A Dual Modality Nanoparticle MRI/CT Contrast Agent with Enhanced T_1 and T_2 Relaxivity

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Introduction: Protein based nanoparticles have been developed as high relaxivity contrast agents for molecular MRI [1-4]. With T_1-weighting, agent concentrations of μM-mM are typically detected [6]. To monitor the delivery of therapeutic agents, there is also interest in bimodal particle CT/MRI contrast agents [5-7]. Dual CT/MRI agents have been reported to be used with concentrations greater than 47mM of Gd [7]. In this work, we present a tungsten-iron (W-Fe) ferritin nanoparticle, with a 4,497$mM^{-1}s^{-1}$ and 458,143$mM^{-1}s^{-1}$ per particle T_1 and T_2 relaxivities respectively, with visibility in CT at concentrations of 20mM of tungsten (343nM of particle).

Methods: Nanoparticle Synthesis: The nanoparticles were synthesized from 2μM native horse-spleen apoferritin (Sigma Aldrich, St. Louis), 48mM Fe(II) Chloride (Sigma Aldrich, St. Louis), and 48mM Sodium Tungstate Dihydrate (Sigma Aldrich, St. Louis) in 0.05M MES buffer (pH 8.5). The temperature of the apoferritin solution was monitored and maintained between 55 and 60ºC in a water bath and allowed to acclimate for 10min prior to the initiation of synthesis. The solutions were continuously de-aerated with N_2 gas (50psi) the flow pressure was adjusted to reach a steady state until it gently bubbled thorough the solutions. Alternating 125μL additions of Fe (II) Chloride and Sodium Tungstate Dihydrate were made at 5 minute intervals for a total of 20 additions of each compound. After approximately 10 additions the solution became rust in color and towards the end of synthesis the solution was yellow-brown. The solution was then dialyzed for 24 hours in a 10000MW cut-off dialysis bag (BioDesignDialysis Tubing, Carmel, NY) against 3L of 0.15 M Nail buffer. Using a 1.5T micro magnetic column (Miltenyi Biotech, Glad Bach, Germany), the dialyzed was magnetically filtered under a 0.15M NaCl buffer wash. The resulting protein concentration was obtained using the Better Bradford Assay (Thermo Scientific, Rockford**)**. To confirm the presence of tungsten a sample of the solution was stained using 20% w/v Tin(II) Chloride (Sigma Aldrich, St. Louis) in 1.0M HCl. Upon confirmation, the concentrations of iron and tungsten were determined using inductively coupled plasma – optical emission spectroscopy. Electron Microscopy: Samples were imaged using a Philips CM12 electron microscope on Cu-C grids. Relaxometry: Several dilutions of sample suspended in 1% low-melt agarose gel were scanned in a 0.5T Bruker relaxometer. The Bruker's minispec software and exponential curve fitting feature were utilized to determine the T_2 (Inter pulse τ = 20ms, 200 points) and T_1 values (pulse separations ranging from 5 to 20000ms, 4 scans, 7 points). CT: The sample was lyophilized and the concentrate imaged against native ferritin (Sigma Aldrich, St. Louis) using a Siemens AXIOM Sireskop SD System (60kV, 2.5mAs). ImageJ software was used to analyze the image. In vivo Imaging: Using stereotactic injection, W-Fe contrast agent and magnetoferritin control were administered into the striatum of an adult male Sprague Dawley rat. To confirm the detectability in vivo, a 7T Bruker scanner and a surface RF coil was used, with a FLASH sequence (TE/TR= 5.31/11.911ms).

Results and Conclusions: TEM visualization of the synthesized alloyed nanoparticles is shown in Figure 1 particles range in size from 9 to 13nm. Relaxivity measurements demonstrated a 58 fold increase in T_1 relaxivity compared to magnetoferritin and a similar T_2 relaxivity (Figure 2), as obtained from the relaxivity curves (Figure 3). Results of CT imaging demonstrated W-Fe ferritin contrast intensities on the order of 1.5 times greater than that of native ferritin for the same concentration, as shown in Figure 4. In vivo results are shown in Figure 5. W-Fe ferritin nanoparticles offer a high relaxivity, and show promise for dual modality CT/MRI applications.

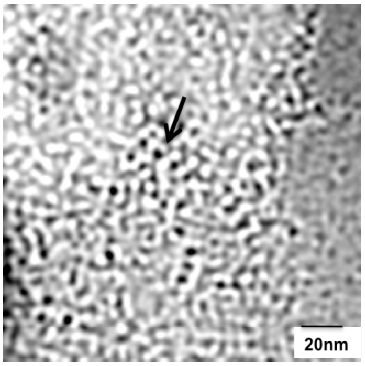

Figure 1: TEM image of W-Fe nanoparticles (indicated by arrow) at a magnification of 110k.

		R_1 [$mM^{-1}s^{-1}$]	R_2 [$mM^{-1}s^{-1}$]
Magneto-ferritin	Particle	78	404045
	Iron	0.07	407
W-Fe Ferritin	Particle	4497	458143
	Iron	0.19	14.1
	Tungsten	0.15	12.9

Table 2: Relaxivity values compared between Magnetoferritin and W-Fe Ferritin. Values are an average of 3 experiments.

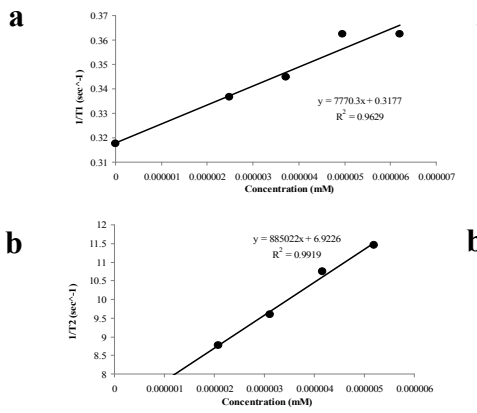

Figure 3: (top left) Relaxivity curves of W-Fe ferritin particles of (a) r1 and (b) r2.

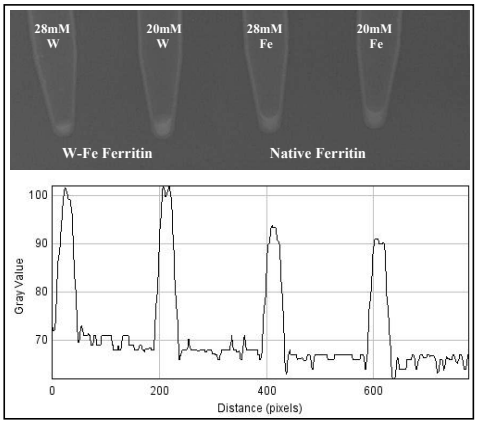

Figure 4: (top right) CT image of W-Fe ferritin compared to native ferritin (a) with intensity map of contrasted regions (b).

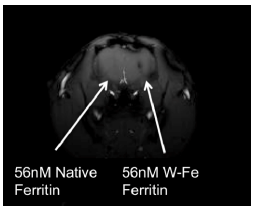

Figure 5: (left) In vivo T_2 MRI image of rat striatum with injections of magnetoferritin and W-Fe ferritin as indicated.

References: **[1]** Merchant et al. IJRI 14(3).2004 **[2]** Uchida M, et al. Magn Reson Med 2008;60(5):1073-1081 **[3]** Bulte JW et al. JMRI. 4(3) 1994 [4] Bennett KM, BioPhys Journal. 95(1) 2008 **[5]** Elleaume et al. Phys. Med. Biol. 2002 47:3369-3385 **[6]** Caplan MR et al. ABME 33(8), 2005 Elleaume et al. Phys. Med. Biol. 2002 47:3369-3385 **[7]**Regino et al. CM&MI 2008.