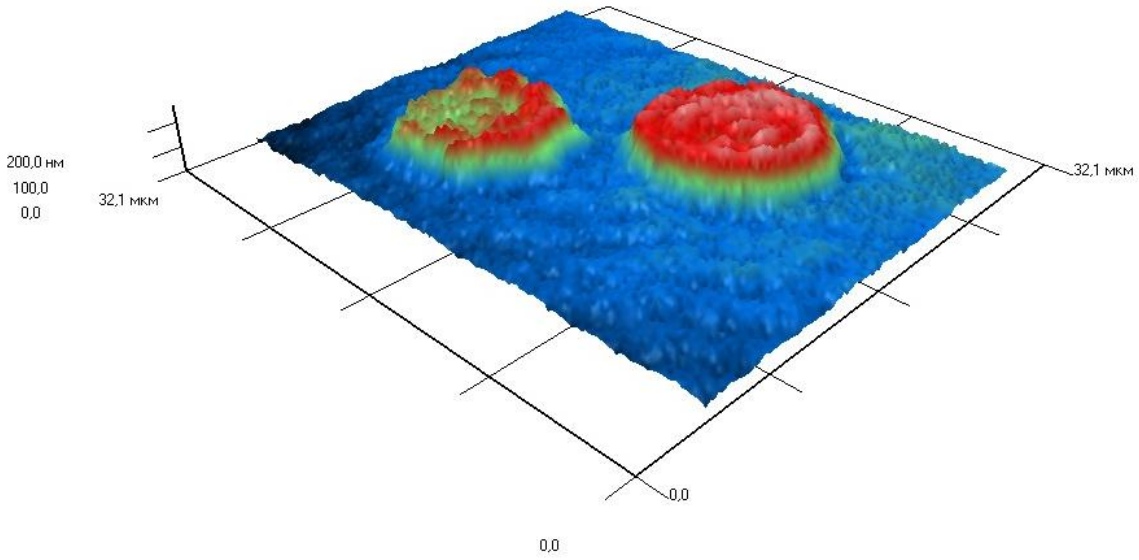


B

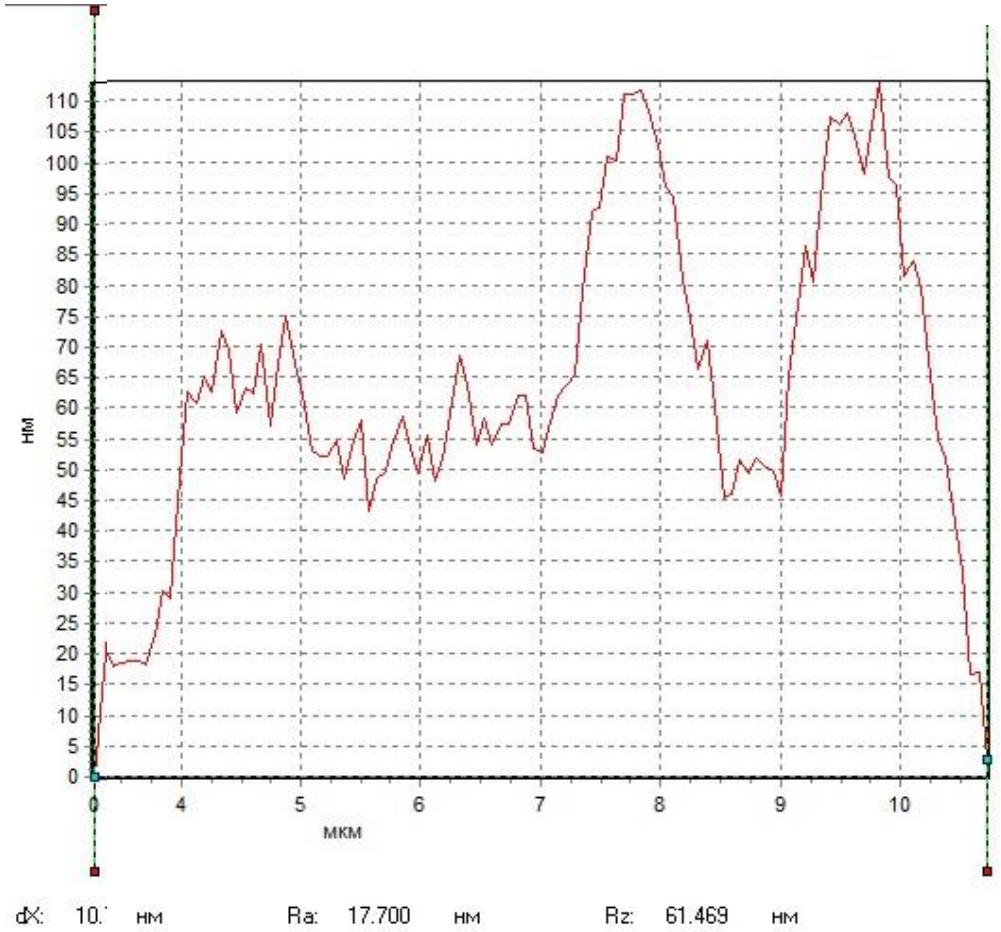


C

Fig. 2. 3Dphaseportrait (A) and phase profile of erythrocytes (B, C) during incubation with cortisol. Incubations of erythrocytes with cortisol reduced erythrocyte dual-curvature which manifested itself in dwindled curvature and depth of the central dimple as well as roughness with outgrowths and bulges appeared on the surface

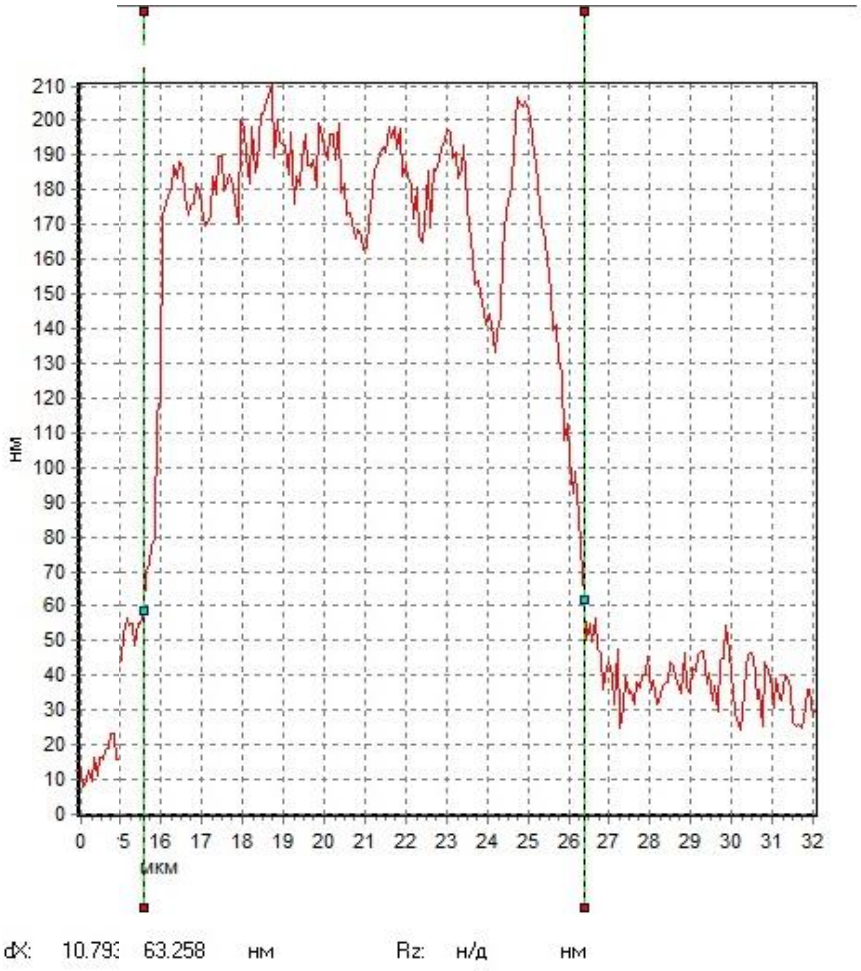


A



B

Fig. 3. 3Dphaseportrait (A) and phase profile of erythrocytes (B, C) during incubation with adrenaline. Incubation of erythrocytes with adrenaline caused greater deformation of erythrocytic membranes and the appearance of spikulas (continuation of the drawing on page 10)



C

Fig. 3. 3Dphaseportrait (A) and phase profile of erythrocytes (B, C) during incubation with adrenaline. Incubation of erythrocytes with adrenaline caused greater deformation of erythrocytic membranes and the appearance of spikulas

has an antioxidant effect and leads to less significant changes in the nanostructure and microorganization of the surface of erythrocyte membranes.

Conclusion

Laser interference microscopy is an informative method for analyzing of the erythrocyte membrane structure. The main advantage of the method is high-quality images accompanied by quantitative data on the height and diameter. Detailed morphological parameters and the use of a qualitative assessment of the morphology of erythrocytes allow you to comprehensively characterize the morphological and functional state of the cells.

Conflict of interest statement

Nothing declared.

Acknowledgements

This study was supported by the Russian Foundation for Basic Research, research project № 18-016-00195.

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