Tracking of mesenchymal stem cells in a rat stroke model using a novel multimodal, plasmid-functionalized nanoparticle

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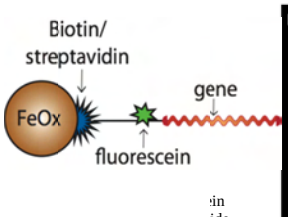
Introduction: Cellular therapies for neuronal injuries have shown therapeutic potential. Of particular interest are mesenchymal stem cells (MSCs), which are suggested to secrete proteins for the protection and regeneration of brain tissue [1]. However, little is known about the actual fate of transplanted stem cells and whether such protein secretions have extended therapeutic potential for ischemic stroke. In this study, we have fabricated an iron oxide based MRI contrast agent with a fluorescein tag and DNA plasmid for expressing mCherry. These modifications impart multimodality to both the nanoparticle and transfected cell, while hinting at the potential for genetically modifying transplanted cells to alter their secretory profiles. These agents were transfected into rat MSCs and imaged in tissue mimicking phantoms. Labeled rMSCs were utilized in a middle cerebral artery occlusion (MCAO) model for MRI detection and *ex vivo* optical verification of the fluorescein-tagged nanoparticle with mCherry expression of implanted cells.

Methods: Strepavidin-coated, 350-nm diameter iron oxide particles (Bangs Laboratories, Fisher, IN) were functionalized with a 35-mer DNA with a biotin tag and an opposing C6 thiol modification. The plasmid (mCherry) was linearized, ligated to a C6 thiol-modified linker strand and mixed to form a disulfide assembly. To ensure pure populations of MSCs, rat femur marrow MSCs (rMSCs) were harvested directly and treated with biotinylated CD54 and CD90 antibodies, followed by incubation with IMag particles and repeated cell separation runs (IMagnet, BD Biosciences, San Jose, CA). Following purification, nanoparticles were transfected into rMSCs for 24 hrs, followed by three washes to eliminate external particles and contamination. The cells were incubated for 72 hrs to initiate plasmid incorporation and mCherry expression. For *in vitro* experiments, cells were harvested and mixed with an equal volume of 2% agarose, set in a 10-mm NMR tube and image at 11.75 T using T_2- and T_2*-weighted spin echoes and a 3D gradient recalled echo (GRE) with 50-µm isotropic resolution. *In vivo* transplantation was in conjunction with a middle cerebral arterial occlusion following Longa et al [2] and Uluç et al [3]. One hour after MCAO, 1×10^6 cells were transplanted using a 10-µL microsyringe (Hamilton, Reno, NV) and injected in the exposed common carotid artery (CCA) [4] of a Sprague-Dawley rat. Six hours after transplantation, the animal was sacrificed and transcardinally perfused with 4% paraformaldehyde. The entire rat head was imaged at 11.75 T with a 25-mm birdcage coil using a 3D GRE sequence at 100-µm isotropic resolution. Fluorescent images of the fluorescein and mCherry were acquired *in vitro* and *ex vivo* from cell culture monolayers and 60-µm thick preserved brain sections, respectively, with a Nikon C1si Laser Scanning Confocal Microscope (Nikon Instruments Inc., USA).

Results: Increased nanoparticle exposure to rMSCs reveals increased uptake as evidenced by progressively increasing hypointense contrast in the tissue phantom of Fig. 2 displaying 150,000 rMSCs per layer exposed to increasing Fe masses. As shown in the confocal images of Fig. 3, the nanoparticles are localized in the perinuclear region. After arterial injection, 3D GRE images clearly reveal the labeled rMSCs as hypointensities in the ipsilateral side of the stroked brain (Fig 4). These dark signal voids can be seen throughout this hemisphere of the brain. The corresponding confocal image (Fig5), with red and green channels overlaid, reveals the green fluorescein signal from nanoparticles within a blood vessel where they are likely to reside due to the short time from transplant to animal sacrificing. This overlay confirms that nanoparticle-labelled cells have migrated intact to the site of injury as seen by the non-native mCherry expression. In addition, there is evidence of cells starting to move outside the vessel, as indicated by spectral deconvolution (orange signals).



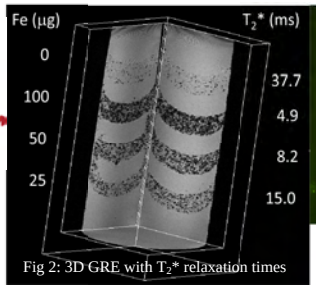


Fig 2: 3D GRE with T_2* relaxation times

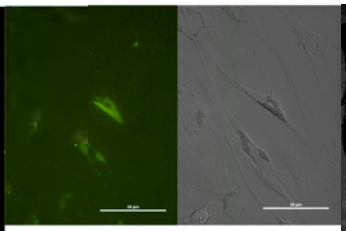


Fig 3: Fluorescent (left) and DIC (right) images of in vitro labeled rMSCs at 40x magnification. Scale bar = 50µm

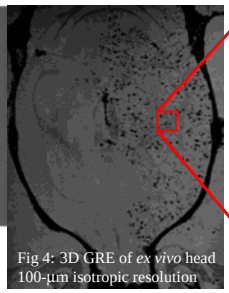


Fig 4: 3D GRE of *ex vivo* head 100-µm isotropic resolution

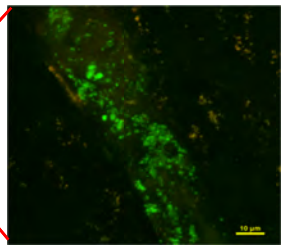


Fig 5: Fluorescent confocal image of red and green overlay. Scale bar = 10 µm

Discussion: This preliminary study shows that a nanometer-sized iron oxide particle functionalized with fluorescein and a plasmid for mCherry expression can be incorporated into rMSCs without addition of cell penetrating peptides (CPPs) or transfection agents. The fluorescein allows for bimodal imaging of nanoparticle location while the mCherry expression provides independent verification of the cell type specific to the transplanted cell line and simultaneously demonstrating that rMSCs can be modified genetically. Beyond cell identification, these techniques demonstrate that a MRI contrast agent can be engineered to follow the transport and fate of transplanted cells while introducing genetic alterations to enhance protein expression. Using this nanoparticle construct, future efforts will modify, profile and characterize the secretory nature and therapeutic timeline of MSCs as a valid stroke therapy.

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1. Caplan,A.I. *et a, J. Patol* (2009); 2. Longa, E. Z., *et al Stroke* (1989); 3. Uluç, K. *et al* J.Vis.Exp. (2011); 4. Chua,J.Y. *et al J.Cereb.Blood Flow Metab* (2011).