In-Vivo Positive Contrast Tracking of Bone Marrow Stem Cells Labeled with IODEX-TAT-FITC Nanoparticles

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Introduction

Bone marrow-derived mesenchymal stem cells (BMSCs) possess tremendous therapeutic potential because of their capacity to differentiate into multiple functional lineages. To assess the efficacy of BMSCs cell therapy, MRI methods are being developed to monitor their bio-distribution in longitudinal studies. Although, labeling BMSCs with iron-oxide nanoparticles can facilitate their *in vivo* detection, cellular function and mobility might be compromised due to toxicity. TAT-peptide derivatized ultra-small super-paramagnetic iron oxide nanoparticles coated with dextran (IODEX-TAT-FITC) have demonstrated their potential for BMSCs labeling without detrimental effects on cell viability or function [1]. In order to increase the detection limits, the susceptibility effect of the iron cores can be exploited to enhance sensitivity.. However precision in negative contrast detection could be reduced by partial volume effects and local magnetic field inhomogeneities. Positive contrast offers a more practical detection scheme with high contrast-to-noise ratio [2]. In this study, we explore the suitability of positive contrast MRI for *in vivo* tracking of labeled BMSCs.

Materials and Methods

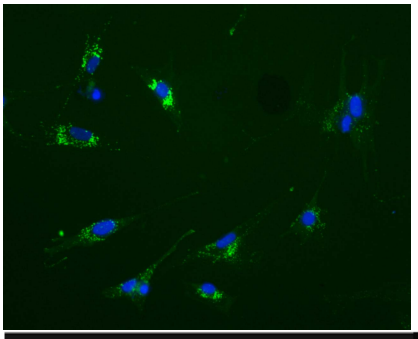
BMSCs preparation: Bone marrow-derived BMSCs were harvested from femurs and tibias of adult male Wistar rats. The cells were seeded in high-glucose Dulbecco's modified Eagle's medium with L-glutamine supplemented with 10% foetal bovine serum and 25µg/mL gentamicin on poly-l-lysine coated flasks. Culture media was replaced every two days. Haematopoietic and other non-adherent cells were removed during medium changes. After 10 to 14 days of primary cultivation, the adherent cells were nearly 80% confluent and were dissociated with 0.25% trypsin and 1mM [1]. **Cell labeling:** BMSCs were incubated with IODEX-TAT-FITC for 6h at 10µg/ml (iron) in culture media [1]. After removal of extracellular nanoparticles with PBS, approximately 50,000 labeled BMSCs were stereo-tactically injected into the striatum of rats at a rate of 1µl/min. ***In vivo*** **MRI:** Injected rats were anaesthetized at 1.5% isoflurane. Positive contrast detection of BMSCs was carried out with a multiple-echo ultra-short echo time pulse sequence (MUTE) at 9.4T [2], with a single loop surface receiver coil for reception. Images were reconstructed offline with regridding using Kaiser-Bessel interpolation. Scan parameters were: TR=100ms, TE=0.3ms (UTE)/8ms (ECHO), flip angle=45°, FOV=30x30mm, matrix=256x256. **ROI analysis:** Regions-of-interest were drawn at the labeled cells and at the unlabeled contra-lateral position. Using the measured signal intensities (SI), contrast-to-noise ratio (CNR) was calculated according to $(SI_{IODEX} - SI_{UL})/\sigma_{noise}$ and compared between UTE, ECHO and subtracted (UTE-ECHO) images.

Results and Discussion

Figure 1 displays a fluorescent microscopic image of labeled BMSCs with DAPI staining. Green fluorescence indicates the presence of particles, which have been efficiently endocytosed. The UTE image in figure 2 demonstrates the feasibility of obtaining positive contrast of IODEX-TAT-FITC labeled cells with a very short echo-time. This is due to the induced T1 relaxation effect of iron. We observe that although the relatively low iron content (~9.5pg / cell) resulted in a diminished T1 effect, contrast was still respectable at +26. To boost the CNR further, a subtraction was made between UTE and ECHO images, resulting in 30% improvement. This scheme to enhance contrast exploits the induced T2* relaxation effect of iron. The signal intensity pattern of typical susceptibility effect of iron is also clearly depicted in the positive contrast images.

Conclusion

We have shown that IODEX-TAT-FITC labeled bone marrow stem cells can be detected with high positive contrast using the MUTE sequence. Subtraction between UTE and ECHO images could further enhance positive contrast by 30%. Thus, conflict between cell viability and high detection sensitivity can be avoided with these nanoparticles. Further work includes studying the effects of lower cell concentration and incubation time on iron content per cell and the resulting MR sensitivity.



References
[1] Jackson et al. J. Nueroscience Meth. 183, 141-148 (2009)
[2] Lee et al. WMIC 2008. Abstract 1048.

Figure 1: Fluorescent microscopic image (Nikon 90I with FL/DIC/Phase) of IODEX-TAT-FITC labeled BMSCs. Green fluorescence indicates nanoparticles. Nuclei are stained blue with DAPI.

Figure 2: In-vivo coronal UTE (TE=0.3ms), ECHO (TE=8ms) and subtracted (UTE-ECHO) images acquired with MUTE. White arrow depicts position of labeled cells in the striatum.