ATOMIC FORCE MICROSCOPY STUDY OF HUMAN FIBLOBLASTS

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Abstract. In the study we have investigated human fibroblasts by combined fluorescence microscopy and atomic force microscopy. The pulsed force mode for atomic force microscope was used to determine fibroblasts mechanical properties (local stiffness and adhesion). The fluorescence microscopy was used to study the fibroblasts cytoskeleton organization.

Fibroblasts morphology and locomotion is one of the most difficult phenomena to be explained from complex interaction among cytoskeleton, membrane, integrins and extracellular matrix. The atomic force microscopy (AFM) has a great potential to provide fundamental insights into these phenomena because of the combination of high resolution imaging and the ability to obtain information about the mechanical properties (stiffness, elasticity, and hardness) of cells in relation with cellular functions [1,2]. Here the mechanical properties of human skin fibroblasts and their cytoskeleton were studied with atomic force microscopy and fluorescence microscopy.

Primary skin fibroblasts were obtained from healthy donors by minimal invasive 3-mm punch biopsy. Small pieces of skin were incubated in the appropriate medium (Dulbecco's Modified Earle's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic in 6-well plates under the cover slides at 37˚C. Fibroblast's growth was observed after 10-14 days of cultivation. For AFM investigation monolayer of fibroblasts was detached using 0,25% trypsin-EDTA. Then cells were transferred into the culture dishes with glass slides at the bottom. After 24 hour of incubation at 37˚C cells were fixed with 2% glutaraldehyde for 30 min. Thereafter fibroblasts were washed five times in phosphate-buffered saline (PBS), dehydrated in a graded series of ethanol and air dried.

A MultiMode Nanoscope (R) IIIa AFM (Digital Instruments/Veeco) was used in all experiments. The AFM imaging was performed under ambient air conditions using tapping-mode. Silicon nitride cantilevers (NSC12/50) with a nominal force constant of 0.65 N/m (NT-MDT, Zelenograd, Russia) were used. To study mechanical properties (local stiffness and adhesion) of the fibroblasts force modulation mode (FMM) was used. The AFM images were processed with the Nanoscope software (Digital Instruments/Veeco).

To stain actin and tubulin fibroblasts were grown into the chambers on slides. After exposure cells to irradiation slides were washed and fixed in 4 % paraformaldehyde solution for 30 minutes at +4˚C. Then samples were washed twice and permeabilized in 0.1% Triton X-100 for 15 minutes. Then samples were incubated with Alexa Fluor 488 anti-α-tubulin antibody (1:1000) and Alexa Fluor 633-Phalloidin (1:500) (Molecular Probes) for 1 hour at 37˚C, washed twice in PBS. Slides were additionally stained with propidium iodide to detect nucleus. Fluorescence was analyzed by confocal laser scanning microscope Leica TCS SPE.

Fibroblasts demonstrate a well-developed cytoskeleton, comprising mainly arrays of parallel actin stress fibers extending the long axis of the cells as revealed by Alexa Fluor 633-Phalloidin staining (Fig.1). To study the organization of the microtubule network, fibroblasts

were stained with Alexa Fluor 488 anti-α-tubulin monoclonal antibody. As seen from Fig.1 microtubules radiate from a perinuclear location and more often appear curved in form filling the large regions of the cell body.

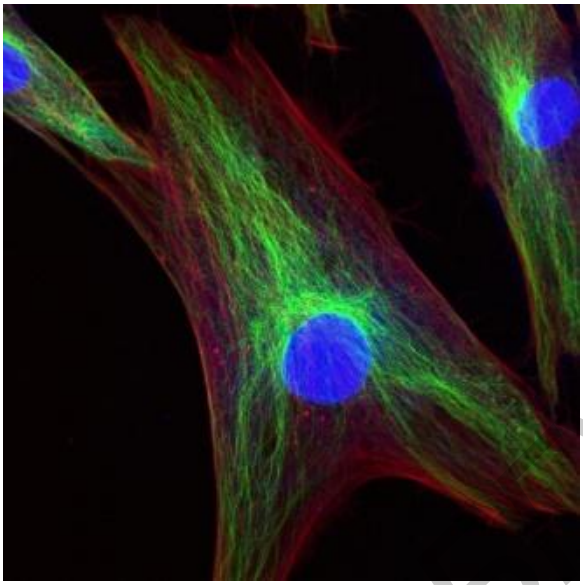

Figure 1 - Fluorescent images of skin fibroblasts stained with Alexa Fluor 633 Phalloidin to identify F-actin (red) and anti- α -tubulin Alexa Fluor488-MAT to identify tubulin (green), propidium iodide to detect nucleus (blue)

The fibroblasts images obtained with AFM (Fig.2) showed a characteristic spindle shaped cells with irregularly shaped flat lamellipods. Fibroblasts cellular length varied from 75 to 150 μm. The AFM images demonstrate that actin stress fibers form densely packed parallel arrays with lateral size from 30 to150 nm traversing the nucleus area. The structure of actin stress fibers appears better defined in the error signal image.

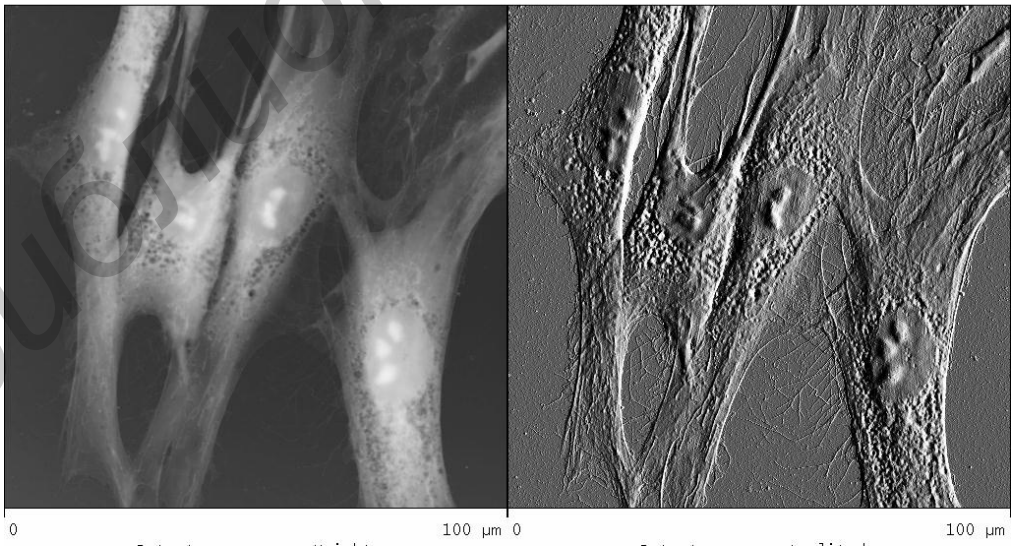

Figure 2 - AFM images of human skin fibroblasts (a- height; b – amplitude)

Using the pulsed force mode adhesion and stiffness images of fibroblasts were obtained simultaneously with its topographic image. The topographic, adhesion and stiffness images of

fibroblasts are presented in Fig.3. Darker parts in the adhesion and stiffness images correspond to low adhesion and stiffness value on fibroblast membrane.

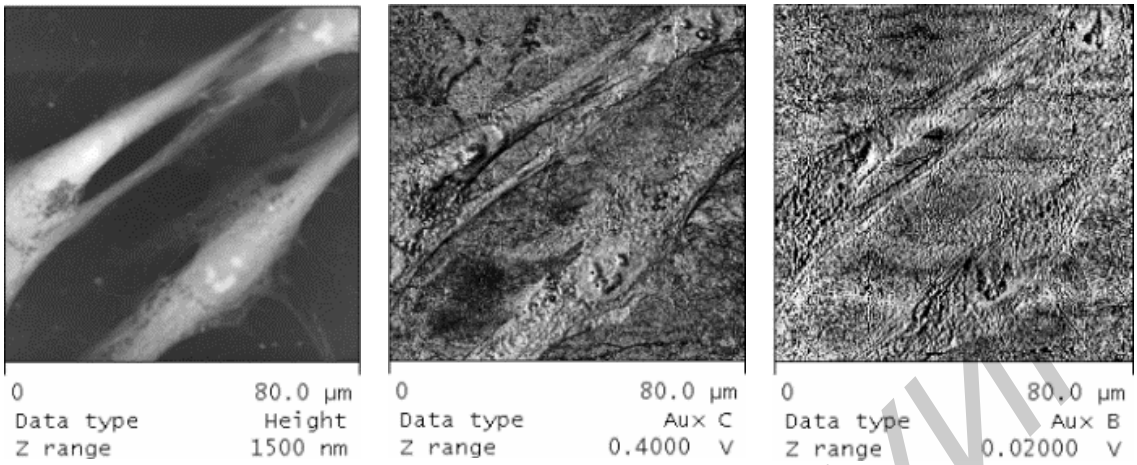

Figure 3 - AFM images (a- height, b- adhesion, c- stiffness) of human skin fibroblasts

As follows from the AFM images of the fibroblasts (Fig.4) their nuclei are more adhesive and less rigid than the surrounding nucleus region and the peripheral (lamellipodial) regions. The stiffest part of the fibroblasts corresponds to the lamellipodial region of cell. Since the lamellipodium is very thin, probably the underlying substrate can affect the fibroblast stiffness.

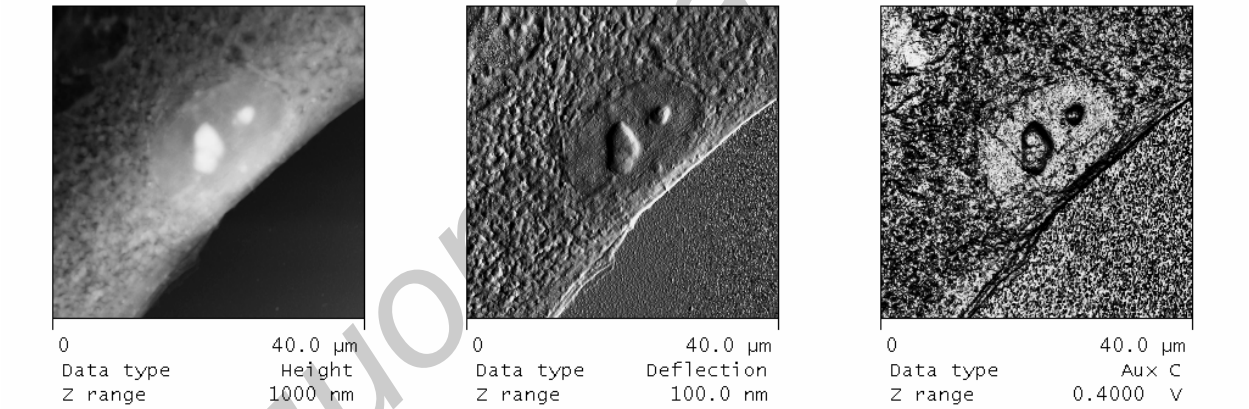

Figure 4 - AFM images (a- height, b- deflection, c- adhesion) of the fibroblast nucleus area

The AFM measurements of cellular topography, adhesion and stiffness combined with fluorescence microscopy open up possibilities for the mechanical properties investigations of the fibroblasts in relation with their cytoskeleton organization.

References

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