Positive Contrast MRI of Magnetically Labeled Human Cervical Carcinoma Cells and Tumor Monitoring

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Introduction: There are rapidly increasing interests in cellular and molecular magnetic resonance imaging (MRI) techniques using contrast agents for the visualization of the migration, and growth of various types of cells, including stem cells and tumor cells. Several MR contrast agents have been suggested for use in cellular MRI. Among them, superparamagnetic iron oxide (SPIO)-based contrast agents are currently being evaluated as potential markers for the monitoring of cellular growth, migration, or proliferation since SPIO's can remain internalized for more than several weeks [1]. As a result the technique is applicable to monitoring tumor growth. However, SPIO-loaded cells cause signal reduction (negative contrast) associated with spin dephasing. This negative-contrast visualization is sometimes problematic since it can be confused with the signal voids caused by tissue inhomogeneity (low intensity). Several researchers have proposed positive-contrast imaging (PCI) methods using either spectral selection of an off-resonance region near the labeled cells [2, 3], suppression of non-labeled regions by dephasing [4] or spectral selection using different echo times [5]. We were motivated to investigate a method to generate positive-contrast by using a susceptibility weighted echo-time encoding technique (SWEET) [6]. We demonstrate the SWEET method can be useful to detect the magnetically labeled human cervical carcinoma (KB) cells implanted in mice, and to monitor tumor growth in mice *in vivo* for a duration of 2 weeks.

Methods: We verified the SWEET method as a PCI approach from *in vitro* gelatin gel phantom and animal (mouse) imaging. Furthermore, we monitored the tumor growth from injected cancer cells for 2 weeks and confirmed this growth with histological sections. **Cell preparation:** Human cervix carcinoma (KB) cells were used for tumor monitoring and a combination of a SPIO (Feridex, Berlex laboratories Inc) and a PLL (Poly-L-lysine) was used for efficient cell transfection. KB cells (20,000 cells/cm^2) were cultured in a 10% fetal bovine serum (FBS) RPMI (Roswell Park Memorial Institute) medium (supplemented with 100U/ml of penicillin and streptomycin) at 37°C. The cell labeling solution was prepared using 750ug of Feridex, 11.25ug of poly-L-lysine (PLL, Sigma) and 15ml of serum-free RPMI medium. The cells were incubated for 1 hour at 37°C for uptake, and then were washed three times with fresh phosphate-buffered saline (PBS) to remove the residual extracellular SPIO or PLL. ***In vitro* phantom imaging:** In order to verify the feasibility of the SWEET method, gelatin phantoms were constructed using 35 mm diameter vials. Phantoms containing eight different concentrations of cells (1x10^6, 5x10^5, 2x10^5, 1x10^5, 5x10^4, 1x10^4, 5x10^3 labeled, and 5x10^5 unlabeled KB cells) were prepared to image various concentrations of SPIO-labeled cells within a fixed area (selected data shown Fig. C to E). All MR images were acquired on a 3.0T MRI scanner (ISOL Tech. Korea) with a customized animal coil. Positive-contrast images were obtained from a conventional spin-echo sequence and the SWEET method with an echo-time shift (Imaging parameters: TR/TE=500/40ms; shifted echo-time=22ms; 256x256 matrices; 8x8 cm^2 Field-of-View (FOV); 1.5mm slice thickness with no gap; 2 number of excitations: NEX). ***In vivo* mouse imaging:** For the examination of the efficacy of our method in an animal model as well as the monitoring of the proliferation of SPIO-labeled cancer cells, we performed an *in vivo* animal study using nude mice. Either SPIO-labeled or unlabeled KB cells were harvested and then resuspended in PBS with a concentration of 3×10^7 cells/mL. One hundred microliter aliquots of the cell suspension were injected at two different sites; (1) subcutaneously onto the dorsal side and (2) intramuscularly into the hind legs of a BALB/c nude mouse (n=3). The left side was injected with SPIO-labeled KB cells and the right side with unlabeled KB cells acting as a control. For all mice, MR images were acquired prior to injection of the cells and at 8 subsequent time points (day 0; 1 hour after injection, day 1, day 2, day 4, day 7, day 9, day 12, day 14) after injection in order to monitor the proliferation of the cancer cells as well as tumor growth. The timing parameters were; TR/TE=500/20 ms, 256 x 256 matrix, 10 x 10 cm^2 FOV, slice thickness=3 mm. An echo-time encoding step (dT) in SWEET imaging used 4.48 ms and a total acquisition time was 4 min with 2 NEX.

Results: The effective internalization of SPIO particles was observed from fluorescent and the microscopic images as shown in Figures (A) and (B), respectively. The SWEET method selectively enhanced the effect of the field inhomogeneity caused by SPIO-labeled KB cells from the gel-phantom (Fig. C through E) and mouse (Fig. F) imaging. Figure (C) shows the image from a conventional spin-echo sequence and (D) illustrates the susceptibility effect due to the SPIO-labeled cells by the SWEET method on the same sample. In Figure (E), a positive contrast image was generated from the difference between (C) and (D) (note that effective removal of the background signals is evident). Figure (F) shows the *in vivo* mouse MRI results (see figure caption for more information). Labeled cells were marked in red arrows and unlabeled control cells in white arrows. Through the longitudinal MRI of the implanted tumor over a 2 week period, a solid tumor mass was observed to have grown from the cancer cells at a subcutaneous flank (panels on upper row) and intra-muscular space (bottom row) in Figure (G); After 2-weeks of MR imaging, we assessed histology sectioning in order to verify the correlation between positive-contrast in the MR images and the existence of SPIO particles (data not shown).

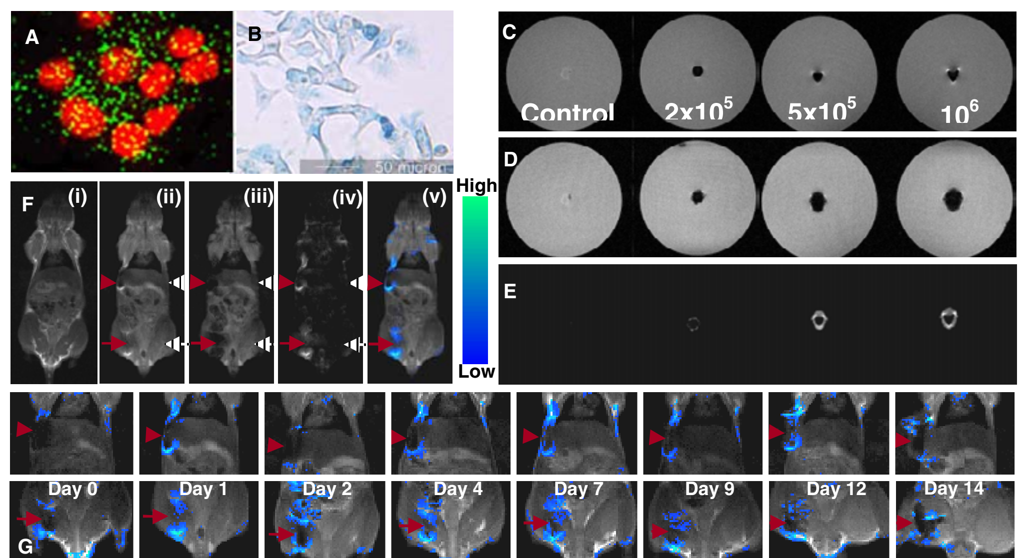

Figure (A) and (B): Labeled KB cells fluorescent and Prussian blue stained microscopy, (C): Selected gelatin phantom image by spin-echo and (D): SWEET, (E): Positive contrast image from (C)-(D). Selected images of Feridex labeled KB cells injected nude mouse: (F); (i) reference spin-echo image before injection, (ii) Spin-echo and (iii) SWEET, (iv) Positive-contrast image at 1 day after injection, and (v) Pseudo-coloring on positive-contrast. Figure (G): Monitoring of tumor growth for 2 weeks

Discussion: We have demonstrated that a proposed SWEET scheme has enabled *In vitro* and *in vivo* positive-contrast visualization without the use of complicated pulse sequence programming. However, a fundamental limitation of the proposed method is that susceptibility artifacts originate from sources other than labeled cells, such as the air-tissue interface of organs or the body, as seen elsewhere [2]. Additionally, the positive-contrast is shown as an enhancement around the edge of SPIO labeled sites as the tumor mass enlarges (in Figure G: Day 14). Recently, the simultaneous use of fluorinated nano-contrast agents ^{19}F and ^{1}H MR imaging has been validated on clinical MR scanners [7, 8]. The optimization of the SWEET technique and integration with the novel contrast agents constitutes further direction of our future investigation.

References: [1] J. Jackson et al. ESMRMB 2005, [2] C.H. Cunningham et al. (2005) Magnet Reson Med 53:999-1005, [3] M. Stuber et al. (2005) ISMRM, Miami, USA, p.2608, [4] J-H Seppenwoolde et al. (2003) Magnet Reson Med 503:784-790, [5] W. Chen (2006) ISMRM, Seattle, USA, p.1825, [6] HW Park (1988) Phys. Med. Biol. 33(3):339-349, [7] F.S. Fischer Circulation. (2001) 11;104(11):1280-5, [8] J. Keupp et al. (2006) ISMRM, p.102