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The Effect of Cell Pretreatment on Cell Surface Topology Studied via Atomic Force Microscopy

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Abstract: Atomic force microscopy allows distinctively interpret the results of cell structure research together with another techniques using, at that, the same biological preparations. However, there are still discussion situation concerning application of atomic force microscopy with fixed cells. In this work, using spectroscopy and atomic force microscopy techniques, we investigated morphometric parameters of cells in conditions of fixation with various chemical preparations as well as at drying and staining. We calculated phenomenological parameters and obtained histograms of distribution of adhesion forces of the probe toward a surface of cell membranes. The minimal disturbing effects were detected in the cases of a simple cell drying without fixation and staining.

Key words: Atomic Force Microscopy · Cell Membranes · Fixed Cells

INTRODUCTION

A broad application of scanning probe microscopy for investigation of cell morphology allowed to obtain data on features of cytomembrane both at qualitative and quantitative levels. Atomic force microscopy allows distinctively interpret the results of cell structure research together with another techniques using, at that, the same biological preparations [1-3]. However, there are still discussion situation concerning application of atomic force microscopy with fixed cells. In this work, using spectroscopy and atomic force microscopy techniques, we investigated morphometric parameters of cells in conditions of fixation with various chemical preparations as well as at drying and staining.

The aim of this research was to reveal the role of cell pretreatment with dye and fixing chemicals in changes of cell membranes using phenomenological parameters of cell surfaces [4].

MATERIALS AND METHODS

Thymocytes were used as experimental objects. The cells were placed at glass surface using drying and fixation with glutaraldehyde (0.6, 1, 2.5%), ether-ethanol (1:1), methanol, Romanovsky-Gimsa dye.

Atomic force microscope (AFM) images were acquired in air by a Solver P47H atomic force microscope (NT-MDT, Moscow, Russia) operated in the tapping and contact modes using NSG11 and CSG11 cantilevers (r = 10 nm, NT-MDT, Moscow, Russia). The height, Mag (signal from lock-in amplifier), RMS (signal from RMS detector) and phase (signal from the phase detector) were performed with the Nova 1.0.26 RC1 software (NT-MDT, Moscow, Russia). Resolution scan was 512x512 points.

To describe surface structure, phenomenological parameters were used [4] classify microgeometry of the surface. We obtained a set of scans from different areas of the surface and the parameters of undulation were detected according to formulas:

- arithmetical average roughness height that determinates the center of symmetry:

$$S_a = \left(\frac{1}{N^2}\right)\sum_{i,j=1}^{N}|z(i,j) - z_{mean}| \quad (1)$$

Where *z(i, j)*-height of the surface in point with coordinates (*i, j*) detected via AFM, *N*-number of points in scan line; $z_{mean} = \frac{1}{N^2}\sum_{i,j=1}^{N} z_{i,j}$ -average value for height of the surface at the image.

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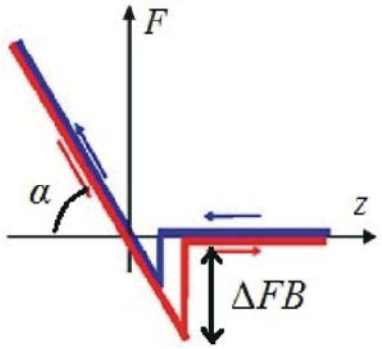

Fig. 1: Lines of force for advance-outshoot of cantilever from the surface of the sample under study

- root-mean-square roughness height that determinates dispersion of random value and characterizes a dissipation of its separate values from the center of distribution:

$$S_q = \left\{ \left(\frac{1}{N^2} \right) \sum_{i,j=1}^{N} (z(i,j) - z_{mean})^2 \right\}^{\frac{1}{2}} \quad (2)$$

- Height amplitude that determinates a difference between maximal (z_{max}) and minimal (z_{min}) values of roughness heights at the image:

$$S_y = z_{\max} - z_{\min} \quad (3)$$

To obtain additional information and to identify the material of surface, we analyzed adhesion forces between the probe and studied surface using atomic force spectroscopy (AFS). We registered lines of force for advance-outshoot of cantilever from the surface of the sample under study (Fig. 1). In majority of cases, adhesion forces F are combinations of electrostatic forces, Van der Waals forces, capillary forces and chemical forces.

Using formula (4) we calculated adhesion force (F):

$$F = ctg\alpha \cdot k \cdot \Delta FB \,, \quad (4)$$

Where k-coefficient of cantilever inflexibility, Nm; ΔFB-signal jumping from optical receiver, nA; α-degree of curve inclination at contact of cantilever with the surface.

RESULTS AND DISCUSSION

We obtained AFM images (15x15 mcm and 3x3 mcm) for separate thymocytes adhered to a glass surface and their membranes were studied. Examples of various AFM images with different methods of sample preparations are presented at Fig. 2.

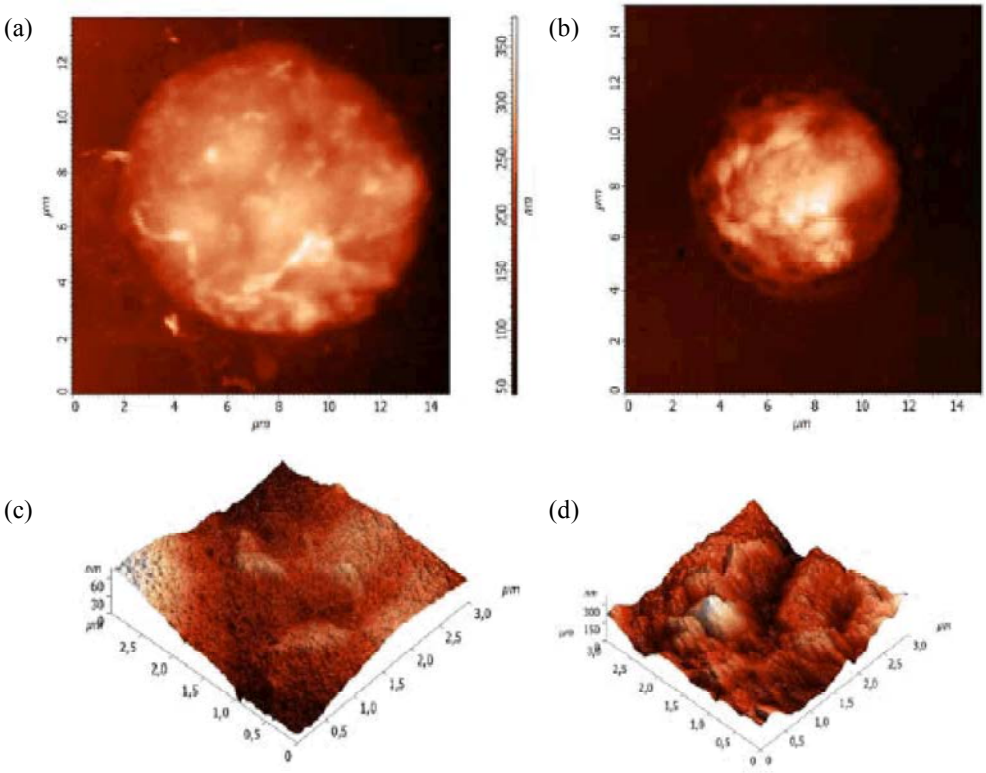

Fig. 2: 2D and 3D AFM images of cell and its membrane. a and b-dried and unstained variants; c and d-variants fixed with glutaraldehyde and stained with Romanovsky-Gimsa dye.

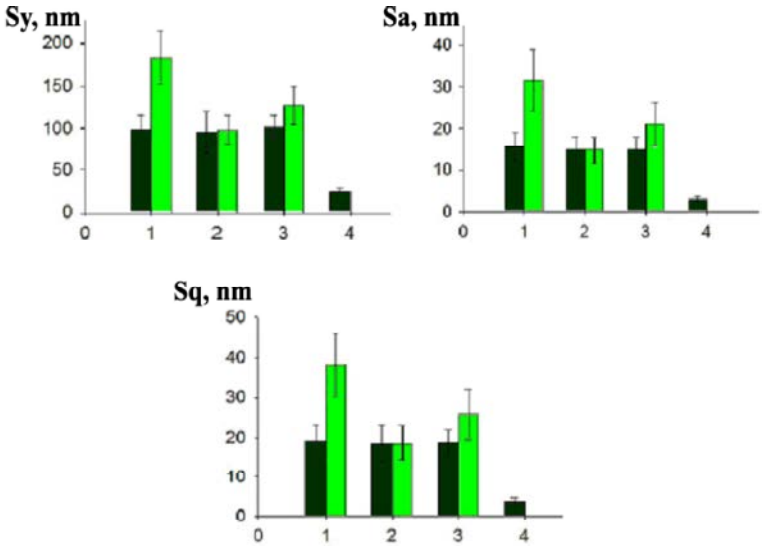

Fig. 3: Sy, S_a and S_q parameters of surface undulation of a cell. 1-0.6% solution of glutaraldyhyde; 2-methanol; 3-ether-ethanol (1:1); 4-dried (unmodified) sample. Dark green-stained samples, apple green-unstained samples.

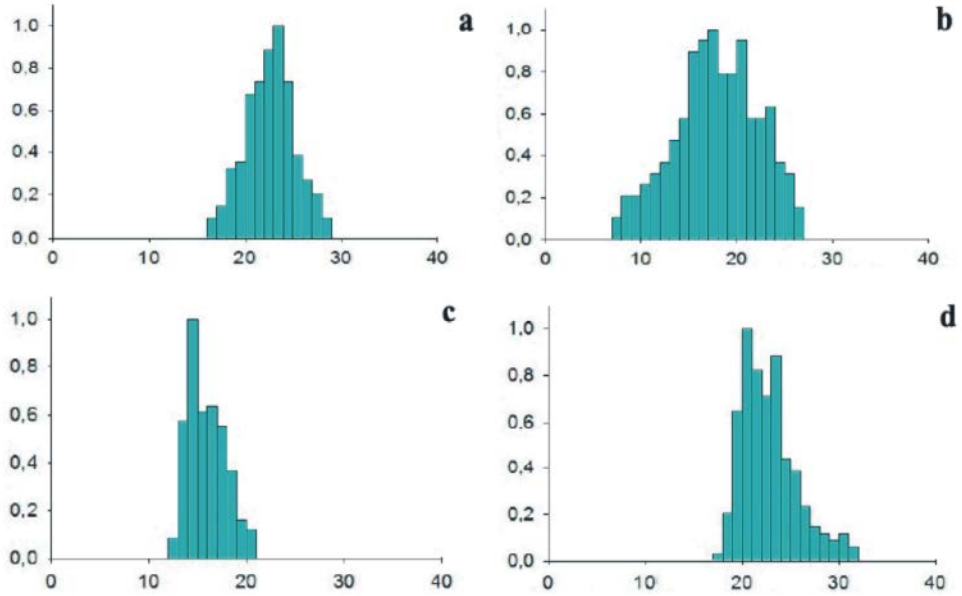

Fig. 4: Histograms of adhesion force of the probe toward a surface. a-dried and unstained cells; b-fixed with glutaraldehyde and stained; c-fixed with methanol and stained; d-fixed with ether-ethanol and stained. Axis Y-number of values, %; axis X-adhesion force of the probe toward cell surface, nN.

It was detected that diameter and height of cells is significantly depended on a way of cell fixation. Diameter of fixed cells is about 6 mcm while the same parameter for unfixed cells-12 mcm. The average height of unfixed cells-0.18 mcm while heights of modified cells varied from 0.49 to 0.98 mcm. In modified cell, practically all cytoplasm is concentrated near to the nucleus that results in decreasing of image sharpness and grains of cytoplasm are not identified (Fig. 2).

We calculated parameters of surface undulation (S_y, S_a and S_q [4]) to assess quantitatively structural and morphological changes of the cell surface; 1x1 mcm areas were used in these calculations. It is clear from Fig. 3 that parameters of surface undulation of unfixed cells are significantly less in comparison with fixed cells. At that, the values of surface undulation (S_y, S_a and S_q) for unstained samples but fixed with different chemicals are practically equal. At staining of cells fixed with

glutaraldehydeand ether-ethanol, there was a sharp increase in parameters of surface undulation (Fig. 3). At staining of cells fixed with 0.6%glutaraldehyde, there was a partial modification of cell membrane owing to binding with some part of amine groups of membrane proteins. Due to this process, glutaraldehyde does not protect lipids of cell membrane from the following ablation with spirit that is incorporated to dye. At that, there is a sharp increase in values of surface undulation in comparison with unstained cells (Fig. 3). At fixation with methanol, the parameters of surface undulation were equal for stained and unstained cells (Fig. 3). While staining, there was an additional loss of lipids owing to the action of the same spirit that was used at fixation.

Adhesion forces of cells were investigated via AFM method. Type of cell fixation induced changes in the distribution of adhesion forces (Fig. 4). There was fixation of proteins within cell membranes while fixation with glutaraldehyde. With increase in concentration (0.6%, 1%, 2.5%), there was a decrease of adhesion forces of probe to cell surface. At low concentration of glutaraldehyde (0.6%), only some part of protein amino groups are bound to this compound. Hydrophobic properties of cell membranes are increased with increase of concentration ofglutaraldehyde.

Fixation with ether-ethanol results in more pronounced adhesion in comparison with methanol. It might be explained ether-ethanol may increase viscosity of lipids and adhesion forces respectively.

Staining with Romanovsky-Gimsa dye might reduce adhesion forces for the probe toward cell surface in cells fixed with ether-ethanol and methanol. Spirit might fluidize lipids and glycerin from the dye might favor to production of hydrophobic film-all these two events might give input to reduction of adhesion forces.

CONCLUSION

Thus, drying and action of fixing and staining compounds act on cell surface and change its function. Atomic force microscopy allowed revealing cell reactions to various ways of cell fixation and staining. The less altering effect was detected in the case of simple drying. Clarification of AFM results needs to take into account the methods of cell preparation.

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