MRI Quantification of USPIO-Labeled Cell Density and Number

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Introduction

MRI is capable of tracking cells after transplantation in cell-based therapies, assessing anatomical and functional recovery in vivo with high spatial resolution. Quantitative analysis of labeled cells after transplantation may allow more accurate assessment of cell delivery and subsequent distribution and migration, which can lead to more effective monitoring and optimization of therapeutic paradigms. So far, efforts in MRI quantitation of labeled cells have been focused on the theoretical formulations and numerical simulations of MR signal decay and transverse relaxation induced by magnetically labeled cells [1-3]. In this study, we present a MRI technique to quantify magnetically labeled cells that were injected in agarose gel phantoms, mimicking the in vivo transplantation.

Methods

Cell labeling: Mouse embryonic stem cell (mESC) and human embryonic kidney (HEK293) cells were labeled with MION-47 and transfection agent Poly-L-lysine (PLL; Sigma, St Louis, MO). PLL of concentration 2.0 μg/mL was mixed with MION of concentration 50.0 μg/mL for 60 min in cell culture medium at room temperature. These culture media were then added to the cells and kept overnight for 24 hours at 37 °C in a 95% air per 5% CO_2 atmosphere. Cells were washed twice with PBS, trypsinized, washed, and resuspended in PBS at respective concentrations for making phantoms. Viabilities of mESCs and HEK293 cells after labeling were >92% using Trypan blue staining. Intracytoplasmic iron uptakes of labeled mESCs and HEK293 cells were confirmed by the Perls' Prussian blue staining.

Phantom Preparations: Uniform gel suspensions (1% agarose gel) of mESCs and HEK293 cells were prepared with cell concentrations ranging from 0 to 5.0×10^6 cells/mL in separate 4-cm long, 1-cm diameter cylindrical phantom tubes. For injection phantoms, different numbers of mESCs and HEK293 cells (5.0×10^4 to1.0×10^6 cells) in 5μL PBS were injected into cylindrical holes of 3-mm diameter inside the agarose gel tubes. Immediately after the injection, the holes were filled with agarose gel. This procedure allowed control of the numbers of cells injected with accuracy, without being interfered by the pressure and backflow problems often encountered by cell injections in vivo.

MRI: All MRI experiments were performed at 3.0 T (Philips Intera Achiva) using a wrist coil with cylindrical phantom tubes placed parallel to the main magnetic field. All T_2*-weighted images were acquired axially by 3D gradient echo (GE) sequence with TR = 50 ms, FA = 30º, FOV = 77 mm, acquisition and reconstruction data matrix = 128×128×32 and 256×256×32 respectively. The final reconstructed resolution was 0.3×0.3×0.6 mm^3. For gel suspensions of labeled cells, 3D GE images were acquired with TE = 4, 8 12, 16, and 20 ms. R_2* maps were computed on a pixel-by-pixel basis by linear least-squares fitting of log signal intensity vs. TEs. Images were analyzed using a software toolkit developed in IDL (RSI, Boulder, CO). R_2* measurements were made in a circular ROI encompassing each phantoms, and taken from the average in three contiguous slices. Values of relaxation rate enhancement ΔR_2* in each tube with labeled cells were calculated by subtracting R_2* of that tube by that of gel-only tube. To quantify the labeled cells injected in gel, 3D GE images with TEs of 2 and 4 ms were acquired. ΔR_2* maps were computed on a pixel-by-pixel basis. By assuming an approximate linear relationship between local ΔR_2* and cell density, the total number of cells in each 5 μL injection was estimated by:

$$\text{Estimated Cell Number} = \iiint_{\text{Injection region}} \{\Delta R2^*/C\}\,dV \approx \sum_i \sum_j \sum_k \Delta R2^*(i,j,k)\cdot\Delta x\cdot\Delta y\cdot\Delta z / C$$

where C is the calibration constant in unit of s^{-1}/(cells·mL^{-1}) that were to be derived from MRI measurements of relaxivity ΔR_2* vs. cell concentration in uniform cell suspensions. Δx·Δy·Δz is the reconstructed voxel size in unit of mL, and i, j, and k are the indexes of voxels within injection region that were hypointense due to labeled cells. During the analysis, 3D volumes of interest (VOIs) were first manually defined to encompass the hypointense injection regions in T_2*-weighted images. Then the total cell numbers were estimated using Eq. [1] three times independently, and results averaged.

Results

Fig. 1 shows the plots of ΔR_2* against cell concentration of mESCs and HEK293 cells. Despite the nature of inhomogeneous clustering of USPIO nanoparticles inside the cells, linear relationships were clearly seen for the cell concentration range studied. Such linear relationships were consistent with a recent study of SPIO-labeled human melanoma cells by others [4]. The slope or calibration constant C of mESCs was 7.51×10^{-6} s^{-1}/(cells·mL^{-1}) and that of HEK293 cells was 1.24×10^{-5} s^{-1}/(cells·mL^{-1}) for the labeling procedure used. Fig. 2 shows six contiguous slices covering an hypointense region due to the injection of 5.0×10^5 labeled mESCs at TE = 2 ms, together with the corresponding VOI used in the total cell number calculation. Note that hyperintense boundaries were seen around the ROIs. They were likely caused by the susceptibility effect arising from the inhomogeneous interface between the gel and 5 μL cell suspension volume injected. Fig. 3 demonstrates the excellent agreement between actual cell numbers and MRI estimations for cell numbers from 5.0×10^4 to 1.0×10^6 cells with the percentage discrepancies ranging from 1% to 14%.

Discussion

The results illustrate the feasibility to quantify local cell density and number after transplantation by calibrating the relaxivity of labeled cells in vitro and quantifying local ΔR_2* in vivo. The calibration constant C in unit of s^{-1}/(cells·mL^{-1}) depends on the cell type, contrast agent concentration, transfection agent and incubation procedure, and it is desirable to determine it using the same batch of labeled cells prior to transplantation at the same field strength. The technique demonstrated here is potentially applicable for accurate assessment of local retention of donor cells in specific tissue or organ after systemic and local injection in cell-based therapies.

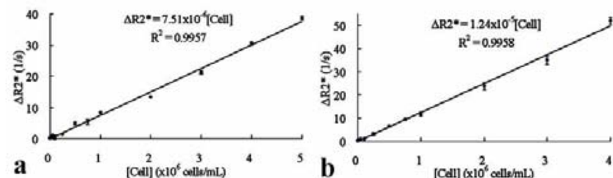

Fig.1 Approximately linear relationship was founded between ΔR_2* and mESC concentrations (**a**) and HEK293 cell concentrations (**b**).

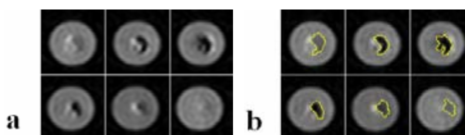

Fig.2 Six contiguous slices of 3D gradient echo images at TE=2ms showing the hypointense volume where 5.0×10^5 labeled mESCs were injected in agarose gel (**a**) and delineation of VOI used to calculate total number of cells injected (**b**).

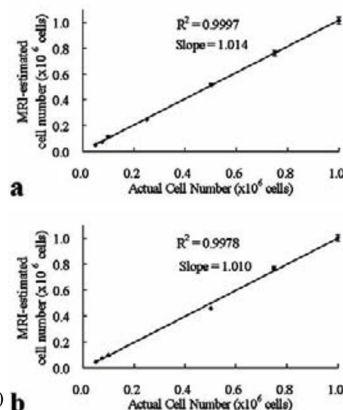

Fig.3 Numbers of labeled cells injected in agarose gel estimated by MRI vs. the actual cell numbers in mESC (**a**) and HEK293 (**b**) cell injection phantoms.

References [**1**] CV Bowen et al, MRM 2002;48:52-61. [**2**] CH Ziener et al, MRM 2005;54:702-706. [**3**] J Pintaske et al, MAGMA 2006;19:46-53. [**4**] J Pintaske et al, MAGMA 2006;19:71-77.