Investigation of mechanical properties of transformed living cells by means of atomic-force microscopy with high aspect ratio probes

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Abstract. Fabrication of high aspect nanowhisker structures at the apex of standard probes by the focused electron beam was carried out. The protocols of cultivation, structural modification and transportation of the cell structures of the selected line (fibroblasts) were performed. The mechanical properties of living cells in normal and malignant transformation (cancer cells) in a liquid were investigated by high aspect nanowhisker probes and compared with standard probes results. Elevation of the adhesion force and reduction of the elasticity modulus on cells with pathology was detected.

1. Introduction

Atomic force microscopy (AFM) is a widely known tool for biological research. AFM allows to obtain images of the topography of biological objects with a resolution of up to several angstroms, investigate interactions between single molecules and measure the mechanical characteristics of various biological samples at the micro- and nanoscale level.

One of the directions of AFM development in the field of biological research is the creation of specialized probes. The quality of the AFM data and the resolution of images directly depends on the cantilever and its tip [1, 2]. Despite the fact that there are currently many different cantilevers on the market for studying biological objects in liquids, the issue of creating new types of probes remains the most important task since there are still disputes about the advantages and disadvantages of different types and shapes of the tips [3, 4].

Thus, there is an obvious demand for specialized probes, which would allow high-resolution visualization of the surface of the studied objects. One of the most suitable materials in this case are high-aspect structures such as nanotubes [5], nanowhiskers [6], nanorods, etc.
Therefore, the aim of this work was to fabricate high-aspect probes to study mechanical properties of living cells in normal and malignant transformation by means of atomic-force microscopy and give a comparison with standard tip.

2. Experimental setup
The nanowhiskers (NW) were fabricated and measured at the top of the standard probes by electron beam induced deposition of precursor gases in a vacuum chamber of scanning electron microscope.

The investigation of cellular structures was carried out by scanning probe microscope combined with an inverted optical microscope. The investigation of native cells was carried out in PBS (phosphate-buffered saline) liquid by QNM PeakForce technique [7]. Heating platform in the main block of the microscope allowed to thermostating the cells during the measurements. The confocal laser scanning microscopy was used to study the location of the cells by using a TRITC fluorescent dye and a 100x/1.4 N.A lens.

Normal and transformed (cancer) mouse fibroblasts cells were cultured in a DMEM (Biolot, Russia), containing 10% of fetal bovine serum (Biowest, France) and 80 μg/ml gentamicin (Biolot, Russia) in an incubator at 37 °C and 5% CO₂. Cells were dissected in a multiplicity of 1:3 - 1:5, confluence of the cell line was 70-80%. The temperature maintenance during transportation of cells was carried out by Petri dish, covered (inside and outside) with several layers of aluminum foil.

3. Results and Discussions
Formation of a single NW was carried out by means of electron microscope with an integrated gas inlet system with C9H16Pt precursor (figure 1, a). Optimal parameters for the formation of NW structures were: accelerating voltage 5 kV, exposure time 5-10 s, aperture of electron microscope 20 μm. The composition of NW was about 30% of platinum, the rest is carbon. The working pressure in the vacuum chamber of the microscope was about 10⁻⁶ mBar.

The typical geometric parameters of the Pt/C whisker were about 400 nm in length, about 50 nm in diameter, and about 20-25° of the growth angle, which allowed to achieve orthogonality orientation of the probe relative to the substrate and obtain stable AFM images of cells (figure 1, b). The angle was set to compensate the angle of the probe holder in AFM microscope.

![SEM image of NW formed at the top of the standard probe (a), and AFM image of single fibroblast (b).](image)

**Figure 1.** SEM image of NW formed at the top of the standard probe (a), and AFM image of single fibroblast (b). The scale bar on SEM image is 30 μm.

Before studying biological objects a calibration grid with array of small rectangles of constant height 20nm±1.5 nm and period 3.0±0.05 μm (TGQ1, NT-MDT) was investigated (figure 2, a, b). The
edges of rectangles are close to vertical orientation, therefore deviation from vertical at the edge of rectangles on AFM image shows lateral resolution of the probes. The measured value of deviation from vertical position at the edge of rectangles in the PBS buffer was about 28±9 nm for standard probe and 8±4 nm for NW probe (along X and the Y axis). Thus, improvement of lateral resolution by 3-5 times compared with standard probes was found. The roughness of the rectangles surface on the 1x1 μm region was 0.32 nm for the standard probe and 0.89 nm for NW probe, which shows an increase in the accuracy of the vertical measurement (along Z axis) and indicate an improvement of image contrast.

![AFM image of the edge of a single calibration grid rectangle in the PBS buffer obtained by the standard probe (a) and the NW probe (b).](image)

**Figure 2.** AFM image of the edge of a single calibration grid rectangle in the PBS buffer obtained by the standard probe (a) and the NW probe (b).

In the study of fibroblast membranes an enlargement of adhesion force on fibroblast membranes was found using NW probes (figure 3, b). The maximum value of the adhesion force was about 2.4 nN for NW probe and about 1.1 nN for standard probe. It should be noted that the native cell change during the study, therefore study of adhesion forces should take place at flat surface, where adhesion force was about (~ 0.9 nN) for NW probe, that 2 times bigger compared to standard probe (~ 0.45 nN) (figure 3, a).

![AFM images of the adhesion force distribution and the cross-section of the surface measured in the central region of fibroblast using the standard probe (a) and the NW probe (b) in PBS buffer.](image)

**Figure 3.** AFM images of the adhesion force distribution and the cross-section of the surface measured in the central region of fibroblast using the standard probe (a) and the NW probe (b) in PBS buffer.

The adhesion forces in both cases increase with the transition from normal fibroblasts to fibroblasts with pathology, while the adhesion for the NW probe are large (table 1). This can be an additional factor along with a high aspect ratio explaining the better penetrating ability and hydrophilic properties of NW probes, which corresponds to past data on the adhesion strength of HB to dry erythrocyte membranes [8].
The Young's modulus for the standard probe decreases with the transition to cells with pathology, whereas for the NW probe, on the contrary, it slightly increases. That can be explained by the bending of the whisker during the approach to normal fibroblast and sticking of the whisker, having better hydrophilic properties, to the object surface.

It should be noted that increasing the adhesion force and reducing the Young's modulus is usually characteristic of cells with pathologies that acquire great softness and elasticity (cancer cells have greater elasticity for incorporation into normal cells). Wherein the deformation of the cell has lower values using NW probes, what can be explained by the elasticity of NW and the comparable sizes of the whisker tip and the tip of pyramid of the standard probe (~10 nm), which contact with the investigated surface.

### 4. Conclusions

Thus, the optimal parameters for the nanowhisker formation at the axis of the standard probes was found. It was shown that specialized nanowhisker probes give better results when measured objects in liquid medium. The protocols of cultivation, structural modification and transportation of the fibroblast cells in normal and malignant transformation were fulfilled. An increase of the adhesion strength and a decrease of the elastic modulus at cells with pathology was revealed. The results obtained in this study can be used for a more detailed and accurate study of native objects in liquid by means of atomic force microscopy.

### Acknowledgments

The work was carried out with the support of Russian Foundation for Basic Research (16-32-00806), the Federal Agency for Scientific Organizations (AAAA-A16-116041110123-5), the leading universities of the Russian Federation (grant 08-08).

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