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The use of atomic force microscopy for human mesenchymal stem cells study

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The AFM use opens up exciting new possibilities for investigation of mechanical properties (stiffness, elasticity, and hardness) of a wide variety of biological cells in relation with dynamic processes and cellular functions [1]. In this work the actin cytoskeleton and mechanical properties of human mesenchymal stem cells (hMSC) were studied using fluorescence microscopy and AFM. Study in details of hMSC mechanical properties such as elasticity, adhesion and stiffness, cytoskeleton organization and cell shape is required to realize their promising potential for development of new therapies for regenerative medicine and stem-cell-based tissue engineering.

For AFM investigations the hMSCs were fixed with 2% glutaraldehyde for 30 min. All data were obtained on Nanoscope (R) IIIa MultiMode AFM. Force modulation mode was used to study mechanical properties (local stiffness and adhesion) of the hMSCs. The images were acquired by using silicon nitride cantilevers (NSC12/50) with a nominal force constant of 0.65 N/m (NT-MDT, Russia). The measurements were performed in air at RT. AFM investigations of hMSCs exhibited a considerable range of morphologies as well as spreading and the lengthened shape of the cells. Cells possess irregularly shaped flat lamellipods. For the spindle shaped cell the nuclear region height varies from 400 nm to1 μm, whereas lamellipodia thickness varies from 150 to 340 nm. For the star shaped cell nuclear region height is about 400-800 nm with the lamellipodia thickness 180-300 nm. Lamellipodia contain orthogonally arranged actin networks at the hMSC peripheries. The nucleus zoomed area of star shaped cell is shown in Figure 1. The area around nucleus looks like a smooth fiber mesh. Zooming in on the nucleus the granular structure of elongated bundles of actin filament with 20-70 nm granule size is visualized. AFM images demonstrate many parallel actin bundles extending throughout the nuclear region. Darker parts in adhesion image correspond to low adhesion value. The nucleus appears to be distinctly softer than the flat lamellipodia.

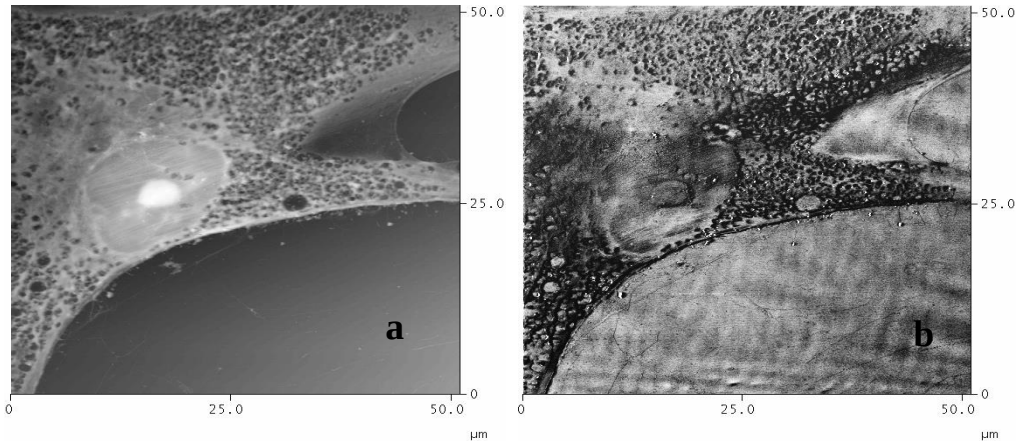

Figure 1. AFM images of human mesenchymal stem cell: (a) contact mode topography and (b) force modulation image (adhesion).

According to the hMSC fluorescent images the microfilaments are linear in form and mostly is localized over the nucleus. The microtubules more often appear curved in form and span large regions of the hMSCs. Mechanical properties of hMSC most likely are regulated by the actin cytoskeleton, its structure and dynamics. This study demonstrates that the pulsed force mode for atomic force microscope combined with fluorescence microscopy opens up possibilities for investigation of the mechanical properties of hMSCs in relation with cytoskeleton organization.

1. N.I. Nikolaev, T. Müller, D.J. Williams, et al., *J. Biomech.* **47**, 625 (2014).