Simplified synthesis and characterization of magnetoferritin for convection enhanced delivery

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Introduction: Recent advances in the development of targeted and smart molecular contrast agents have made it possible to detect cellular molecular events *in vivo* (1). Recently, convection-enhanced delivery (CED) has been proposed to deliver agents with very high specificity to the brain to detect glioblastoma multiforme, but the technique requires the detection of agent concentration differences of tens of nM (2). Also, because these agents must be delivered by convection, they must have size ranges similar to biological molecules. There is thus a clear need to develop agents with high relaxivity which are also very small and functionalizable. Many superparamagnetic iron oxides nanoparticles (SPIOs) have high relaxivity (3), but may be too large to diffuse rapidly through the parenchyma. Ferritin, a 13 nm iron storage protein, has been used as an MRI contrast agent (4,5), and is small enough to be delivered rapidly by CED. Because it is only partially filled with iron in its native form, ferritin has been reconstituted to fill completely with iron to increase its MRI transverse relaxivity (6,7). Recently, the production of such "magnetoferritin" has been reported using a genetically modified ferritin expressed in bacteria, to obtain about a 93-fold increase in relaxivity (7). In this work, we developed a simple chemical procedure to obtain a ferromagnetic magnetoferritin from commercially-available apoferritin, with about a 96-fold increase in relaxivity over native ferritin. By magnetically filtering the magnetoferritin, the nanoparticles were made monodisperse and readily transferred between buffers for functionalization. The protein's perfusion characteristics were compared to targeting peptides intended for CED. This makes it practical to use magnetoferritin for CED to detect malignant glioma cells.

Methods: Magnetoferritin Synthesis: 2μM apoferritin (Sigma Aldrich, St Louis) buffered in 0.05M MES pH 7.4 and 48mM Fe (II) Chloride (Sigma Aldrich, St. Louis) were deaered with N_2 gas (50psi). The apoferritin solution was kept in a water bath at a constant temperature of 60°C, the pressure of the N_2 gas was regulated until no overflowing of apoferritin solution bubbles were created, this process lasted for approximately 30 min until a steady N_2 blowing was achieved. Every ten minutes 125μl of Fe(II)Chloride was added to the deaering apoferritin solution and mixed with a pipette to ensure mixing after addition, the solution was then covered with a rubber bung. This procedure was repeated a total of 20 times until the solution turned a slightly gray-brown color. Upon completion the sample was removed and filtered magnetically with a 1.5T micro magnetic column (Milteney Biotech), and washed with 0.15M NaCl buffer. The resulting protein concentration was obtained using the Better Bradford Assay (Thermo Scientific, Rockford). Electron Microscopy: Samples were adsorbed on Cu-C grids and transmission electron microscopy (TEM) images were obtained using a CM12 electron microscope. Relaxometry: Relaxivity measurements were performed utilizing a 0.5T Bruker relaxometer. Bruker's minispec software and the exponential curve fitting feature were utilized on several different dilutions of magnetoferritin to find the corresponding T_2 values (Inter pulse τ = 20ms, gain = 70, 200 points). Inductively coupled plasma mass spectrometry was used to determine total iron concentration. Perfusion Studies: Native ferritin was functionalized to FITC and infused in a 0.6% Agarose gel using a syringe pump (Harvard Instruments) for 2 h at a rate of 1μl/min. The perfusion distance was measured using Adobe Photoshop where the intensity at the injection site was normalized to the inlet concentration. Mathematical perfusion models were used to fit the data and provide with the diffusion (D) and retardation (*f*) coefficients.

Results and Conclusions: Partially-filled apoferritin particles, prepared using published protocols, are shown in TEM images in Fig. 1a. Using our protocol, and after magnetic filtration, monodisperse, filled particles were seen (Fig. 1b). The particles had core diameters ranging from 8nm to 11nm. Relaxivity measurements indicate a 96-fold increase in relaxivity for magnetically filtered magnetoferritin when compared to native horse spleen ferritin. Figure 2 shows the R_2 measurement as a function of iron concentration. Our magnetoferritin had a relaxivity of 95.9 $mM^{-1}s^{-1}$. Figure 3 illustrates the perfusion characteristics of the multivalent construct, ($D = 6 \times 10^{-6}$ cm^2/s and f= 1 where 1 represents no retardation) and native ferritin ($D=3.8\times10^{-6}$ cm^2/s and f = 0.85). Thus the retardation and diffusion coefficient was comparable to that of the construct. Magnetoferritin is thus practical as a molecular imaging agent for CED in the brain, and nM differences in magnetoferritin concentrations should be detectable *in vivo*.

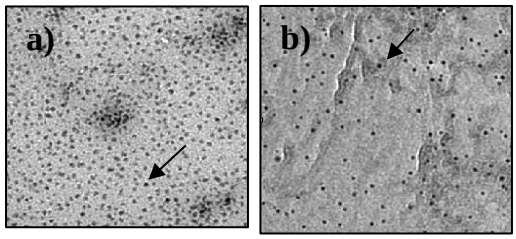

Figure 1. TEM images of apoferritin with (a) partial loading and (b) complete loading and magnetic filtration. The images show that partially filled, unfiltered ferritin is polydisperse (aggregated), and filtered magnetoferritin is monodisperse. Images are 88k magnification. Arrows point at ferritin molecules.

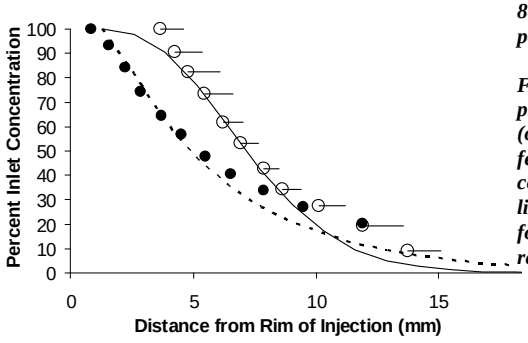

Figure 3. Perfusion profiles trivalent construct (open circles) (8), native ferritin (black circles) continuous and dashed lines depict model results for trivalent and ferritin respectively

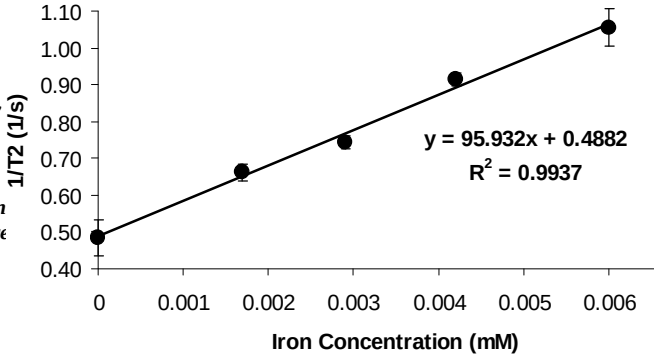

Figure 2. Magnetoferritin relaxivity curve for 5 different dilutions of protein. (T_2 exponential curve fit. 4 scans, τ = 20ms, 200 points, 37°C)

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