Positive Contrast Imaging of Iron-Oxide Labeled Human Embryonic Stem (hES) Cell and Fibroblast using SWEET

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Introduction: The MRI detection of superparamagnetic iron-oxide particles (SPIO) -labeled cells is important in cell-replacement therapies, which can help monitoring the migration/proliferation of cells following the transplantation. Cells loaded with SPIOs cause signal reduction (negative contrast) associated with dephasing due to the magnetic field inhomogeneity near the cells, therefore the signal void are typically used as the means for detection of the cells. Negative contrast agents have drawback of being confused with the signal void caused by other sources such as tissue inhomogeneity or partial volume effects [1, 2]. Selective and positive contrast for SPIO-labeled cells is warranted with suppression of background tissue, and recently, several methods to elicit positive contrast have been suggested [2-4]. Off-resonance imaging using spectrally selective RF pulse has the advantage of detecting the positive signal proximal to the cells [2, 3], however, requiring special sequence programming. We were motivated to provide simple means of detecting SPIO-labeled cells by using susceptibility weighted echo-time encoding technique (SWEET) whereby the subtraction of two sets of image volumes acquired at slightly-shifted echo time generates positive contrast at the cell position [5].

Methods: In this study, we investigated in-vitro, positive contrast imaging of the SPIO-labeled cells using a SWEET method [5]. **Type of SPIOs:** Two different types of SPIOs were tested; ultra small dextran coated iron-oxide particles (USPIO; Feridex, Berlex Laboratories Inc. Wayne, NJ, USA) and micrometer-sized iron-oxide particles (MPIO; Bangs Laboratories, Fishers, IN, USA). Two different sizes of MPIOs (0.96um and 1.63um diameter; tagged with green fluorescence) were tested. **Cell Preparation:** Human fibroblasts and embryonic stem cells were derived and cultured using standard method [6]. The day before the labeling, fibroblasts were counted by hematocytometry and seeded into 100mm dishes. Human embryonic stem (hES) cells were expanded for 6 days and transfer to culture dishes at day 7. **Feridex Labeling:** 2.2ul of Feridex stock solution as provided by the manufacturer (11.2mg Fe/ml) was added per ml culture medium. For the efficient Feridex-uptake, Poly-L-lysine (PLL) was then added as a transfection agent. For fibroblast, $5x10^4$ human fibroblasts were incubated within the medium containing the Feridex-PLL mixture with 25ug Fe/ml Feridex and 375ng/ml PLL for 24 hours. Following labeling, the cells were washed three times and trypsinized, and counted by hematocytometry. For hES cells, twenty colonies were expanded for 5 days and labeled at day 6. **MPIO Labeling:** For cell labeling with MPIO, $1.6x10^9$ (0.96um) and $2.8x10^8$ (1.63um) MPIOs were added to the growth medium, and incubated with $5x10^5$ fibroblasts for 18 hours. After labeling, all cells were washed three times with sterile phosphate-buffered saline (PBS) to remove free particles. **Image phantom preparation:** To demonstrate that SPIO particles can be detected by the SWEET method, agar-gel phantom (35mm-diameter vials) was constructed. The vial was filled with 0.5% agarose gel except for a tube space (~15 ul) positioned top-middle portion of the vial. After removing the tube, SPIO particle dilutions and labeled cells were injected into the remaining tube-shaped space, and then sealed with agarose. To investigate positive contrast effect of SPIO particle, MPIO particles of 40ug, 28ug, 20ug, 12ug, and 4ug iron content per each vial were used. For labeled cell study, the agarose phantoms containing SPIO-labeled cells were composed of various numbers of labeled cells. $2x10^4$, $1x10^4$, $7x10^3$, and $5x10^3$ Feridex labeled fibroblasts were contained in tube-shaped gel vessels respectively. The phantoms of MPIO labeled cells consisted of $5x10^5$, $1x10^5$, $5x10^4$, and $1x10^4$ labeled fibroblasts. For hES cell phantom, a million of labeled hES cells were collected. **MR imaging:** MR images were acquired on a 3.0T MRI scanner (ISOL Tech. Korea) with custom surface coil. Positive contrast images were acquired from conventional spin-echo sequence and SWEET method with echo-time shift. Imaging parameters were used as follows, TR/TE=500/40ms; shifted echo-time=22ms; 256x256 matrices; 80x80mm^2 Field-of-View (FOV); 1.5mm slice thickness with no gap; and scan time=4.3 minutes with 2 averages. The difference from conventional images and the echo-time shifted image using SWEET means the susceptibility effect due to superparamagnetic iron-oxide, which manifest as the positive contrast in the presence of SPIO labeled cells.

Results: The effective internalization of SPIO particles was observed from the microscopic images is shown in Figure (A) and (B). Figure (C) shows the image from conventional spin-echo sequence and (D) illustrates the susceptibility effect due to the SPIO particles by the SWEET method on the same sample. In Figure (E), a positive contrast image was generated from difference of (C) and (D) (note that effective removal of the background signal). SPIO-labeled cell images (lower row in the figure grid) showed positive contrast in the labeled cell positions (H through J).

Discussion: We have shown that positive contrast visualization by SWEET scheme was possible without the use of dedicated pulse sequence programming. The method can separate out the magnetic susceptibility effect from main field-inhomogeneity and chemical-shift artifact [5]. The method can provide simple means of positive contrast cell-detection, readily available for the immediate use in clinical setting due to use conventional pulse sequence without specific sequence implementation. In spite of these advantages, limitations of the current approach include; (1) relatively long T2-weighting (spin-echo) to get similar T2* effect of gradient-echo pulse sequence, and (2) the generation of the positive contrast requires the acquisition of two sets of images, thus lengthening the data acquisition time. The adoption of parallel imaging techniques, steady-state free precession (SSFP)-type imaging, combined with reduced field-of-view approach would warrant further investigation.

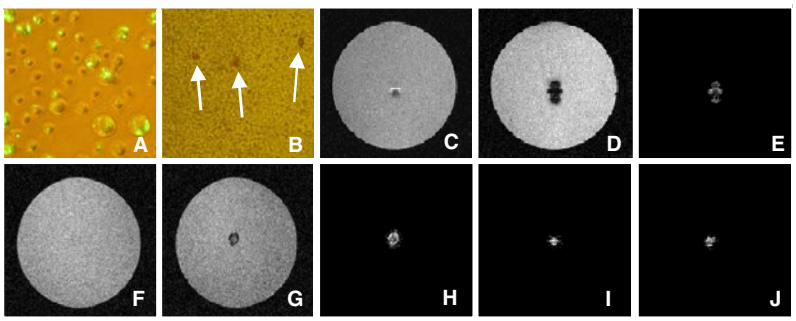

Figure (A): Labeled cells stereoscopic microscopy of MPIO (0.96um) labeled fibroblasts (fluorescent), (B): Feridex labeled hES cells, (C): Selected MPIO particle image by spin-echo and (D): SWEET, (E): Positive contrast image from (C)-(D). Selected images of Feridex labeled hES cells: (F) Spin-echo and (G) SWEET and (H) Positive contrast image on hES cells. Examples of positive contrast for iron-oxide labeled human fibroblasts (I): Feridex labeling (J): MPIO, were also shown.

References: [1] Shapiro *et al. PNAS*, 2004, [2] Gilson *et al. ISMRM*, 2005, [3] Cunningham *et al. MRM*, 2005, [4] Coristine *et al. ISMRM*, 2004, [5] Park *et al. Phys. Med. Biol.*, 1988, [6] Noboru Sato *et al. Nature Medicine,* 2004