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The use of pulsed force mode atomic force microscopy to study the human mesenchymal stem cells

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The atomic force microscopy (AFM) is increasingly applied to human mesenchymal stem cells (hMSCs) study. The AFM has been widely used for imaging hMSCs because of the combination of nanometer scale resolution and the ability to obtain time-dependent dynamic information about the cells under physiological conditions. In addition to high resolution visualization, elastic properties of hMSCs can be detected with the AFM. The pulsed force mode (PFM) is a non-resonant, intermediate contact mode of AFM. The AFM capabilities can be extended by using PFM, which enables to obtain information about relative difference in cell surface elasticity with nanometer-scale resolution. The PFM 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.

Detailed characterization of hMSCs mechanical properties 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 PFM of AFM. All data were obtained on a Nanoscope (R) IIIa MultiMode atomic force microscope. 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 PFM 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. 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 PFM for mapping the local mechanical properties (elasticity, adhesion and stiffness) to hMSCs surface topology. The PFM combined with fluorescence microscopy opens up new possibilities for investigation of the hMSCs mechanical properties in relation with the cytoskeleton organization.

References

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