

PRECISE LOCALIZATION AND QUANTIFICATION OF MAGNETIC/FLUORESCENT NANOPARTICLES INSIDE MESENCHYMAL STROMAL CELLS BY CONFOCAL MICROSCOPY

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Abstract

In vivo tracking of magnetically labeled stem cells by non-invasive Magnetic Resonance Imaging (MRI) is a favorable diagnostic solution for longtime studies of patients after cell transplantation [1]. Generally, after addition of SPIO (Superparamagnetic Iron Oxide) nanoparticles into the cultivation medium they pass through the cell membrane by endocytosis [1]. For the safe in-vivo application of magnetically labeled cells it is indispensable to evaluate not only viability of cells but also to precisely characterize the fate of internalized SPIO inside the cells. The aim of this study was to investigate the qualitative and quantitative localization of magnetic/fluorescent SPIO in internal organelles and cytoplasm in mesenchymal stromal cells (MSC). MSC were exposed 12 and 24 hours to maghemite-rhodamine (named SAMN/RITC) nanoparticles in concentration of 50 µg/ml. Visualization and quantification of SAMN/RITC were evaluated in nucleus, mitochondria and lysosomes by confocal microscope (Leica TSC SP8 X). Scanning mode of MSC was optimized and semiautomatic software utility for analysis of confocal scans was developed (in Matlab R2010a). SAMN/RITC showed excellent uptake by MSC. Confocal microscopy displayed accumulation of SAMN/RITC clusters in all parts of cytoplasm. By Matlab utility it was exhibited that nanoparticles were not localized in mitochondria or cell nucleus. This localization was not changed after one week of cultivation. Statistical results demonstrated very minimal pathophysiological effect of SAMN/RITC based on quantification volume and shape of labeled /unlabeled MSC. In conclusion SAMN/RITC nanoparticles are not cytotoxic, do not enter the cell nucleus and mitochondria and do not disintegrate cell metabolism.

Keywords: Stromal cell labeling, magnetic/fluorescent nanoparticles, confocal microscopy

LITERATURE

- [1] Berry (2003) J Phys D Appl Phys, 36:198-206

Author did not supply full text of the paper/poster.