Positive Contrast Visualization of SPIO Labelled Cells by Diagonal-SPRITE

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Introduction

Superparamagnetic iron oxide particles (SPIOs) have been used to label a range of cells [1]. However, the presence of SPIOs, possessing high positive susceptibilities in the cell, leads to magnetic field inhomogeneities that lead to significant signal dephasing. The subsequent signal loss is comparable to that observed at tissue-air interfaces except that the susceptibility mechanism occurs at a smaller scale, typically within the dimensions of the imaging voxel. Susceptibility contrast enhancement necessitates MR techniques that demonstrate T2* contrast. Conventional spin-echo and inversion-recovery sequences are not optimal for susceptibility imaging and gradient-echo based pulse sequences are commonly employed. However, signal voids arising from susceptibility agents such as SPIOs cannot be distinguished from bulk susceptibility differences occurring at air-tissue interfaces or air-bone interfaces. This can be overcome by the application of ultra-short TE, UTE [2] method such as Diagonal-SPRITE [3] which is also able to generate positive contrast from the presence of susceptibility agents. In this study, Diagonal-SPRITE MR methods were used to provide positive contrast of SPIO labelled cells *in vitro* and *in vivo*.

Method

IGROV1 cells (human ovarian epithelial cancer cell line) were obtained (Cancer Research UK) and labeled with SPIOs [4]. For the *in vitro* experiment, control and SPIO labeled cells (~2 x 10^4) were pelleted and suspended in 1% agarose (50µl) into 250µl tubes. The agarose cell pellets were allowed to set and the tubes filled with phosphate buffered saline (PBS). The tubes were placed inside a tube containing water and located centrally inside a 40mm ID birdcage rf coil (Magnetic Laboratories, Oxford UK) and MRI performed on a 4.7T Varian Inova scanner (Palo Alto, CA). Parameters used for Diagonal-SPRITE: TR = Tp + 0.5ms; Tp = 0.25-6ms; FOV = 30x30x30mm; matrix size = 120x120; flip angle = 5°; 11 coronal slices of 2.7mm thickness and 5 averages. For the *in vivo* experiment, SPIO labeled cells (10^5, PBS) were injected into the leg of a B6 mouse (Harlan UK) and unlabeled cells (10^5, PBS) into the contralateral leg. Diagonal-SPRITE was performed as before except 21 transverse slices of 1.4mm thickness were obtained and only Tp values of 0.25 and 0.6ms employed.

Results and Discussion

Fig. 1 shows the variation in signal intensities of control and SPIO labeled cell pellets at different values of Tp using the Diagonal-SPRITE pulse sequence. The SPIO labeled cell pellet possess a shorter T2* value than the control cell pellet. At Tp=0.25ms, the signal intensity of both the control and SPIO labeled cells are similar (Fig. 1A) but at higher values of Tp, eg, 5ms, the signal intensity of the SPIO labeled cells is attenuated (Fig. 1B). The attenuation of the signal intensity of the SPIO labeled cells at high Tp values compared to the control cells is consistent with the shorter T2* value of the former cells relative to the latter. Positive signal enhancement of the SPIO labeled cells can be obtained by subtracting the image obtained at ultra-short Tp (0.25ms) from that at short Tp (0.6ms, Fig. 1C). For the *in vivo* experiment, a mouse was injected SPIO labeled cells in one leg and control cells in the contralateral leg. Again, negative enhancement was observed at the longer Tp of 0.6ms (Fig 2B) compared to the shorter Tp of 0.25ms (Fig. 2A). Positive contrast of the SPIO labeled cells using Diagonal-SPRITE *in vivo* was readily visualized by the difference image obtained by subtraction of the images at the two values of Tp (Fig. 2C).

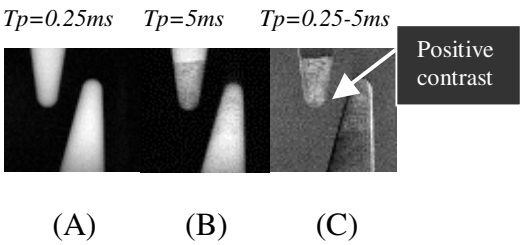

Fig. 1: Diagonal-SPRITE MRI images of tubes containing control and SPIO labelled cell pellets at (A) Tp of 0.25ms and (B) Tp=5.0ms. Positive contrast of SPIO labelled cells was achieved by subtraction of image (B) from image (A) to give image (C)

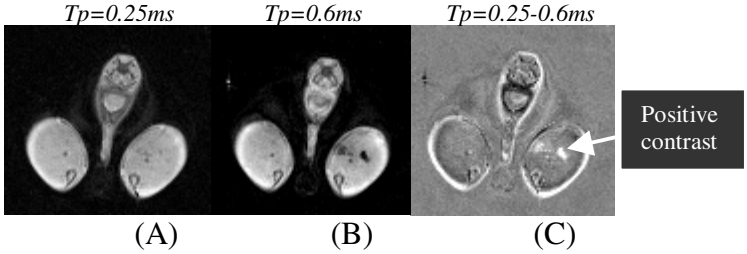

Fig. 2: Transverse diagonal-SPRITE MRI images of the hindlegs of a mouse in which the leg on the right received unlabeled cells and the contralateral leg, SPIO-labeled cells at (A) Tp=0.25ms and (B) Tp=0.6ms. By subtraction of image (B) from image (A) to give image (C), positive contrast from the SPIO labelled cells were readily visible.
(The apparently higher signal intensity of the fat in (C) arises from a slight (3-4%) difference in resolution between images (A) and (B) and as such, only readily apparent on the edges of images.)

Conclusion

Employment of Diagonal-SPRITE methods with ultra-short and short Tp values allows the visualization of SPIO labeled cells by positive contrast *in vitro* and *in vivo*. Furthermore, signal voids from the presence of SPIOs may be distinguished from those arising from bulk susceptibility by Diagonal-SPRITE MRI.

References

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