In vivo Implementation of REACTION (Release Activation of Iron Oxide Nanoparticles)

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INTRODUCTION: A major advancement in MRI based cell tracking would be to not only detect the position and movement of cells, but also gene expression. This will be particularly important for monitoring stem cell transplants and detecting their differentiation into appropriate cell types. Here we describe a new enzyme/contrast agent paradigm which can achieve enzymatically responsive changes in cellular T_2 and T_2*. Cells are labeled with particles coated with a biopolymer that restricts the approach of water to the particle, preventing the magnetic core from relaxing protons. Cleavage of the coat by a specific enzyme reveals the magnetic center thus increasing the relaxivity of the agent. In an *in vivo* demonstration of this paradigm, large enhancements of dark contrast volume and CNR within the contrast regions were measured, for injected cells co-labeled with enzyme and particles versus cells labeled with particles alone. The success of this proof of principle demonstration justifies research towards a genetically controlled system, whose expression is regulated during cellular differentiation.

MATERIALS AND METHODS: *Cell free system:* Cleavage of the dextran coating from Feridex nanoparticles (1mM iron) by 25nM dextranase was established in acetate and citrate buffers, pH 6.0 and 5.5 respectively. Dextran cleavage occurred within minutes as evidenced by precipitation of brown magnetic cores. Reaction mixtures were incubated for 3 days at 37°C on a turning wheel to achieve complete reaction. T_2 and T_2^* relaxation times were mapped at 4.0T at 0.1mM iron.

In cellulo: Feridex (1mM iron) was pre-incubated with poly-l-lysine (1nM) at 37°C for 1 hour and added to cells in suspension ($1*10^6$ cells/ ml) for 2 hours of labeling, with occasional mixing. For enzymatic cleavage of the particles directly in cells, dextranase (25nM) was added to the labeling mixture. Samples were prepared immediately after labeling ($5*10^6$ cells/ ml, in 1% agar) and T_2 and T_2^* relaxation times were mapped. Confocal fluorescence microscopy of cells that were double labeled with a prepared green fluorescent Feridex and Texas Red labeled dextranase was performed to confirm double labeling. Anti dextran staining was performed on labeled and control cell cultures to determine cleavage of dextran coat.

In vivo: Two sets of MCF7 cells were prepared. One set was labeled only with Feridex, the other set labeled with both Feridex and dextranase as above. Following treatment with the anti-mitotic agent mitomycin C (10µg/ml), cells ($2.5*10^6$ cells, mixture of 80% unlabeled and 20% labeled cells) were injected into the hind limb muscles of CD1 nude mice (n=6). 3D T_2* gradient echo MRI (TE 5 ms, TR 50 ms) of the mouse legs were obtained at 1 day post injection. 3D data sets were analyzed using both BioImageSuite and Amide software to determine the volume (mm^3) of the susceptibility effect and CNR.

RESULTS: *Cell free system:* The T_2 of Feridex samples at 0.1 mM iron in acetate and citrate buffer in agar was 115 ± 8 ms and 109 ±12 ms, respectively. Following incubation with dextranase, T_2 dropped to 67 ± 4 ms and 77 ± 18 ms. These changes following enzymatic cleavage of the Feridex were 42% (P=0.05) and 28%, respectively for acetate and citrate. T_2* significantly decreased ($p<0.005$) from 15 ± 0.3 ms to 8 ± 0.2 ms and 15 ± 0.2 ms to 10 ± 0.3 ms in acetate and citrate buffers, manifesting a 55% and a 65% drop, respectively.

In cellulo (Figure 1): The T_2 of cells alone was 153 ± 22 ms. T_2 dropped to 77 ± 9 ms after labeling with Feridex. When cells were co-labeled with Feridex and dextranase for 2 hours, the T_2 further dropped to 50 ± 4 ms, a 35% decrease ($p<0.05$). T_2* was reduced from 17 ms ± 4 for cells labeled with feridex to 8 ms ± 1 for cells treated with dextranase, a 50% drop ($p<0.05$). Confocal fluorescence microscopy confirmed co-localization of dextranase and Feridex in intracellular compartments. Dextran staining on feridex labeled cells revealed a condensed pattern of staining that considerably differed from the diffuse pattern observed in dextranase treated cells.

In vivo (Figure 2): T_2* weighted MR images acquired at 1 day post injection demonstrated that a greater magnetic susceptibility effect was generated by cells treated with dextranase and Feridex relative to Feridex only labeled cells. The volume of the dark contrast was 680 mm^3 ± 380 and 200 mm^3 ± 140 for double labeled hindlimb and for feridex only, respectively ($p = 0.04$). CNR of the region containing Feridex and dextranase co-labeled cells was 7.24 ± 0.35, while the CNR of the region containing Feridex only labeled cells was 4.88 ± 0.61, a 48% difference ($p = 0.001$).

DISCUSSION: Increase in effective nanoparticle relaxivity by way of aggregation of nanoparticles to report on molecular events has been well demonstrated (Bogdanov, A, et al, Mol Imaging (1) 2002). Here we introduce a different approach to increasing nanoparticle relaxivity, potentially reporting on molecular events. We demonstrate *in vitro* and *in vivo* modulation in T_2 and T_2^* relaxation times of Feridex-labeled cells in the presence of dextranase, due to enzymatic digestion of the coat. Removal of the thick polymer coat reduces the distance of approach for water molecules to the magnetic core, thus allowing water to experience higher magnetic field inhomogeneities. This reveals itself as shorter T_2 and T_2^* relaxation times and larger susceptibility weighted signal voids in the *in vivo* gradient echo images. In all cases, T_2* relaxivity changes were greater than T_2 changes, especially *in vivo*. This may be due to further aggregation of naked iron oxide cores following release from particles. Changes in particle relaxivity require colocalization of enzyme and particles in low pH environment, enabling optimal enzymatic activity. Confocal microscopy revealed that upon endocytosis by cells, both magnetic particles and enzymes are shuttled into the same low pH intracellular compartments, such as endosomes and lysosomes. Moreover, the diffuse pattern of dextran staining associated with dextranase treatment, versus the very punctate staining seen with intact Feridex particles, further supports cleavage of the coat. These experiments form the backbone for new classes of potential in vivo MRI reporter genes/agents that can report on cellular changes at the molecular level, such as stem cell differentiation.

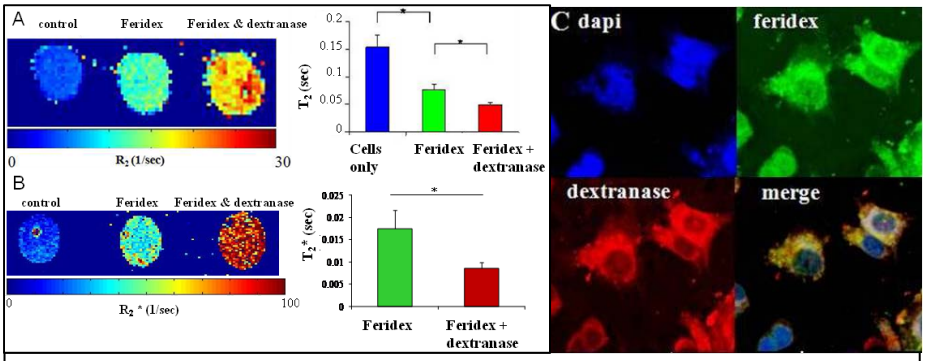

Figure 1: *In cellulo* reduction of transverse relaxivity following enzymatic cleavage of Feridex coating. (A) R_2 and (B) R_2* mapping of cell samples, accompanied by corresponding T_2 and T_2* for each sample studied. (C) Confocal fluorescence microscopy of cells co-labeled with green fluorescent feridex and red fluorescent dextranase (upper).

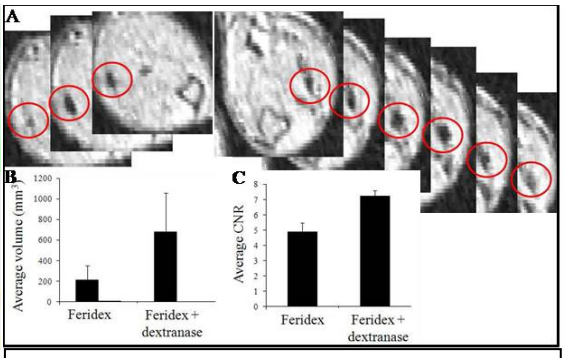

Figure 2: *In vivo* REACTION**.** A) MRI of legs injected with labeled cells (left, Feridex only; right, Feridex with dextranase). Dark contrast regions are circled in red. B) Measured volume of dark contrast regions. C) CNR from cells labeled with Feridex only or co-labeled with Feridex and dextranase.

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