Characterization of soft and heterogeneous surfaces by mechanical properties map obtained with atomic force microscopy

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Characterization of soft and heterogeneous surfaces by mechanical properties map obtained with atomic force microscopy

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Abstract. In this research, mechanical properties of soft materials were estimated using atomic force microscopy methods. Different regimes of the nanoindentation process were used. Surface mapping and calculation by Jonson-Kendell-Roberts model allowed obtaining elastic modulus of the platelets heterogeneous surface, minimizing experimental deviations, and identifying the difference between the studied surfaces.

1. Introduction

In biomedical studies, the atomic force microscope (AFM) is used not only to visualize the topography of biological materials and cells, but also to determine their mechanical properties. One of the tasks is to select a correct model for calculating the elastic modulus. A review of the literature data on the nanoindentation of biological cells in the liquid phase by atomic force spectroscopy showed that there was no single technique for determining the elastic characteristics of the membranes of such cells. More experimental works on determining the elastic modulus of fixed biological cells in the liquid phase are based on the simplest model of contact interaction – Hertz or Johnson – Kendell – Roberts (JKR). In the present research, we did not take into consideration different types of influences on calculation of elastic modulus. Unlike the JKR, the Hertz model does not take into account the adhesion forces. But it is valid only for a perfectly elastic sample. Both models do not take into account the contribution of the viscous friction force that occurs during deformation of not fixed cells.

The elastic-viscous nature of the sample can be taken into account when analysing the nanoindentation data on the basis of the model developed by Ting [1]. It describes interaction between the tip of an axisymmetric probe and a flat elastic viscous sample both during the approach and retraction of the device working platform. Note that the Ting model does not take into account the adhesion forces and it is difficult to address them.

The general method for determining the viscoelastic characteristics of the material is to record the stress relaxation after the predetermined or known displacement (deformation of the sample) [2]. In this study, based on the atomic force spectroscopy, a force response was measured on time after the given depth of cell indentation. Analysis of the cells viscoelastic characteristics during measurements in liquid phase was carried out according to the Hertz model taking into account the finite thickness of the cell with assumption that it had a planar shape and the probe deformation could be neglected.

In the Hertz model approximation, under the assumption that the rate of deformation of the sample is constant, using the Voigt-Kelvin model, as well as the direct and inverse Laplace transforms, the authors of Ref. [3] obtained an expression for the magnitude of the interaction force between the probe

and the sample. But this model does not take into account adhesion forces. To solve this problem, we used the Tabor parameter. For example, if we calculate using JKR model we can take into consideration adhesion forces between cantilever and sample surfaces [4]. This model is more suitable for the polymeric materials as well as fixed and living biological cells [5].

One of the important problems is to determine local mechanical properties of heterogeneous surface by AFM [6]. As usual, objects are characterized by convexities and concavities. Characteristics may also vary in different points of studied objects. It is important to make an amendment for shape of the probe that is well described in F. Borodich's article [7]. Other corrections to be made are the angle, under which the presser on the convexity is applied, and the probe interaction with the concavity.

Another important problem is to obtain the statistically reliable data on the base of mechanical properties values distribution on the surface and their changes from cell to cell.

Therefore, the aim of our research is to establish availability of viscoelastic properties of fixed platelets membranes similarly with homogeneous materials polydymethylsiloxane using elastic properties map obtained by AFM method. Consideration of all these corrections, as well as elasticity maps constructing studies, allows us to minimize variance of elasticity for the surface under research.

2. Experimental

In our research we used two types of materials – fixed biological cells (heterogeneous surface) and polydimethylsiloxane (PDMS, homogeneous surface). PDMS was made by deposition a polymer solution onto planar substrate and then drying it in air.

Platelets were separated from stabilized whole blood by sodium citrate. Obtained cells precipitation was washed with phosphate buffered saline (PBS) 3 times. Then suspensions of cells were fixed with 0.5% buffer solution of glutaraldehyde during 30 min. Suspension twice washed by PBS and water precipitation with cells was used to make a smear on mica plates. The method of preparing cells for AFM researching was described in Ref. [8].

For research, we used an AFM device NT-206 (produced by MicrotestMashines, Belarus) with standard silicon probes of V-shaped type (produced by "Mikromacsh", Estonia) with stiffness 0.35 N/m (CSC 11) and 3 N/m (NSC 11) and curve radius of probes 10 nm. The stiffness of probe is indicated according to the technical passport of manufacturer. Elastic modulus was calculated by JKR model.

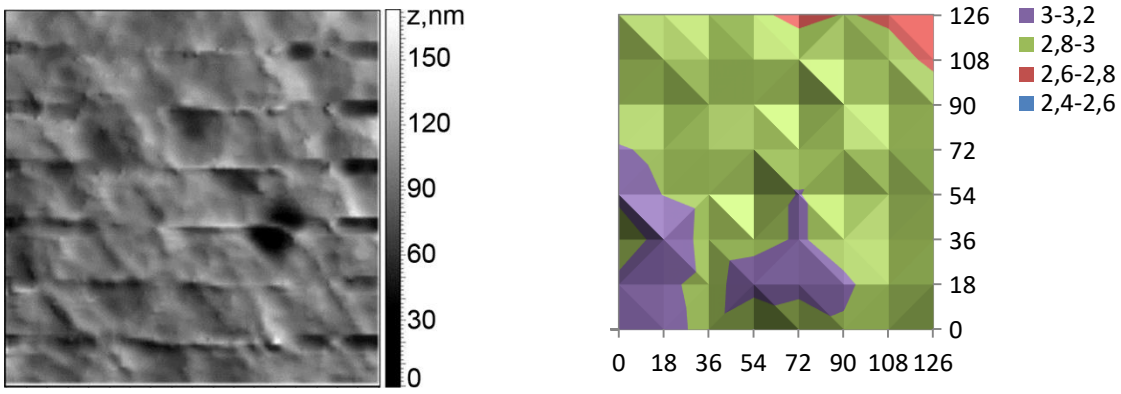
Process of point spectroscopy was made with different indentation times. This makes it possible to identify the viscous properties exhibited by the materials.

3. Results and discussion

The research surface of PDMS was characterized by homogeneous structure with low value of RMS, about 2.3 nm for scan sizes 4x4 μm^2 (Fig. 1a) and 5.5x5.5 μm^2 (Fig. 2a). In Figure 1, the lines and points of nanoindentation were demonstrated. Similar size of fixed platelets surface is interesting too, because it has the heterogeneity in the form cells (about 3 μm) on the mica plate surface (Fig. 3). Map of mechanical properties allowed identifying platelets on the surface. AFM images of research platelets are presented in Figure 4a.

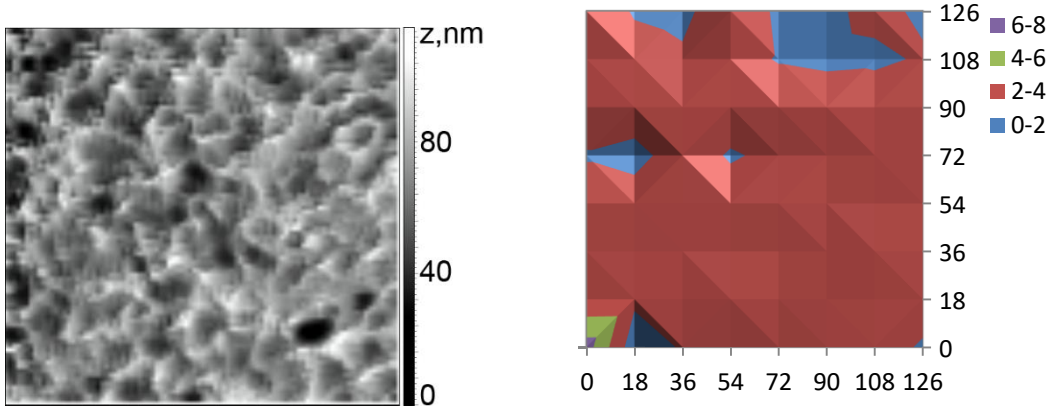
It was found that with increasing of indentation time for PDMS in the range from 12 to 99 s, the elastic modulus did not change and constituted 2.6 MPa in average (Fig. 1b). It should also be taken into account that values of elasticity did not depend on probe stiffness or nanoindentation loads of probe on sample surface and remained constant (Fig. 2b). Depth of nanoindentation increased with increasing load from 80 to 106 nN. The obtained results confirm that this material does not have viscosity properties.

According to Figures 3 and 4, the platelets laid more in the form of conglomerates rather than single cells. The average elastic modulus of the cells is 163.5 MPa ± 15%. Deviations are due to various properties of the surface membranes platelets and include device error. During mapping mechanical properties and correlating with surface morphology, we can reject data not connected with the platelets properties (Fig. 4). So, after analysis by mapping we give less deviation, about 10%. On the map of elasticity modulus, one place with higher value was identified, due to hollow on the mica plate.



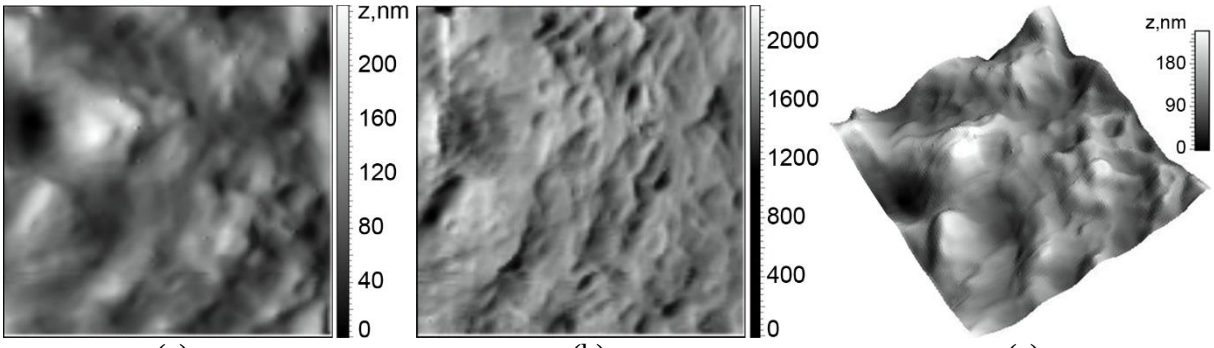
(a) (b)

Figure 1. (a) Structure in Torsion and (b) elasticity modulus (MPa) of PDMS surface 4x4 μm^2 (CSC11 probe, time 61 s, load 106 nN).



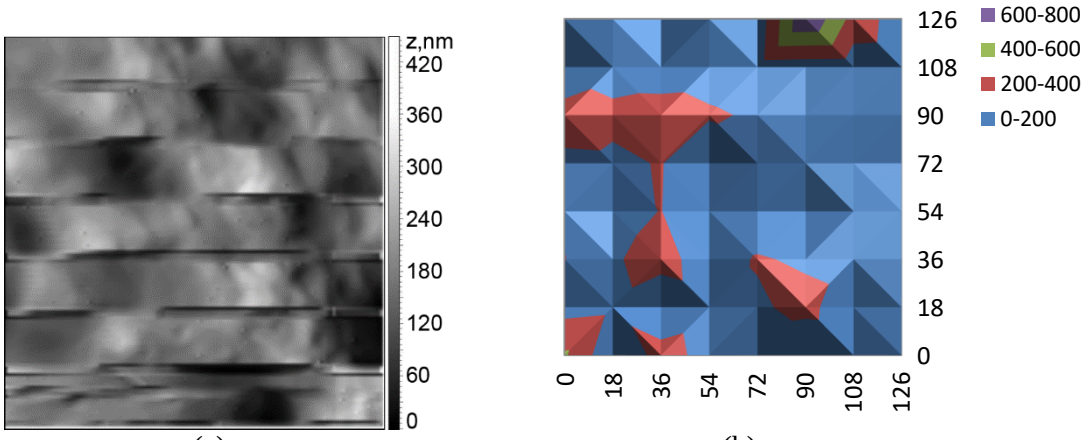
(a) (b)

Figure 2. (a) Structure in Torsion and (b) elasticity modulus (MPa) of PDMS surface 5.5x5.5 μm^2 (NSC11 probe, time 37 s, load 106 nN).



(a) (b) (c)

Figure 3. AFM-images of fixed platelets on mica substrate: (a) surface topography, (b) torsion, (c) 3D. Scan size 6.3x6.3 μm^2.



(a) (b)

Figure 4. (a) Structure and (b) elasticity modulus (MPa) of fixed platelets, surface 7x7 μm^2 using NSC 11A probe, load 40%.

The different indentation times in point were applied for fixed platelets too. It was found, that with increasing of nanoindentaion time the elastic modulus increased from 114.3 to 210.6 MPa. This is due to insignificant demonstration of platelets viscous properties. More optimal time of indentation is 70 s with load 80 nN.

4. Conclusion

In this research, mechanical properties of soft materials were estimated by AFM methods. Different regimes of the nanoindentation process were used. It was demonstrated that mapping mechanical properties allowed minimizing values deviation. It was found that fixed platelets had moderate viscous properties.

Acknowledgments

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