Investigation of native cells in liquid using the high aspect ratio nanowhisker probes by means of atomic force microscopy

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Abstract. Specialized Pt/C single nanowhisker probes for atomic force microscopy have been used to study the native cellular bio-objects (fibroblasts) in PBS liquid. Optimal parameters of nanowhisker fabrication were found such as accelerating voltage 5 kV, exposure time 5-10 sec, aperture of electron microscope 20 μm. The preliminary results of our study showed an improvement of image contrast and adhesion forces when using nanowhisker probes compared to the standard Si probes.

1. Introduction

Atomic force microscopy (AFM) is one of the few methods that allow to study native (living) cell. Unlike common optical methods for investigation of cellular structures, AFM allows to generate three-dimensional topography images of topology with nanoscale accuracy as well as to obtain the additional information about the specimen (roughness, adhesion force, stiffness, etc.). However, there is a problem of further enhancement of the method resolution to visualize the structure of complex bio-objects, in particular, the fine structure of cell membranes [1, 2].

One of the main elements affecting spatial resolution of the resulting images is the AFM probe (nanoneedle or tip) which interacts with the sample. To increase the AFM spatial resolution for detailed diagnostics of bio-objects the modification of the shape and composition of standard probe are carried out. One of the promising directions in this area is the formation of rod like nanostructures at the apex of the standard probes also known as nanowhiskers [3, 4]. However, there are still few works on controllable fabrication of single oriented nanowhiskers as well as their application in a liquid medium.
Therefore, the purpose of this work was to fabricate and apply specialized probes with single oriented nanowhiskers to study the fine structure of cellular native bio-objects in liquid by means of atomic force microscopy.

2. Experimental setup

The Pt/C nanowhiskers (NW) were fabricated at the top of the standard probes by electron beam induced deposition of precursor gases in a vacuum chamber of electron microscope [5]. The fabrication and visualization of the NW were performed by scanning electron microscope (SEM) CrossBeam Neon 40 (Carl Zeiss, Germany). The investigation of cells was carried out by scanning probe microscope BioScope Catalyst (Bruker, Germany), combined with an inverted optical microscope Axio Observer D1m (Carl Zeiss, Germany). The investigation of native cells was carried out in PBS (phosphate-buffered saline) liquid using the PeakForce QNM mode [6]. TCS SP5 (Leica, Germany) confocal microscope was utilized to study the location of the cells by using a TRITC fluorescent dye and a 100x/1.4 N.A lens.

3. Results and Discussions

Nanowhisker was fabricated at the top of the probe by decomposition and precipitation of the gas precursor C_9H_{16}Pt components under an electron beam in vacuum [6]. The optimal length of NW was about 300-400 nm with average diameter of about 40-60 nm. NW were fabricated with an angle to the tip axis ~ 22-25 degrees to reduce double-tap contact with the surface (compensation of the tip holder angle in microscope).

The optimal growth parameters of NW were follows: accelerating voltage 5 kV, exposure time 5-10 sec, aperture of electron microscope 20 μm (Fig. 1, b). The aperture of 20 μm made possible to achieve a whisker thickness of about 40-60 nm while the exposure value affects the height of the whiskers.

![Figure 1](image-url)

**Figure 1.** SEM image of NW formed at the standard probe apex (a), and SEM images of NW after variation of fabrication parameters of NW - exposure time and aperture (b). The scale bar is 500 nm.

The minimum value of accelerating voltage (5 kV) was chosen to achieve the optimal thickness of NW which allows to increase the penetrating ability (image contrast) and the mechanical stability of NW during scanning process.
To stain actin structures the mouse fibroblasts were plated on coverslips 1-2 days before the experiments. Cells were fixed with 3.7% paraformaldehyde solution (10 minutes, room temperature), permeabilized 0.1%. Fluorescence of the dye was excited by a laser with a wavelength of 543 nm, the emission was recorded in the range of 580-670 nm (Fig. 2, a). Fibroblast cells were studied close to native state by means of AFM in PBS liquid (Fig. 2, b).

![Confocal image of actin cytoskeleton in transformed fibroblasts of SV40 mouse (a) and AFM image of single fibroblast close to native state (b). Staining of F-actin with fluorescent dye TRITC-phalloidin. The scale bar is 30 μm. The contrast of AFM image was increased by peak force error mode, the white frame shows the comparison area of the probes.](image1)

**Figure 2.** Confocal image of actin cytoskeleton in transformed fibroblasts of SV40 mouse (a) and AFM image of single fibroblast close to native state (b). Staining of F-actin with fluorescent dye TRITC-phalloidin. The scale bar is 30 μm. The contrast of AFM image was increased by peak force error mode, the white frame shows the comparison area of the probes.

![AFM topography images of fibroblast visualized by the standard (a) and the NW (b) probes in PBS buffer.](image2)

**Figure 3.** AFM topography images of fibroblast visualized by the standard (a) and the NW (b) probes in PBS buffer.

An improvement of the penetrating ability using NW probes was found in the study of fibroblast membranes (Fig. 3, 4). Thus, the NW probes visualized the same section with a height difference of 3.4 μm while the standard probes showed height difference of about 2.7 μm in the same region. As seen in the cross-sections, the height of the projections is greater when used NW probes (small protrusion ~ 1.3 μm, large protrusion up to ~ 3 μm) compared to standard probes (Fig. 4, a, b).

The maximum value of adhesion force was about 2.4 nN for the probe with NW and of about 1.1 nN for the standard Si probe. It should be noted that the cell can change during the study therefore it is
necessary to measure the flat area. Measurement of flat surface area revealed extension of adhesion force using the NW probes (~ 0,9 nN) in 2 times in comparison to the standard probes (~ 0,45 nN) (Fig. 5, 6).

![Figure 4. Diagonal cross-sections of the native fibroblast surface from the AFM image visualized by the standard (a) and the NW (b) probes in PBS buffer.](image)

![Figure 5. AFM images of adhesion forces distribution (a) and cross-section area (b) measured on the fibroblast (central area) by standard probe in PBS buffer.](image)

![Figure 6. AFM images of adhesion forces distribution (a) and cross-section area (b) measured on the fibroblast (central area) by NW probe in PBS buffer.](image)
Thus, NW probes are shown to improve the accuracy of heights visualization and it corresponds to the previous data of adhesion forces to dry erythrocyte membranes [7] that reveals a better penetration ability and hydrophilic features of NW probes. Application of the specialized NW probes can produce better results in image contrast in comparison to the standard probes while studying the native cells in liquid medium by means of atomic force microscopy. It is difficult to analyze lateral resolution due to modification of cells by tips and relocation of their surface, therefore, it requires additional research.

4. Conclusions
Optimal parameters of nanowhisker fabrication were found to study the fine structure of cellular native bio-objects. It was found that application of probes with nanowhiskers in PBS liquid improve image contrast and adhesion forces in the study of native fibroblast cells. The results obtained in this study can be used for a more detailed and accurate study of native objects in liquid with complex structure by means of atomic force microscopy in such areas as pharmacology and medicine.

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