Study of Morphology and Rigidity of Neutrophilic Granulocyte Membrane in the Real Time Mode by Scanning Probe Microscopy

S. N. Pleskova, Yu. Yu. Gushchina, M. B. Zvonkova, and A. E. Khomutov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 712-714, June, 2006
Original article submitted June 16, 2005

The rigidity of neutrophilic granulocyte membrane was determined: 2.1±0.7 kPa. Scanning in the contact mode did not modify cell morphology, while spectroscopy caused nonspecific swelling reaction and a 3-fold increase in cell volume. Spectroscopy had no effect on rigidity of neutrophilic granulocyte membrane.

Key Words: *scanning probe microscopy; neutrophilic granulocytes*

The main advantage of scanning probe microscopy (SPM) is the possibility of real-time imaging of native cells without using fixatives and without loss of spatial resolution of the sample (the resolution is comparable to that provided by electron microscopy) [1]. However, the probe exerts a mechanical impact on the sample surface. The task of the study was to evaluate the possibility of injury or modification of neutrophilic granulocyte (NG) morphology during scanning as a result of exposure to lateral forces from the probe and to evaluate the NG membrane rigidity by SPM. Neutrophilic granulocytes were selected as the model system due to their capacity to high spontaneous adhesion in the presence of Ca^{2+}.

MATERIALS AND METHODS

Neutrophilic granulocytes were isolated from donor venous blood [2] and washed with buffered saline. The cells in Hanks' medium were transferred into Petri dishes (Corning) in a concentration of 0.5×10^6 cell/ml. NG spontaneously adhered to the surface over 20 min.

Research and Educational Center "Physics of Solid Body Nanostructures", N. I. Lobachevsky Nizhny Novgorod State University. ***Address for correspondence:*** plsn@mail.ru. S. N. Pleskova

The morphology of NG was studied under a scanning probe microscope Solver Bio™ (NT-MDT Co.) using silicon nitride probes with a lever elasticity coefficient of 0.01 N/m and radius of the probe tip 40 nm. The study was carried out in a contact mode. The probe was brought to the NG surface and the membrane was pressed with the force of 0.4-4.0 nN. The cantilever (elastic lever to which the probe was attached) started to percieve the elastic resistance force from the NG membrane. As a result, FS spectroscopy curve was recorded, representing a relationship between applied force and degree of the membrane depression under the probe. The Young modulus was calculated from it according to Herz model [3,4].

The data were analyzed using Origin 5.0 Server software.

RESULTS

The morphology of NG during scanning remained unchanged for a long time (60-120 min; Fig. 1). Thus, the impact of lateral forces during scanning in the contact mode is negligible, and hence, this scanning method can be used for the analysis of various substances modifying neutrophil morphology without risk of corrective effect of the probe.

Measurements of Young modulus were at first carried out on native NG in 25 independent experiments (the cells from different donors were taken; FS spectroscopy of each cell was carried out at least 3 times; Young modulus value was estimated as the mean of these measurements). The rigidity of NG membranes was 2.1±0.7 kPa. Since the impact of the probe during spectroscopy is more significant than during scanning, we hypothesized that it could modify cell morphology. The reaction of NG swelling in response to mechanical exposure to 0.3-1.0 nN force was studied. The size of NG increased significantly: the height increased 2-fold, the volume 3.6 times (Fig. 2). Then we evaluated the relationship between the increase in NG size and membrane rigidity. In order to detect this re-

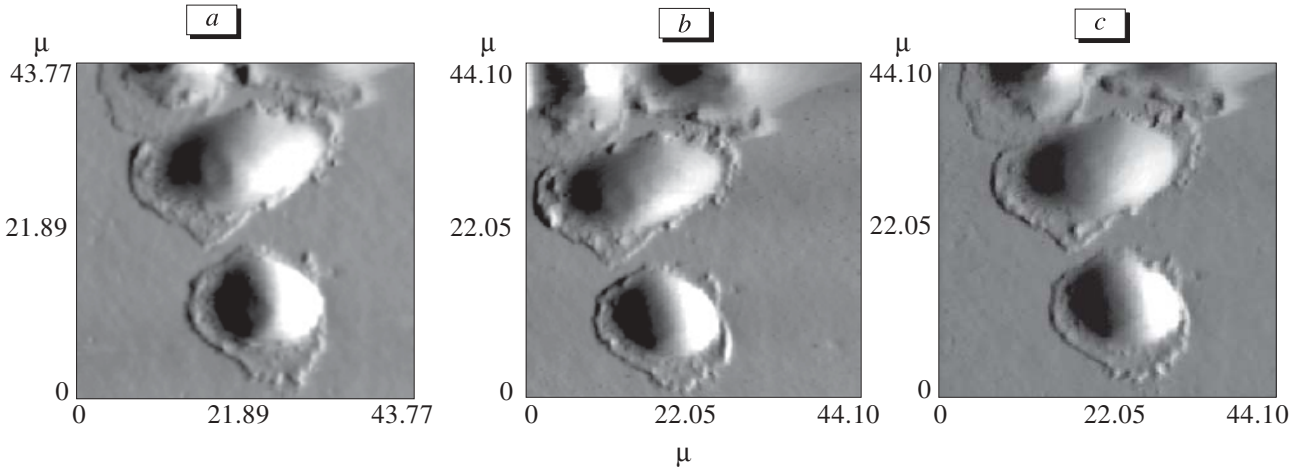

Fig. 1. Images of neutrophilic granulocytes obtained by scanning probe microscopy in contact mode. *a-c*) scanning was carried out every 15 min.

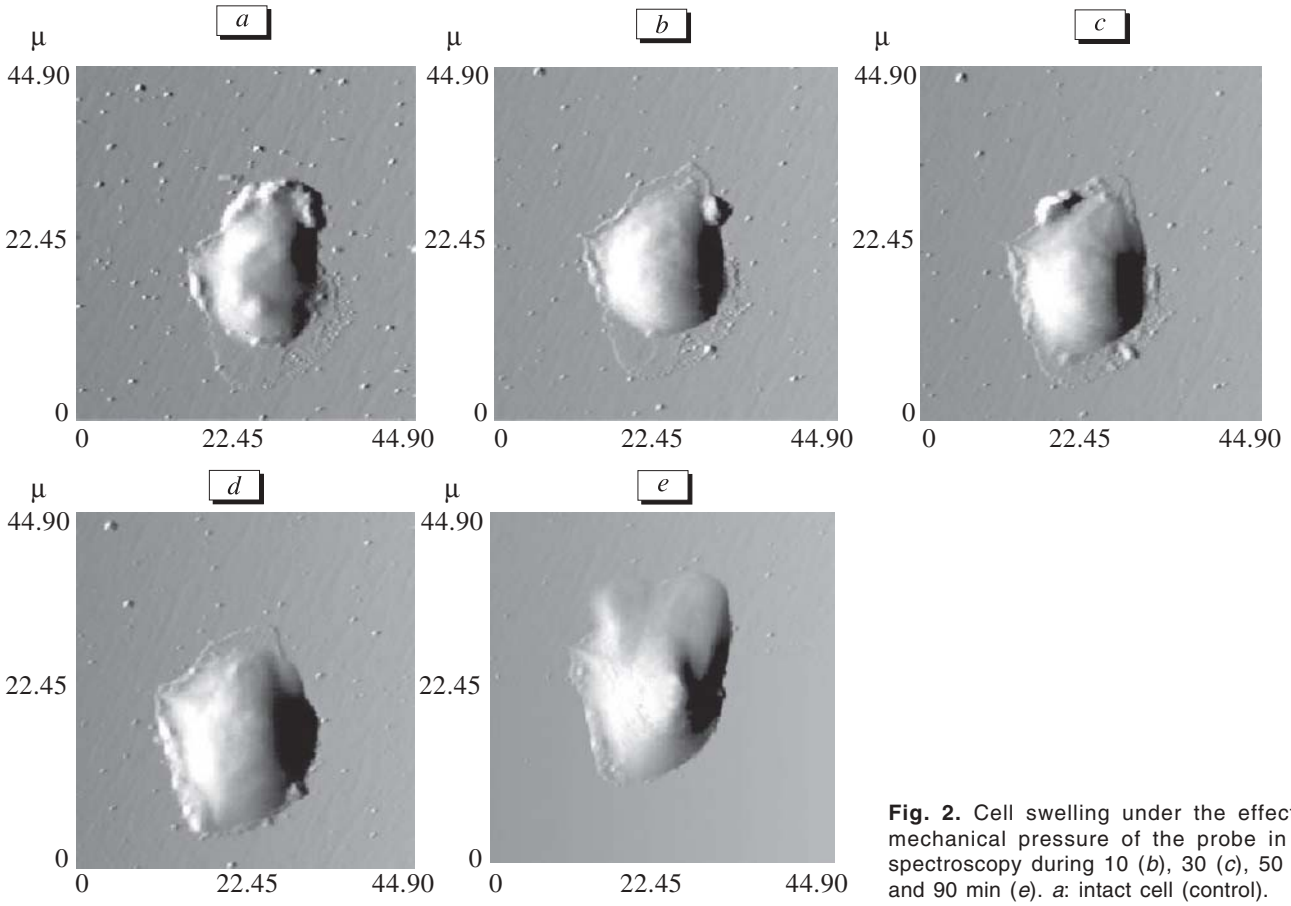

Fig. 2. Cell swelling under the effect of mechanical pressure of the probe in FS spectroscopy during 10 (*b*), 30 (*c*), 50 (*d*), and 90 min (*e*). *a*: intact cell (control).

lationship, spectroscopy was carried out during the entire process of swelling.

Membrane rigidity values were within the confidence interval (1.40-2.23 kPa), the differences between them were statistically negligible.

Hence, spectroscopy significantly modified the morphology of donor NG, but not NG membrane rigidity. In order to evaluate the effects of different substances on the cells, morphological and spectroscopic studies should be carried out in independent experiments.

The study was supported by the US Foundation for Civil Research and Development (grant REC-NN-001-X2:06) and Program of the Russian Ministry of Education "Development of Scientific Potential of Higher School" (code 4600).

REFERENCES

1. A. S. Borovik, O. S. Tarasova, A. V. Bol'shakova, and I. V. Yaminskii, *Uspekhi Sovrem. Biol.*, **120**, No. 2, 217-224 (2000).
2. I. V. Podosinnikov, L. G. Nilova, and I. V. Babichenko, *Lab. Delo*, No. 8, 68-70 (1981).
3. A. A. Bukharaev, A. A. Mozhanova, N. I. Nurgazizov, and D. V. Ovchinnikov, *Phys. Low-Dim. Struct.*, Nos. 3-4, 31-38 (2003).
4. E. A. Hassan, W. F. Heinz, M. D. Antonik, *et al.*, *Biophys. J.*, **74**, No. 3, 1564-1578 (1998).