A Novel Targeted Iron Oxide Nanocolloid Agent for T1 and T2* Imaging of Fibrin Using Conventional MR Techniques

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Introduction: Targeted iron oxide nanoparticles are easily detected with MR by their pronounced signal dropout. Recent advanced techniques[1] allow "hot spot" imaging by exploiting the off-resonance effects from the strong magnetic susceptibility differences of the iron oxides. These techniques rely on $T2^*$ signal distruption rather than T1 signal enhancement, which might be desirable. When iron oxides are used as a targeted molecular imaging agent, a long delay is typically required between contrast administration and imaging to allow for background blood pool clearance. During the wait, the targeted USPIO or MION agents bind endothelial cell surface markers, but they can also further extravasate into plaque, being taken up by macrophages or other intraplaque constituents. This can confound the morphological source of the MR signal. Moreover, in coronary imaging—where high temporal and spatial resolution are required and inherent anatomic magnetic susceptibility heterogeneity can mask that of the agents—dark spot T2-weighted gradient echo imaging of targeted iron oxides is challenging and has not yet been demonstrated. The objective of this research was to develop an intravascular, fibrin-specific iron oxide agent useful for early, rapid detection and quantification of ruptured plaque using $T2^*$ and T1 effects.

Methods: Superparamagnetic nanocolloid particles were developed with the core having multiple magnetite nanoparticles suspended in vegetable oil and encased in a lipid membrane. The MR T1 and T2 properties of the nanocolloid were determined using serial dilutions of the agent and MR acquisitions including Look-Locker (inversion recovery) and multi-echo gradient-echo techniques at 1.5T and 3.0T. To assess signal on both T1- and T2-weighted images, functionalized particles were targeted to clot surfaces with biotin and an antibody targeted to fibrin-rich thrombi (n=7) suspended in saline *in vitro*; one clot was a non-targeted control. Similarly, carotid artery samples (n=2) from symptomatic patients were treated with the fibrin-targeted agent. Imaging of the clot phantoms was performed at 1.5T using a high-resolution ($0.3\text{x}0.3\text{x}1.2\text{mm}^3$) 3D T1-weighted turbo spin-echo (TSE) sequence for ROI analysis and lower-resolution ($1\text{x}1\text{x}5\text{mm}^3$) gradient-echo imaging, both T1-and $T2^*$-weighted, for visual inspection. The endarterectomy specimens were imaged at 3.0T using high-resolution 3D T1-weighted TSE, and T1- and $T2^*$-weighted turbo field echo (TFE).

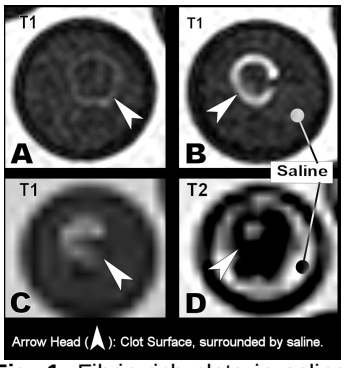

Fig. 1. Fibrin-rich clots in saline. Control (A) is not well seen on high resolution T1-TSE, whereas the clot with fibrin-targeted iron oxide nanocolloid (B) enhances brightly. On lower resolution FFE (C) the T1 effect is lessened, but still detectable, whereas on T2 (D) the typical signal dropout effects are pronounced.

Results and Discussion: The average particle size and zeta potential of the nanocolloid were approximately 140±10nm and -23mV, respectively. The iron concentration, measured by mass spectrometry, was 1240μg iron per g of emulsion. In solution, T1 and T2 effects varied with concentration, as expected. At lower concentrations T1 enhancement was noted and as the concentration of agent increased the $T2^*$ effects dominated making dark distorted images typical to iron oxide agents. The r1 relaxivity was calculated to be 1.112 ± 0.105 $\text{mmol}^{-1}\text{s}^{-1}$ and 0.660 ± 0.064 $\text{mmol}^{-1}\text{s}^{-1}$ at 1.5T and 3T, respectively, based on iron concentration. Calculated as the "particulate relaxivity" (*i.e.*, based on nanoparticle concentration), r1 becomes 18466 ± 1739 $\text{mmol}^{-1}\text{s}^{-1}$ at 1.5T and 10966 ± 1062 $\text{mmol}^{-1}\text{s}^{-1}$ at 3T. When bound to the outer surface of the fibrin-rich clots, the agent produced signal enhancement on T1-weighted imaging (SNR=26); whereas the control clot, which bound no agent, had an SNR=10, similar to surrounding saline. On T2-weighted images, characteristic signal dropout (blooming into many neighboring voxels) was produced by the bound agent but not on the control clot. (See Figure 1.) The carotid samples imaged at 3T also exhibited bright enhancement on T1-weighted TSE; whereas gradient echo images (T1- and T2-weighted) showed marked $T2^*$ effects (See Figure 2). Interestingly, some areas within the thickened carotid wall remained dark on both T1-TSE and gradient echo images (consistent with calcium deposits). Based on pharmacokinetic parameters and models for similar lipid-encapsulated emulsion nanoparticles, the systemic concentration of this nanocolloid agent for a typical *in vivo* application was projected to be less than 1% dilution upon injection and approximately 0.03% of this concentration in 20 minutes, which suggest that, although the particles are constrained to the vasculature, the background levels will be negligible soon after injection leaving only the bound agent visible. It is expected that T1-based detection will work with moderate concentrations (such as for molecular markers), short echo times, and spin echo techniques, but at higher concentrations and/or longer echo times, $T2^*$ effects will dominate. The dual T1 and T2 contrast features of this agent may help obviate the need for pre-contrast baseline images.

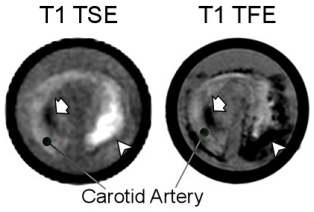

Fig. 2. Carotid Artery with fibrin-targeted iron oxide nanocolloid. Within the plaque (arrow head), bright signal is seen on T1-TSE (Left) but it changes to typical T2* signal void on gradient echo (Right). Some areas (arrow) have low signal on both techniques.

Conclusions: As demonstrated using *in vitro* atherosclerotic carotid arteries, these larger, fibrin-specific superparamagnetic nanocolloids may provide highly sensitive, bright-contrast detection of microthrombi exposed in ruptured plaque. The combination of high MR sensitivity and the imaging speed advantages of short T1-weighted pulse sequences may overcome the cardiac motion barrier to MR coronary molecular imaging.

References: [1]Liu, Dahnke, Jordan, et al., *NMR Biomed* 2007 Jun 13 [Epub].