

Dual Contrast Method for Cellular MRI using Positive and Negative Contrast Agents

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Introduction: MR contrast agents (CAs) are important elements for cellular MRI. Gadolinium (Gd) chelates are used in T1-weighted production of positive signal contrast while superparamagnetic iron oxide particles (SPIOs) produce T2-weighted signal reduction. Although SPIOs provide more sensitivity toward cell labeling compared to Gd-chelates, the negative contrast generated by the SPIOs may be confused with signal voids from tissue inhomogeneity, edema, or local hemorrhage. Combination of both Gd-based and SPIO-based CAs may provide unique opportunity in creating user-tunable contrast mechanism. We examined the utility of using combination of these two CAs in MRI for concurrent cell-labeling and dual contrast visualization.

Methods: MR Imaging: All MR imaging was performed with 3 Tesla MRI system (ISOL tech. Korea) and a conventional spin-echo sequence with the following acquisition parameters; FOV (field of view) of 80x80 mm² (100x100 mm² for aqueous phantom), matrix size of 128x128, flip angle of 90°, a single 3 mm thick slice, and a number of signal average (NSA) of 2. For calculating R1 and R2 relaxation rates, spin-echo images were acquired with TRs ranging from 100 to 5000 ms and a TE of 12 ms for R1, and a TR of 3000 ms with TEs ranging from 12 to 500 ms for R2.

We prepared three types of phantoms to examine the effects of proposed use of composite CAs. **Aqueous phantom:** Gadodiamide (Omniscan, Gd-DTPA-BMA) and ferumoxide (Feridex; SPIO) were used as positive and negative CAs, respectively; both are clinically approved with stable relaxation properties proportional to their concentrations [1]. We assumed that the interaction between the two CAs was negligible and evaluated the composite relaxation property of free dispersion in saline with different concentrations (from 0 to 0.3 mM-Gd and 0 to 21.5 µM-Fe) including range for clinically recommended dosages [1]. **Separately-labeled cell phantom:** To investigate the relaxation characteristics of intracellular contrast agents, rat brain glioma cells (C6, ATCC, Manassas, VA) were separately labeled with each CA. Cells were independently incubated with Feridex-PLL or Omniscan-Lipofectamine solution for 6 hours at 37°C; this protocol was determined from experiments measuring cell viability and quantification of CA uptake. To verify the intracellular presence of SPIO particles, Prussian blue staining was performed. After the intracellular Gd and Fe concentrations of the cell suspensions were measured by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy), labeled cells were suspended in 25 tubes (5x5) of Ficoll solution, which provided even cell distribution [2]. The final concentrations were 0, 0.05, 0.1, 0.15, 0.2 mM-Gd and 0, 5.4, 10.7, 16.1, 21.5 µM-Fe. **Concurrently-labeled cell phantom:** In order to verify the potential application of the proposed concurrent method, we labeled the cells with both CAs simultaneously. Sixteen labeling solutions (4x4) consisting of Omniscan (0, 90, 180, and 270 µL Gadodiamide) and Feridex (0, 1.24, 2.48, and 3.73 µg Fe) were prepared; this concentration of the CAs was determined in an empirical manner. Labeling solutions were mixed with 100 µL PLL and 1.5x10⁵ cells/cm² with 15 mL of DMEM culture medium for 6 hours at 37°C and the labeled cells were suspended in 16 tubes (4x4) of Ficoll solution. Internalization of two CAs was measured by ICP-AES after MR imaging (data not shown).

Results: The relaxation properties of the CAs were observed from the three types of composite phantom environments. The data obtained from the aqueous phantom confirmed the previous investigation [1], suggesting that composites of two CAs create the new relaxation characteristics. The data obtained from separately labeled cells [Fig. 1 A and B], and concurrently labeled cells [Fig. 2 A and B] were shown. The R1 and R2 relaxation rates of both composite cell labeled phantoms increased linearly with increasing CA concentrations, although the concurrent labeling scheme exhibited some variations in intracellular CA concentrations. The images of the composite sample tubes showed both T1 and T2 effects compared to the control sample (dotted circle) in the T1- and T2-weighted images (Fig. 1 C and D for the separately labeled cells, Fig. 2 C and D for the concurrently labeled cells).

Discussion: We demonstrated that the composite contrast scheme using two CAs could visualize dual (positive and negative) contrast characteristics in an intracellular environment, thus providing more constant weighting for visualization of CA-labeled cells without pulse sequences for positive contrast imaging [3, 4] or multi-functional CA [5]. However, a fundamental limitation of the proposed method is that excessive concentration of SPIO will wipe out the T1 enhancement of the Gd-chelate. The optimization of the composite concentration and the verification of *in vivo* characteristics will be a focus of future investigation because the proposed method may overcome the drawbacks of using single CA for cell labeling.

References: [1] Marzelli M. et al. (2008) Int J Imaging Systems & Technol 18:79-84, [2] Graziani-Bowering G.M. et al. (1997) J Immunol Methods 207:157-168, [3] Cunningham C.H. et al. (2005) Magn Reson Med 53:999-1005, [4] Seppenwoolde J-H. et al. (2003) Magn Reson Med 50:784-790, [5] Daldrup-Link H.E. et al. (2004) Eur J Nucl Med Mol I 31(9):1312-1321.

Figure 1. A and B: R1 and R2 relaxation rates for the 'separately' labeled cell phantom respectively. C and D: T1- and T2-weighted images for the 'separately' labeled cell phantom. **Figure 2.** A: R1 relaxation rates with respect to the intracellular Gd concentration, B: R2 relaxation rates with respect to the intracellular Fe concentration for the 'concurrently' labeled cell phantom. C and D: T1- and T2-weighted images of the 'concurrently' labeled cell phantom. (Width of blue/red gradient bars denotes concentration of Gd and Fe, respectively. Dotted circles denote the control with unlabeled cells (white), the single CA labeled cells (blue for Gd, red for Fe), and the solid green circle depicts a targeted concentration for dual contrast.)

