Ex Vivo MRI Assessment of USPIO Uptake in Aortic Plaque in a Mouse Model of Atherosclerosis at 11.7T

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Introduction We have presented MRI volumetric measurement of plaque burden for *in vivo* evaluation of therapeutic agents for treating atherosclerosis [1]. However, alternative imaging methods that are able to characterize and quantify inflammation levels in animal models of the disease would be more specific and sensitive to disease progression and to the effects of treatment than would volumetric measures. Ultrasmall superparamagnetic iron oxide (USPIO) contrast agents have been used for noninvasive MRI assessment of atherosclerotic plaque inflammation in both preclinical and clinical studies due to macrophage uptake of the agents [2-3]. An advantage of quantifying macrophage infiltration is that it is expected to respond more quickly to treatment than vessel wall thickness and volumetric measurements. The purpose of this study is to evaluate quantitative imaging methods for characterizing USPIO uptake in the aortic plaque of an atherosclerosis animal model. To this end, polymer solution phantoms, cell phantoms, and *ex vivo* studies were developed to help understand how different signal features are affected by the contrast agent for a variety of pulse sequences. The goal is to determine which MRI metrics best reflect the contrast agent uptake for the purpose of quantification in living tissues.

Methods

Polymer Solution Phantoms: Polymer solution phantoms were created using USMIO (BioPal, Worcester, MA) [4] diluted with distilled water. USMIO is similar to USPIO in size and MR properties. Their iron-oxide core is doped with Europium for quantification using neutron activation analysis (NAA). Some solutions were mixed with a polymer at a volume fraction of 12.5% or 25% to alter viscosity in order to account for the effects of water diffusion in the vicinity of USMIO particles on quantitative MRI metrics. Polymers included Polyethylene Glycol (PEG) 400, PEG 10,000 and Dextran 8. USMIO concentrations ranged from 0 ~ 0.32 mM Fe.

Cell phantoms: Cell phantoms were created by incubating mouse monocyte macrophages (RAW 264.7 - ATCC, Manassas, VA) for 20 hours with a USMIO dose of 0, 0.675, 1.35, 2.7 µmol Fe respectively. Cells were centrifuged to form a pellet. Samples were sent to BioPal for NAA to estimate USMIO concentrations in cell pellets.

In Vitro MRI Protocol MRI was performed on a Bruker Biospin 500WB spectrometer (Bruker NMR, Inc., Billerica, MA) with an 89 mm vertical bore magnet of 11.7 T. The sequences were implemented for assessing T2/T2*/ADC (apparent diffusion coefficient) parameters. R_2 was measured with a multi-echo SE sequence (TR=2000 ms, TE = 15, 30, …, 120 ms), R_2* with a FLASH sequence (TR = 25 ms, flip angle = 15°, TE = 2.5, 3, …, 10.5 ms), and ADC with a diffusion-weighted SE sequence with TR=2500ms, TE=12ms, gradient duration 1.5ms, gradient separation 7.7ms, and diffusion-weighting factor b = 0, 250, 500, and 750 s/mm^2.

Ex Vivo MRI All experiments were approved by Institutional Animal Care and Use. Fifty-week-old male apolipoprotein E-deficient (apoE$^{-/-}$) mice (n=7) were used in the study. USPIO (BioPal, Worcester, MA) was administered intravenously at a dose of 1000 µmol/kg via tail vein injection. The heart and aortic artery tree were removed 48 hours after injection. Aortas were perfused *in situ* with PBS via the left ventricle. 2% agarose was perfused for arterial and luminal shape fixation. *Ex vivo* MRI was implemented to identify USPIO uptake in the aortic plaque. A control sample (free of USPIO) was used for comparison. The MR sequences included 3D spin echo (SE) with 2 echo times (TR=1000ms, TE1=7.5ms, TE2=22.5ms, resolution 50×50×150µm^3) for R_2 estimation, 3D-SE IRON positive contrast technique (on-resonance saturation with a bandwidth of 245 Hz) [5], 2D FLASH (TR = 100 ms, flip angle 15º, and TE = 3ms) for obtaining phase maps to assess susceptibility effects caused by USPIO and those caused by calcifications [6], and GRASP (gradient echo acquisition for superparamagnetic particles with positive contrast) method [7].

Results Figure 1 shows ΔR_2 values (obtained by subtracting R_2 measured in a corresponding phantom without any USMIO) as a function of USMIO concentration for the polymer solutions and the cell pellets (cell pellet volumes estimated to be 0.10-0.14 mL). Both ΔR_2 and ΔR_2* values increased with increasing of USMIO concentrations. Water had the highest ADC (2.28 × 10^{-3} mm^2/s) as expected, and the cell pellets had similar ADC as the 12.5% polymer solutions (1~1.6 × 10^{-3} mm^2/s), and as the *ex vivo* tissue. Both ΔR_2 and ΔR_2* show good sensitivity to the USMIO concentrations. ΔR_2* values in cell phantoms were closer to those from the polymer phantoms than the water-only phantom, especially those within the same range of ADC. Due to susceptibility effects and field inhomogeneity at high field, the ΔR_2 was more reliable when the USMIO concentration was high. However, it was highly dependent on the environment (i.e. live cells vs. polymer solutions, type of polymer).

Figure 2 shows *ex vivo* MRI of heart and arterial samples (Fig 2a: sample with USPIO; Fig 2e: sample without USPIO), corresponding R2 estimations (Fig 2b, 2f), IRON acquisition (Fig 2c) and smoothed phase map [4] (Fig 2d), and calcification area (in innominate artery) delineated by IRON (Fig 2g) and highlighted by phase imaging (Fig 2h) for the sample shown in Fig 2a. Regions with USPIO uptake and calcification are dark in MR signal, and they both show positive contrast with the IRON method (Fig 2c, 2g). However, using phase information, regions with USPIO uptake and calcifications can be separated because of the diamagnetic susceptibility effect for vessel wall and calcification as opposed to the paramagnetic effect of USPIO. As can be seen, areas with USPIO uptake are dark in the processed phase map in Fig 2d, which show positive contrast in IRON image in Fig 2c. Compared to the R_2 map of a control sample (average R_2 is 40 s^{-1}) shown in Fig 2f, the enhanced R_2 values in Fig 2b (in the range of 40~100 s^{-1}, with ΔR_2 in the same range as phantoms) confirmed the USPIO uptake in tissue and in the aortic vessel wall. The dark region surrounded by the positive contrast signal in the IRON image (Fig 2g) is confirmed to correspond to calcification by the positive phase contrast (Fig 2h).

Conclusions and discussions Chemical and cell phantoms provide a controlled environment where native MR-measurable parameters may be modified in a predictable fashion. Given controlled phantoms having matching native T2 and ADC as biological tissue sample, the presence of USPIO may be quantitatively assessed. *In vivo* MRI quantification of inflammation using USPIO remains challenging. R_2* measurement is limited by susceptibility effects caused by various sources including tissue interfaces, calcification, field inhomogeneity, flow and motion artifacts, and resolution. Positive contrast techniques such as IRON and GRASP, as well as susceptibility imaging techniques based on the phase information are not specific to USPIO and do not quantitatively correlate with USPIO uptake. R_2 measurement is a relatively reliable method for assessing USPIO uptake especially for high field MR applications, even though they are highly dependent on the USPIO environment. Insights gained by experimenting with the controlled environment of the polymer phantoms, with cultured cells, and *ex vivo* studies may help verification and optimization of various MRI techniques for future *in vivo* applications.

References 1. Tang H, Chang CH, et al., 17th ISMRM, April 18-24, Honolulu, Hawaii, USA, 2009; 2. Morris JB, Olzinski AR, et al., *Arterioscler Thromb Vasc Biol.* 28:265-271, 2008; 3. Tang TY, Howarth SPS, et al., J Am Coll Cardiol 53:2039–50, 2009; 4. Groman, E.V. et al., Bioconjug Chem, 18(6): 1763-71, 2007; 5. Stuber M, Gilson WD, et al., MRM 58:1072–1077, 2007; 6. Haacke EM, Mittal S, et al., AJNR 30:19 –30, 2009; 7. Mani V, Briley-Saebo KC, et al., MRM 55:126-135, 2006.

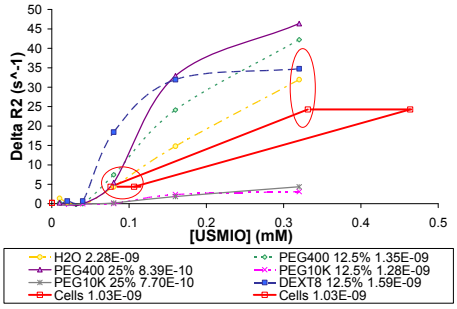

Figure 1. ΔR_2 as a function of USMIO concentration for cell pellets and for polymer solutions

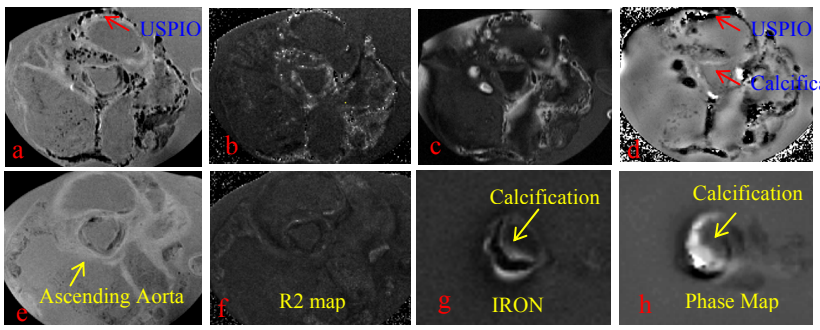

Figure 2. MRI of USPIO uptake in aortic plaque