R_2* Quantification of High Iron Concentrations for Cellular Therapy Applications with TurboSPI

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Introduction: Many MRI techniques are now capable of imaging cells that have been labelled with super-paramagnetic iron oxide (SPIO) [1], and these techniques will be of great benefit to the developing field of cellular therapy. However, the majority of these techniques are not capable of providing quantitative information about cellular density at high iron concentrations, which is critical to monitoring the progress of cellular therapies and assessing the effectiveness of different treatments. Cellular density can be related to the local magnetic dose (LMD) of iron oxide, which affects the relaxation rate $R_2^* = 1/T_2^*$ [2]. To quantify cellular density, an MRI technique must therefore provide spatially resolved measurements of R_2^* over a large dynamic range.

We are exploring the use of an accelerated single point imaging method, TurboSPI [3], to acquire such quantitative R_2^* maps in samples containing SPIO-loaded cells. TurboSPI (Figure 1) uses a train of spin echoes to continually refocus the signal, and samples the rise and decay of each echo with high temporal resolution (2 μs). This sequence is optimal for imaging materials with large R_2^* (> 200 s^{-1}) but small R_2 (< 50 s^{-1}), and it is a prime candidate for imaging cells containing high concentrations of SPIO because, when compartmentalized in cells, SPIO greatly increases R_2^* but has a comparatively small effect on R_2. In this work, we evaluate TurboSPI's ability to quantify R_2^* for the model system of micron-sized particles of iron oxide (MPIO) suspended in gelatin, which offer more consistent results than labelled cell populations while retaining a favourable R_2^*/R_2 ratio.

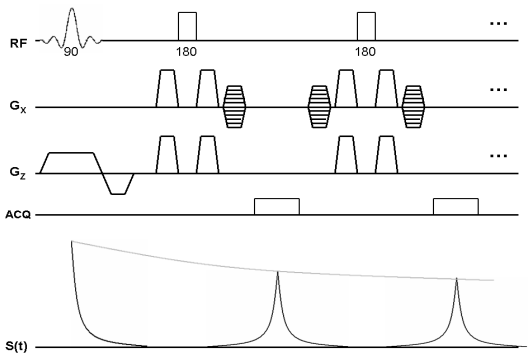

Figure 1: TurboSPI pulse sequence with two echoes shown. The signal envelope decays continually with R_2 (grey line) but rephases with R_2^* (black) during each echo.

Methods: A series of NMR tubes was prepared containing 4% gelatin, MPIO particles (mean diameter 0.96 μm, Bangs Laboratories), and doped with $MnCl_2{\cdot}4H_20$ to approximate biological R_1 and R_2. The resulting iron concentrations ranged from 4 μg/mL to 28 μg/mL. For imaging, these tubes were placed inside a cylindrical phantom filled with Mn-doped water, allowing five tubes to be imaged simultaneously. Data for eight tubes was collected across two sessions. All data were acquired with a 4T Varian INOVA whole-body MRI system, with a body gradient coil (Tesla Engineering, UK) having a maximum strength of 35.5 mT/m.

TurboSPI images were acquired with a 4cm-diameter solenoid RF coil. One 10mm slice was acquired with a 50x50mm FOV, 128x128 matrix size, and TR=200ms. The echo time for the first echo was TE=9ms, and a train of eight echoes (one every 9ms) was used to reduce the imaging time to 7 minutes. Automatic calibration, similar to that described in [4], was used to reduce ghosting from improper alignment of echoes. R_2^* values were calculated for 25-pixel ROIs centered on the middle of each tube, by fitting the time course of each ROI to an appropriate function [5] with IDL 6.2 (ITT Visual Information Systems, Boulder, CO).

For comparison, images were acquired using a standard multi-slice gradient echo (GRE) sequence with TR/TE/α = 200ms/9ms/20º, and a 128x128 matrix (total imaging time 30 seconds). NMR spectra of each tube were obtained using a 5mm solenoid coil and a single-pulse acquisition, to calculate bulk R_2^* values from the linewidth. Measurements of the local magnetic dose were performed with a multi-echo FLASH sequence, using TR/TE/α = 200ms/8ms/15º, 10 echoes with 10ms spacing. LMD for each tube was obtained using a procedure outlined in [2] and implemented in Matlab 7 (The Mathworks).

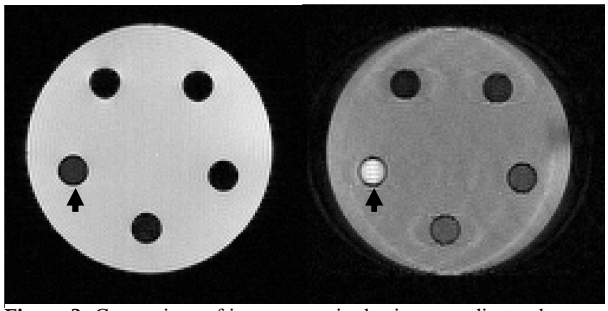

Figure 2: Comparison of images acquired using a gradient echo scan (left), and TurboSPI (right) with matched TR and TE. The ghosting in the TurboSPI image has negligible impact on R2* quantification.

Results and Discussion: A comparison of TurboSPI and a gradient echo (GRE) sequence is shown in Figure 2; MPIO concentration in the tubes increases counter-clockwise starting from the lower left (indicated by the arrowhead). In the GRE image, all of the tubes have little to no signal, but in TurboSPI most have visible signal at the spin-echo centre, and all have enough for R_2^* quantification.

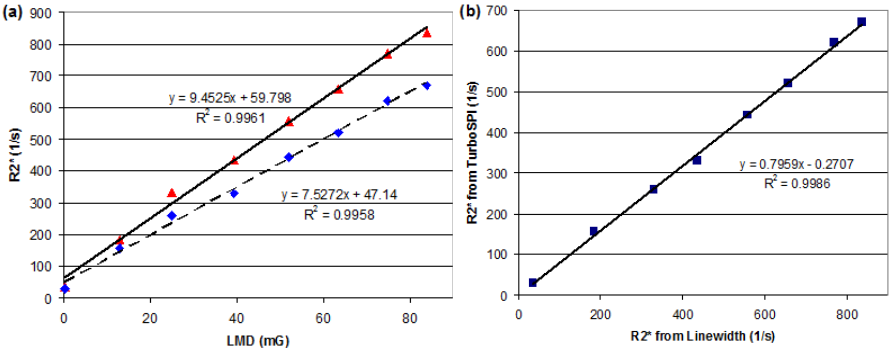

Figure 3: (a) R_2^* from linewidth (red triangles, solid line) and R_2^* from TurboSPI (blue diamonds, dashed line) as compared to LMD. (b) TurboSPI R_2^* as compared to linewidth R_2^*.

LMD values were measured for 8 different tubes, and R_2^* values for each were obtained both from TurboSPI and NMR linewidth. In both cases, R_2^* demonstrates a well correlated linear relationship to the LMD (Figure 3a), as predicted by the static dephasing (SD) regime theory [2]. However, SD regime theory predicts a slope of 10.78 s^{-1}/mG for the line relating R_2^* to LMD, which indicates that these MPIO particles may not have sufficient iron content to satisfy the conditions of the SD regime. At present, the R_2^* values obtained from TurboSPI are approximately 20% lower than those obtained from linewidth (Figure 3b), though it is not clear which represents the more accurate measurement. The spatially resolved TurboSPI value is expected to be less sensitive to large-scale field inhomogeneities from imperfect shim.

In any event, the TurboSPI R_2^* values are well correlated with iron concentration and suitable for quantification of MPIO levels over 2 orders of magnitude, up to 28 μg/mL (LMD=84). For a typical cell density of 4 x 10^6 cells/mL, this concentration corresponds to 7 pg Fe per cell, which is a realistic loading level for clinical applications [6]. For MPIO suspended in gel, higher values than this cannot be quantified by TurboSPI because R_2 is too large (the R_2^*/R_2 ratio is approximately 10:1, meaning T_2 < TE for such samples). For SPIO compartmentalized in cells, R_2^*/R_2 is significantly larger (approximately 70:1 [2]), which will increase the upper limit of quantification to 50 pg of iron per cell or more, or permit visualization of higher cell density applications.

Conclusions: TurboSPI has demonstrated the ability to quantify R_2^* values corresponding to a wide range of iron concentrations, which are inaccessible to traditional GRE sequences but well tolerated by cell populations. This performance will further improve when the technique is applied to quantitative imaging of SPIO in cells.

References: [1] Bulte et. al., *Current Pharmaceutical Biotechnology* 5:567-584 (2004). [2] C.Bowen et. al., *Magn. Reson. Med.* 48:52-61 (2002). [3] S.Beyea et. al., *J. Magn. Reson.* 144:255–265 (2000). [4] C. Bowen et.al, *Magn. Reson. Imaging* 24:857-867 (2006). [5] D. Yablonskiy, *Magn. Reson. Med.* 39:417-428 (1998). [6] J. Frank et. al., *Radiology* 228:480-487 (2003).