Release Activated Iron Oxide Nanoparticles (REACTION) of Cellulose: A Magnetic Relaxation Switch for Environmentally sensitive MRI

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INTRODUCTION**:** While magnetic relaxation switches (MRSws) have been developed using iron oxide nanocrystal cores that cluster together in the presence of an extracellular stimulus [Bogdanov, et al, Mol Imaging (1) 2002], the potential of using such systems to monitor intracellular events *in vivo* via MRI has not been investigated to date. Granot et al [Granot et al, ISMRM, 2009] first demonstrated an MRSw operating in cellulo and in vivo via MRI using a Feridex®/dextranase system. However, the dilute nature of Feridex® labeling makes it a poor choice for any long-term tracking of intracellular processes via MRI. We hereby demonstrate the fabrication of magnetic cellulose particles that are relaxometrically sensitive to cellulase digestion. In the 'off' state, the particle remains intact. When switched 'on' through the cleavage action of cellulase, the iron oxide cores get dispersed, changing the relaxivity of the agent.

MATERIALS AND METHODS:

Fabrication of magnetic cellulose particles: 10-nm magnetite nanocrystals were synthesized via thermal decomposition of iron oleate. Magnetic cellulose particles were then fabricated using an oil-in-water single emulsion method previously described, using cellulose acetate as starting material [Nkansah et al, ISMRM, 2010]. Pure cellulose particles were regenerated via alkaline saponification. Iron content of particles was measured using ICP spectroscopy. Particle size and core distribution within particles were determined by SEM and TEM, respectively.

Activity of cellulase on cellulose particles: Enzymatic activity of cellulase on cellulose particles was assessed by using a hexokinase-based system to measure the initial rate of glucose release from cellulose particles after a 2-hr treatment.

In vitro study: For the *in vitro* study, magnetic cellulose particles (n=3) at a basis [Fe] of 2 mM were treated with 24 μm *T.reesei*/*T.viride* cellulase (pH 5.0, 0.05 M acetic acid) for 3 days at 37°C. Samples were then suspended in agarose gel phantoms at different iron concentrations. r_2 and r_2* relaxivity measurements were made at 4.7 T. Control samples were comprised of particles incubated only in acetate buffer or particles incubated with cellulase in DMEM cell growth medium (pH 7.4).

In cellulo study: MCF-7 human cancer cells (n=3) were labeled for 3 days with DMEM containing magnetic cellulose particles (at basis [Fe] of 2 mM) and cellulase (24 μm) and used to make agarose gel phantoms. T_2 and T_2* relaxation times were then measured at 4.7 T. Control samples were cells incubated only with particles.

RESULTS AND DISCUSSION: Cellulose particles were fabricated at a size of 414 ± 182 nm. Iron oxide nanocrystals were optimally loaded with a magnetite content of 70%. TEM showed good distribution of magnetite cores throughout the particles. Table 1 lists the r_2 and r_2* molar relaxivity of cellulosic nanoparticles, alongside those of inert MPIOs and dextran coated nanoparticles. Cellulose nanoparticles dissolved to produce glucose when treated with cellulase enzyme. The initial rate of glucose formation was found to obey classic Michaelis-Menten kinetics (Fig. 1). Magnetic cellulose particles subjected to a 3-day treatment with 1 mM cellulase at 37°C in 0.05 M acetic acid (pH 5) showed significant degeneration in morphology (Fig. 2a,b). Electron micrographs show what seem to be aggregates of amorphous cellulose fibers, left over from degradation. Furthermore, the reaction mix of the cellulase-treated group was much darker than control (Fig. 2c). Relaxometric measurements of the two reaction mixtures showed a clear difference in r_2 relaxivity with the treatment group having 63% higher r_2 than control with the disparity in r_2* relaxivity not being as high, only 15% (Fig. 3). Because r_2* values are so much higher than r_2 for both treatment and control groups, we hypothesize that these particles exist in the static dephasing regime (where r_2*>>r_2) [Muller et al, MRM 22(2) 1991]. As such, a reduction in the effective size of magnetic cellulose particles in the static dephasing regime should trigger an increase in r_2 with little effect on r_2*. As cellulase acts on the particles, more iron cores get exposed to the surrounding medium, allowing for easier approach of water protons.

A different situation was observed *in cellulo*, where cells labeled with both particles and enzyme exhibited lengthened T_2 and T_2* versus control cells labeled only with particles (Table 2). We interpret this to be due to recycling of the iron particles within the cells as they are released from the cellulose matrix, and hence, a loss in superparamagnetism. To prevent this, future modifications to these particles must include surface protection for the iron cores, such as gold coating. Nevertheless, these results are encouraging and demonstrate the potential of using environmentally sensitive agents to report on intracellular events using MRI. We believe, for example, that the differentiation of a transgenic neural progenitor cell line that expresses cellulase as a reporter gene when differentiating into a neuron could be visualized via MRI, if it is magnetically labeled using a cellulosic MRI agent like the one described here.

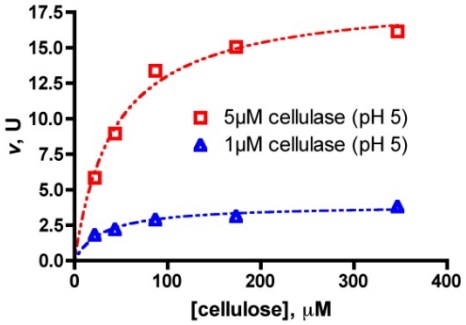

Figure 1. Enzymatic activity of cellulase on cellulose particles. Cellulase displays classic Michaelis-Menten kinetics when exposed to different concentrations of cellulose particles. The initial rate, v (or v_0), shows linear dependence on [substrate] when [substrate] is low, but plateaus at higher [substrate] due to saturation of enzyme binding sites. v_{max} is proportional to [enzyme].

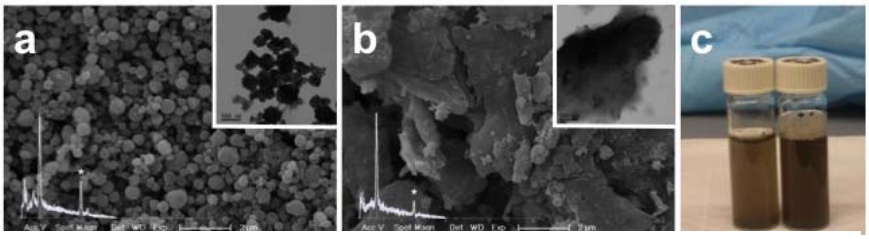

Figure 2. Morphology of cellulase-treated, magnetic cellulose particles. Electron micrographs of cellulose particles incubated for 72 hrs in 0.05 M acetic acid (pH 5) at 37°C in the absence (a) and presence (b) of 1 mM cellulase; set within either SEM picture are the corresponding TEM picture (top right corner) and XPS spectrum (bottom left corner) with asterisks indicating Fe peak in the XPS spectra (c) Photograph of cellulase-treated sample (right) alongside control

	Cellulose NP	Cellulose acetate NP	Feridex®	Bangs®
size (nm)	414	633	160	1600
r_2 ($s^{-1}mM^{-1}$)	69	76	111	69
r_2* ($s^{-1}mM^{-1}$)	399	399	216	466

Table 1. Relaxometry of magnetic cellulose-based agents

In cellulo	T_2/10^6 cells (sec)	T_2*/10^6 cells (sec)
Cellulase	.122±.021	.00848±.0019
Control	.062±.0043	.00439±.000047

Table 2. *In cellulo* relaxometry of magnetic cellulose-based agents

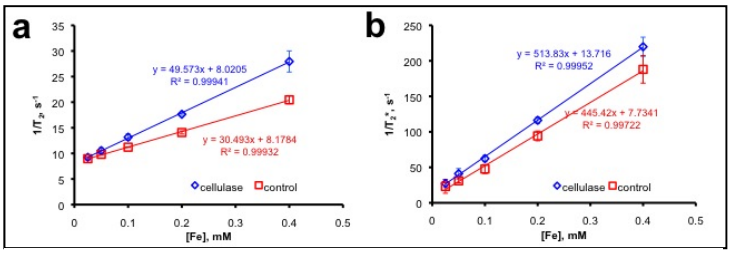

Figure 3. In vitro relaxometric effect of cellulase treatment on magnetic cellulose particles. Measured r_2 (a) and r_2* (b) molar relaxivities (given as slope of the lines drawn) of magnetic cellulose particles following 72-hr treatment by cellulase as described in Fig. 16. Note that $R_2 = r_2[Fe]+R_{2,0}$ and $R_2^* = r_2^*[Fe]+R_{2,0}^*$, where $R_2 = 1/T_2$ and $R_2^* = 1/T_2^*$