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Influence of silicon dioxide nanoparticles on mechanical properties of erythrocyte and platelet membranes estimated by atomic force microscopy

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Abstract. In this research, the influence of silicon dioxide nanoparticles on local mechanical properties of erythrocytes and platelets membranes were investigated by atomic force microscopy. It was found that adhesion force and aggregation properties (sedimentation rate of the second stage) of erythrocyte membranes decreased after incubation of cells with SiO_2 nanoparticles that could be justified by changing the charge of cell membranes.

1. Introduction

Silicon dioxide is widely used as excellent adsorbent for production of different drugs and drug carriers, and also for other applications. The research of nanoparticles' interaction with biological cells is important, because not only the drug itself affects a cell membrane, but also excipients do. Application of modern methods for nanoscale objects research allows getting detailed information about changes after different actions.

Erythrocytes are significant part of blood volume (approximately 45%). Viscosity depends on their concentration and rheological properties. There are some methods usually used to determine viscosity, e.g. viscometer in share rates, flow chambers, biomicroscopic data using tweezers and different indirect techniques [1]. One of these methods, which is perfectly and widely developed now, is atomic force microscopy (AFM) for studying local elastic modulus and adhesion force. Rheological properties of erythrocytes are estimated on the base of local mechanical properties of erythrocyte membranes. Changes of structure and mechanical properties of erythrocytes and platelets membranes are related to type of patient pathologies and different external influences. But these changes may not be obvious and cannot be identified using routing methods. S. Baar studied the effect of high temperature on osmotic properties of erythrocytes [2]. J. W. Choi analysed how to change a number of cells, haemoglobin concentration after thermo action [3].

It is known, that nanoparticles have an effect on viscosity and rheological properties of erythrocytes. For example, polymeric nanoparticles reduce the rate of sedimentation of erythrocytes, which is dilute with connecting functional groups of nanoparticle with carboxyl groups of acetyl derivatives of neuraminic acids. The last defines the charge of erythrocytes membranes, which is one of the main factors in the process of aggregation [4]. The second theory is about depleted layer with low concentration, which forms and changes the erythrocyte aggregation.

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AFM can be used to investigate the cell properties after incubation with different types of nanoparticles and temperature. AFM methods allow studying mechanical properties and structure at the nanoscale that is very perspective and promising.

2. Experimental details

The research group of patients with average age of 55 (50; 59) and type 2 diabetes mellitus (DM 2) was formed. They did not achieve compensation for HbA1c 9.1 (8.30, 10.4). Men and women were in same age, and they had DM 2 during last 5-10 years. Blood sampling was carried out from the cubital vein not earlier than 12 hours after the last meal.

Erythrocytes were separated from whole blood and stabilized with potassium ethylendiaminetetraacetatic acid dipotassium salt (K2EDTA) (Sigma-Aldrich) by centrifugation. Platelets were separated from stabilized whole blood by sodium citrate. Obtained cells precipitation was washed with phosphate buffered saline (PBS) 3 times and was added to physiological solution of silicon dioxide nanoparticles (SiO₂ NP, Sigma-Aldrich, d = 10-20 nm) with concentration C = 0.2 and 1 mg/ml. Cells were incubated with NP suspension at room temperature during 40 and 60 min. Then suspensions of cells were washed and fixed with 0.5% buffer solution of glutaraldehyde during 30 min. After that, suspension was washed twice by PBS and water precipitation with cells made a smear on mica plates. Method of preparing cells for AFM researching was described in Ref. [5].

The influence of silicon dioxide nanoparticles on structure and mechanical properties of membranes blood cells by AFM method has been studied. We used AFM device NT-206 (produced by MTM, Belarus) with standard silicon probe NSC11 of V-shaped type (produced by "Mikromasch", Estonia), stiffness 3 N/m, curves radius of probe 50 nm (for spectroscopy in point procedure) and 10 nm (for scanning procedure). Elastic modulus was calculated by Jonson-Kendall-Robertz model [6].

3. Results and discussion

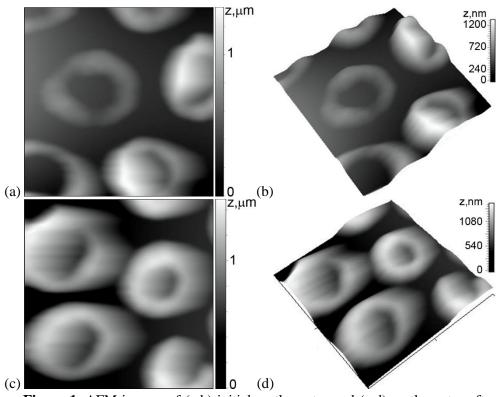
In early research, we showed that gold and polyacrylic acid [5] nanoparticles did not influence the elastic modulus of RBCs membranes; results were based on AFM data. In this research, we found that cell morphology (Fig. 1) and membrane structure did not change after incubation with NP.

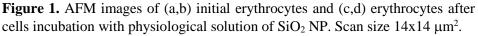
The average sizes of erythrocyte is 6.5-7 μ m. As a rule, the discocyte form of erythrocytes is prevailing in the whole population of investigated erythrocyte cells. In the case of platelets, size of cells are slightly increased from 2 to 3 μ m (Fig. 2). No other changes were observed. The value of roughness of platelets membranes also did not change.

We associate it with NP interaction with membranes of structural element and shaping uniform structure with the cell. It was confirmed that in some cases aggregation stability of RBCs was decreased in presence of NP. It is connected with NP absorbance by cells and as a result changing charge on erythrocytes surface and their aggregation.

The average elasticity modulus of initial erythrocytes membranes is $128.5 \pm 10.0\%$ MPa and for platelets it is $151.6 \pm 10.0\%$ MPa, adhesion force is $23.0 \pm 10.0\%$ and $26.0 \pm 10.0\%$ nN, respectively. After cells incubation with silicon dioxide, the elastic modulus changed within experimental error for both cell types. Changes of adhesion force are minor, however insignificant variations were found in the case of incubation of erythrocytes with NP (c= 1.0 mg/ml) during 40 minutes and reached 19.0% of initial values (Fig. 3). In the Figure 3, data of adhesion and elasticity are presented as relation values compared to initial cells membranes. More detailed calculation is presented in Ref. [5].

In our research, we analysed the aggregation properties of erythrocytes in comparison with AFM data for mechanical properties. Erythrocytes aggregation included three stages. The first and the second ones were more indicative, which allowed interpreting interaction between the NP and cell membrane in different moments. We found that at the first stage sedimentation rate changed in different ways and depended only on the biochemical state of patient's blood. At the second stage, after incubation with suspension of SiO_2 NP, the rate increased as a rule. The most significant increase was observed at the concentration 1 mg/ml (Fig. 4), which was a consequence of the increase in charge and repulsion of cells at the first stage.





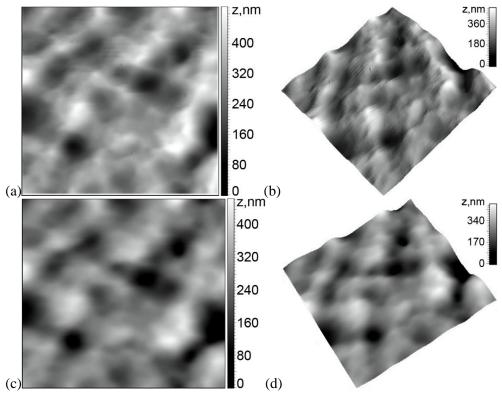


Figure 2. AFM images of (a,b) initial platelets and (c,d) platelets after cells incubation with physiological solution of SiO₂ NP. Scan size $10x10 \ \mu m^2$.

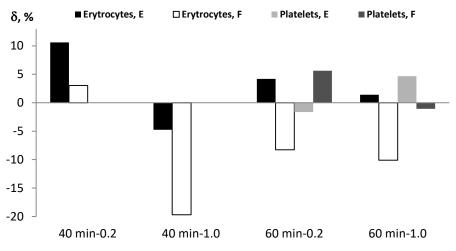
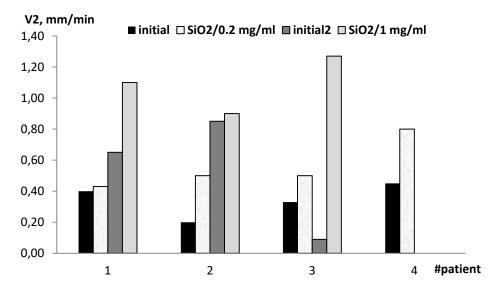
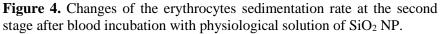


Figure 3. Relative (in percent) changes of elastic modulus (E) and adhesion force (F) of erythrocytes and platelets membranes after cells incubation with physiological solution of SiO_2 NP.





4. Conclusion

It was found that SiO_2 nanoparticles with concentration 1 mg/ml had the most significant influence on aggregation properties of erythrocytes. The obtained results are fundamental and can be used to develop methods to determine properties of blood cells under various affects and pathologies by atomic force microscopy.

Acknowledgments

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