# HUMAN MESENCHYMAL STEM CELLS STUDY WITH ATOMIC FORCE AND FLUORESCENCE MICROSCOPY

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Detailed characterization of human mesenchymal stem cells (hMSCs) mechanical properties (elasticity, adhesion and stiffness) and cytoskeleton organization is required to realize their promising potential for development of new therapies for regenerative medicine and stem-cell-based tissue engineering [1]. In the study the actin cytoskeleton and mechanical properties of hMSCs were studied with fluorescence microscopy and atomic force microscopy (AFM). The AFM capabilities can be extended by using the pulsed force mode, which enables to obtain information about relative difference in cell surface elasticity with nanometer-scale resolution. The pulsed force mode allows a quantitative mapping of hMSCs surface mechanical properties such as adhesion and stiffness, simultaneous with the imaging the cells surface topography in tapping mode.

All data were obtained on a Nanoscope (R) IIIa MultiMode atomic force microscope. The pulsed force mode is a non-resonant, intermediate contact mode of AFM. When working in pulsed force mode, an additional sinusoidal modulation to the cantilever with user-selectable frequency, which is far below the resonance frequency of the cantilever is applied while the tip scans the surface. The images were acquired by using silicon nitride cantilevers (NSC12/50) with a nominal force constant of 0.65 N/m (NT-MDT, Zelenograd, Russia). AFM images were processed with the Nanoscope software. The hMSCs fixed with 2% glutaraldehyde were studied in air at room temperature.

The AFM investigations of hMSCs exhibited a considerable range of morphologies as well as spreading and the lengthened shape of cells. The AFM studies revealed that lamellipodia contain orthogonally arranged actin networks at the hMSC peripheries. 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 granule size of from 20 nm to 70 nm is visualized. AFM images demonstrate many parallel actin bundles extending throughout the nuclear region. The nuclei appear to be distinctly softer than the flat lamellipodia. The pulsed force mode revealed that nuclei are more adhesive and less rigid than the lamellipodial regions. It was determined that the stiffest part of the hMSC corresponds to lamellipodia. The hMSCs were stained with Alexa Fluor 633 phalloidin to identify F-actin and anti- α -tubulin Alexa Fluor 488-MAT to identify tubulin. According to the hMSC fluorescent images microfilaments are linear in form and mostly are localized over the nucleus. Microtubules more often appear curved in form and span large regions of hMSCs. Mechanical properties of hMSC most likely are regulated by the actin cytoskeleton, its structure and dynamics. In this paper we present the potential of the pulsed force mode for mapping the local mechanical properties (elasticity, adhesion and stiffness) to hMSCs surface topology. In conclusion, the pulsed force mode of the atomic force microscopy combined with fluorescence microscopy opens up new possibilities for investigation of the hMSCs mechanical properties in relation with the cytoskeleton organization.

## References

1. Han I., Kwon B., Park H., Kim K. Differentiation Potential of Mesenchymal Stem Cells Is Related to Their Intrinsic Mechanical Properties // J. Int. Neurourology 2017. Vol. 21. S. 24-31.