In vitro MR assessment of proliferating SPIO-labeled mouse embryonic stem cells

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Introduction. Mouse embryonic stem cells (mESCs) are derived from the inner cell mass of pre-implantation mouse embryos representing precursor cells capable of pleuripotential differentiation in the embryo. We recently reported that mESC transplantation may salvage infarcted myocardial tissue in mouse [1]. One of the findings from this study was the ability to track magnetically labeled mESCs for the duration of 4 weeks using a 1.5 T clinical MR scanner. However, several questions regarding the imaging characteristics of proliferating cells need to be determined: 1) effect of multiple cell divisions on cell labeling, 2) quantitative correlation between the number of cells and magnetic signal intensity, and 3) signal detection at higher magnetic field. In order to address these issues, we conducted an *in vitro* investigation of proliferating SPIO-labeled mESCs by studying T2* and T1 effects of the mESC undergoing 4 cell divisions using fast GRE and inversion recovery-GRE (IR) sequences at 1.5 T and 4.7 T.

Methods. Cell labeling solution was prepared by incubating 25 μg/ml of Feridex (Berlex Laboratories, Wayne, NJ) with 375 ng/ml of poly-l-lysine (Sigma, St. Louis, MO) for 60 minutes [2]. The mESC line, TL-1, derived from 129Sv/J mice, was cultured in DMEM supplemented with 10% fetal bovine serum, L-glutamate, penicillin, streptomycin, 0.1mM B-mercaptoethanol (Sigma, St. Louis, MO) and 10^3 μ/ml leukemia inhibitory factor (Chemicon International, Temecula, CA). The cell labeling solution was incubated with 1 cc of mESC medium (approximately $2x10^6$ cells) for 12-24 hours. The labeled mESCs were propagated for a period of 0-day, 2-day, 4-day, 8-day, and 16-day (1 cell division/2days). At each time point, 4 different populations of approximately $1x10^6$, $0.5x10^6$, $0.25x10^6$, and $0.1x10^6$ mESC were selected. Four samples of each population group were suspended in a vial with agar gel filling at each time point and the 4 different quantities of labeled mESC were scanned using a conventional 1.5T Signa MRI Scanner (GE, Milwaukee, Wisconsin) and a Unity Inova console (Varian, Inc., Palo Alto, CA) controlling a 4.7T, 15cm horizontal bore magnet (Oxford Instruments, Ltd., Oxford, UK) with GE Techron Gradients (12G/cm) and a volume coil with an inner diameter of 3.5cm (Varian, Inc., Palo Alto, CA). For 1.5 T, signal intensity using inversion recovery sequence (TE: 2.5, TR: 500) was measured for inversion time (TI) of 100-500. The GRE sequence consisted of 20 ms TR, 8.0 ms TE, and 60° flip angle. For 4.7 T, the GRE sequence consisted of 20 ms TR, 6.0 ms TE, and 60° flip angle. The T1 effects were determined by measuring the signal enhancement and T2* effects were assessed by measuring the area of susceptibility signal.

Results. This study demonstrates that the effects of cell division reduced the T1 and T2* effects. At 1.5 T, the T1 effects were seen up to 8 days (3 cell divisions) with more T1 shortening effects in larger cell population as shown in Figure 1. The T2* effects were seen up to 8 days (3 cell divisions) in $1x10^6$ cells as shown in Figure 2. The T2* signal area and the number of cells demonstrated a statistically significant correlation at day 0 and 8, r=0.87 and 0.78 ($p<0.05$), respectively. In addition, there was a significant difference in the signal area between 0.1 vs $1x10^6$ and 0.25 vs $1x10^6$ up to 8 days (3 cell divisions) as shown in Figure 2. In 4.7 T, the T2* effects were seen in all 4 mESC populations up to 16 days (8 cell divisions) as shown in Figure 3.

Conclusion. This study demonstrates that there are quantitative and temporal limitations on the persistence of T1 and T2* effects on cell labeling. However, these limitations may be overcome by higher magnetic field as shown by persistence of signal in all 4 populations up to 16 days (8 cell divisions) at 4.7 T. Finally, the measured areas of susceptibility artifact appear to correlate with the quantity of labeled cells. However, accurate quantitation of the USPIO-labeled cells with susceptibility signal may be difficult.

References.

[1] Arai T, et al. J Am Coll Cardiol 2004 (accepted)

[2] Frank JA, et al. Radiology 2003;228:480-487.

Figure 1. Inversion recovery signal of PLL-Fe labeled cells, 0-day/1 million, 0.1 million, and 8-day/1 million, 0.1 million cells

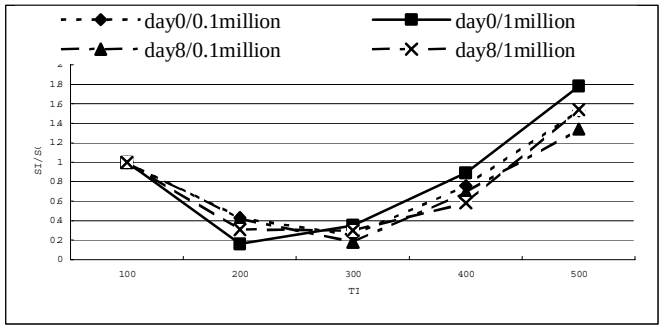


Figure 2. The area of T2* effect (mm^2), 0-day and 8-day/1 million, 0.5 million, 0.25 million, and 0.1 million cells

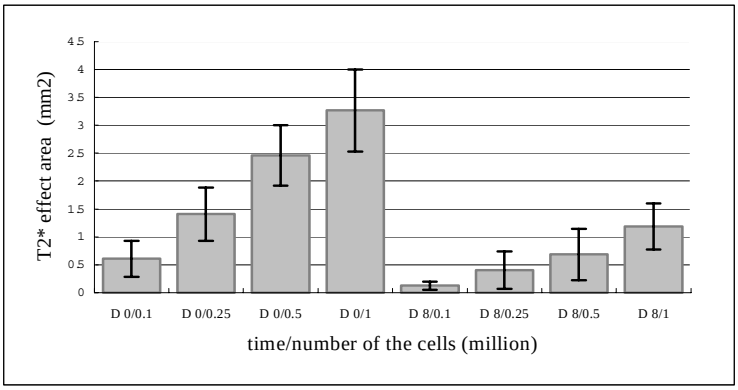
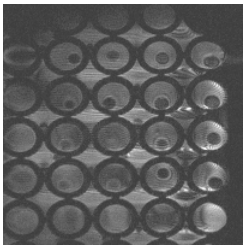


Figure 3. PLL-Fe labeled cells 16 days after labeling at 4.7T



← $0.1x10^6$ cells
← $0.5x10^6$ cells
← $0.25x10^6$ cells
← $0.1x10^6$ cells