Magnetic Resonance Imaging to Potentially Monitor Migration of Magnetically Labeled Mesenchymal Stem Cells in a Murine Model of Myocardial Infarction

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Introduction

Cellular Magnetic Resonance Imaging (MRI) is developing rapidly, having found widespread applications in biology and medicine (1). The approach of prelabeling of cells followed by in vivo imaging has begun to be applied clinically (2). Various methods for magnetically labeling stem and other cell types with superparamagnetic iron oxide particles (SPIOs) or micrometer-sized particles of iron oxide (MPIOs) for detection by in vivo MRI have been well investigated (3). In this study, mesenchymal stem cells (MSCs) were labeled with MPIOs (Bangs) and introduced via bone marrow transplantation. MPIOs are well known to cause T_2* and T_2 shortening following uptake by stem cell and other cells, therefore we hypothesize that MSCs labeled with MPIOs, will potentially infiltrate and engraft to the site of injury such as myocardial infarction and that a hypointense signal will be evident in their vicinity by noninvasive MRI due to cellular engrafting. In this study, we examined pronounced signal intensity attenuation due to cells infiltrating into the infarction site and surrounding area following myocardial injury, which potentially suggests accumulation of MSCs with Bangs particles. The MRI findings were further correlated to *ex vivo* fluorescent imaging and histological analysis.

Methods

Bone-marrow-derived MSCs were isolated from 18 month old C57BL/6 mice (National Institute for Aging). The MSCs were infected with a reporter retrovirus that expresses enhanced green fluorescence protein (EGFP) under the control of the CMV promoter (Delta-U3-GFP). Bangs particles (Bangs laboratories Inc. Cat# ME03F) were opsinized in 50% FBS for 1 hour at 37 ºC. These particles are large styrene/divinyl benzene-coated magnetic microspheres with a mean diameter of 1.63 µm, encoded with suncoast yellow fluorescent label. Beads were washed in PBS then $1x10^8$ beads were resuspended in 1 ml of expansion media (DMEM (MediaTech with glucose & L glutamine Cat#10-013-cv), 10% FBS (Atlanta Biologicals), 1% antibiotic/antimicotic (Penn/Strep with amphotericin B, CellGrow Cat# 30004CL)). Media was changed and 100 µl of beads were placed in 10 ml media with cells 48 hours after being split.

Following incubation with the paramagnetic beads for 48 hours the MSCs were washed in PBS, fixed for 1-2 hr at room temperature (RT) in 0.1 M sodium cacodylate (NaCac) with 4% formaldehyde and 2% glutaraldehyde. Cells were postfixed in 2% OsO4 in 0.1 M NaCac for another 1 hr, then washed, dehydrated and embedded. Thin sections (75 nm) were cut and collected on Pioloform-coated slot grids, stained with uranyl acetate and lead citrate. The cells were examined in a JEM 1230 transmission electron microscope (JEOL USA Inc., Peabody, MA) operating at 110 kV and imaged on UltraScan 4000 CCD camera & First Light Digital Camera Controller (Gatan Inc., Pleasanton, CA).

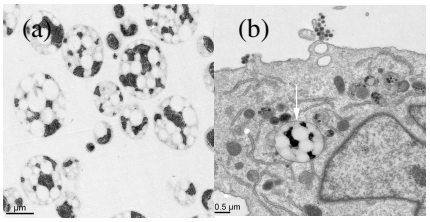

Figure 1. Electron Microscopy (EM) pictures for free Bangs particles (a) and particles taken up by MSCs (b). White arrow indicates Bangs particle taken up by MSC.

MSCs in tissue culture were imaged using an inverted Zeiss Axiovert 10 with a FITC filter for the EGFP expressed by the MSCs, a Rhodamine filter for the Suncoast Yellow paramagnetic beads and phase contrast for total cell identification. The images were captured with a SPOT camera (Diagnostic Instruments) and SPOT Advanced imaging software. Cells were imaged following 48 hours of incubation with the beads, immediately before trypsinization and replating, or after an additional 24 hours following 1:6 replating. Additional images were taken after each replating just prior to the next passage.

Mice were irradiated with 8 Gy and then received a rescuing bone marrow transplantation of whole bone marrow from a C57BL/6 donor 24 hours later via injection into the retroorbital plexus (1/5 – 1/10 of the bone marrow from both tibias and femurs of a single donor which provides approximately 5×10^5 - 2×10^6 nucleated cells). The MSCs, labeled with Bangs particles and expressing EGFP, were transplanted intra-bone marrow (I-BW) by injected into the tibial modulary pace (30-100 µl of 1.3×10^6 cells/ml in PBS).

Adult mice (body weights 22.7 $\pm$ 0.8 g) were used in this study. Myocardial infarction (MI; n=2; open chest with Left Anterior Descending (LAD) coronary artery ligation) and sham (n=2; open chest without myocardial injury) groups underwent surgery 14 days following MSCs transplantation, which allowed MSCs to integrate into bone marrow. MRI was performed 1 day before surgery as baseline, 3 days, 7 days and 14 days post-MI.

Images were acquired on a horizontal 7.0-T, 20-cm MRI spectrometer (Bruker Instruments, Billerica, MA). A 35mm inner diameter volume coil was used to transmit and receive at ^{1}H frequency. Animal setup procedure followed those previously described (4). ECG and respiratory signals were monitored and used for gating (SA Instruments, Inc., Stony Brook, NY). Short-axis heart images were acquired using a cardiorespiratory-gated Gradient Echo sequence with Flow Compensation (GEFC; TR = 120 ms, TE = 4 ms, FA = 30^o , FOV = 30 mm, Matrix Size = 256 x 256) and Multiple Slices Multiple Echoes T_2 Mapping sequence (MSME; TR = 1000 ms, Average TE = 20 ms, FOV = 30 mm, Matrix Size = 256 x 256). Region-of-interest (ROI) analysis was performed on Paravision 4.0 software (Bruker Instruments, Billerica, MA). A contrast-to-noise ratio (CNR) showing the difference between MI site and chest wall was calculated.

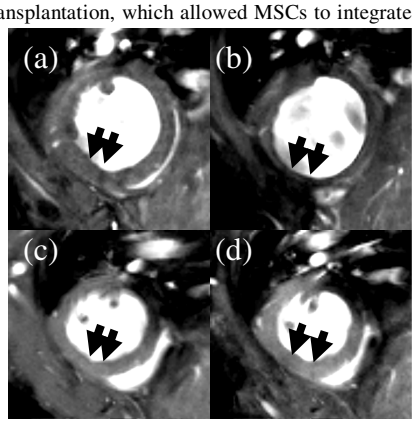

Figure 2. GEFC T2* weighted short-axis heart images 4 days (a), 7days (b) after MI surgery; 4 days (c), 7 days (d) after sham surgery. Black arrows point to MI site (an assumed MI site in sham group was used for comparison).

Hearts were excised at the terminal MRI point and fluorescent imaging was performed on a 2-D IVIS 100 Imaging System (Xenogen Corporation, CA). The heart was cut into sections for staining and histological analysis once MRI and fluorescent imaging were finished. H&E staining, MSC, macrophage and iron staining were performed.

Results

MSCs were labeled with Bangs particles with an efficiency of 98% plus, while their viability and proliferation function were preserved. Most Bangs particles were internalized into MSCs (**Figure 1)**. Signal intensity attenuation post-MI was observed (**Figure 2**) in MRI, potentially due to accumulation of labeled cells. This finding was correlated to the strong signal in *ex vivo* fluorescent imaging of localized suncoast yellow and GFP labeling. Further validation by histopathology would be warranted. There might be immune cells, which contain Bangs particles from dead MSCs, presenting in the ischemic myocardium.

Conclusions

MSCs were successfully and efficiently labeled with MPIO Bangs particles. Hypointense signal was observed in T_2* and T_2 weighted images of mouse myocardium, suggesting the infiltration of labeled MSCs. This finding may support an approach in cell therapy to monitor migration and trafficking of labeled cells post myocardial injury.

References

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