NanoIron Phantom to Validate In-Vivo Iron Mapping

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Purpose: Iron is an important biomarker for many injury and disease processes, such as blunt vascular and neurological trauma and neurodegenerative diseases such as Alzheimer's and Parkinson's disease. The key to diagnosing these injuries and diseases includes not only quantifying the local iron concentration but also identifying the form of the iron [1]. TEM studies have indicated that ferritin, which has a multiphasic core, changes from predominantly ferrihydrite to magnetite and other cubic phases in Parkinson's and Alzheimer's patients [2]. In addition to iron concentration and mineral form, proton relaxivity and susceptibility depend on how iron is distributed within the tissue. To properly map iron one requires a multi-parametric approach which needs to be validated with phantoms with well characterized biomimetic nano-iron.

Methods: We investigated several materials for use in the nano-iron phantom including Fe chelates, hemoglobin, recombinant human ferritin, horse spleen ferritin (HSF), Feraheme, Molday ion, nanoComposix iron oxide, textured (chained) nanoparticles. The range of concentrations was selected to match brain iron concentrations in healthy and diseased tissue (100-200 ppm). A key advance was to develop recombinant human ferritin by obtaining H-chain DNA sequences, amplifying with PCR, splicing them into pET30a(+) plasmids, and transfecting E. Coli. Various mineralization techniques were applied to mimic pure phase, healthy, and pathologic forms of human ferritin. The nano-iron materials were incorporated in polymer matrices, polyvinylpyrrolidone (PVP), to increase stability and to better mimic tissue diffusion and relaxivity properties. T1 relaxation (inversion recovery), T2 star relaxation (gradient echo) and T2 relaxation (spin echo) measurements were made using variable field NMR and small-bore MRI systems. The materials were characterized using X-ray diffraction, SQUID magnetometry and electron paramagnetic resonance and were assessed for long term stability.

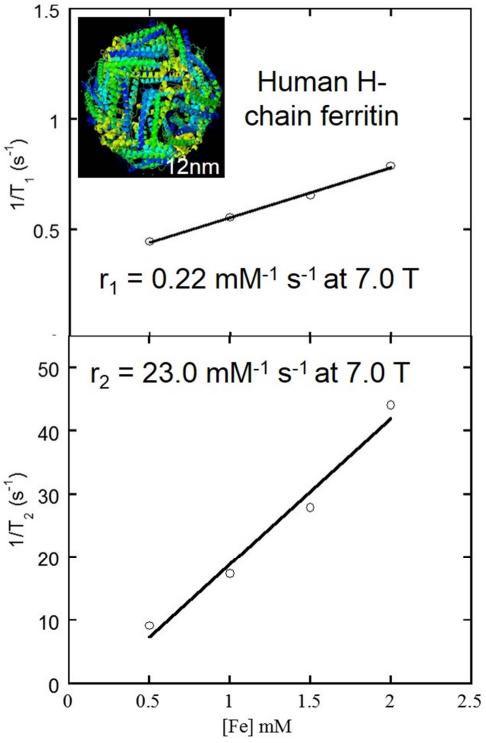

Figure 2 T1 and T2 7T relaxivities of synthetic human H-chain ferritin mineralized with ferrimagnetic cores.

Results/ discussion: An example relaxivity measurement for synthetic H-chain human ferritin (HFn) is shown in Fig. 1. The 7 T relaxivity values are r1=0.22 mM^{-1}s^{-1}, r2 = 23.0 mM^{-1}s^{-1} as compared to native HSF relaxivities of r1 = 0.027 mM^{-1}s^{-1}, r2 = 1.66 mM^{-1}s^{-1}. The individual relaxation rates themselves cannot be used to distinguish these two types of ferritin in-vivo where the concentration is unknown. However, the relaxivity ratios are quite different, r2/r1 = 104, 61 for HFn and HSF respectively, and can potentially help determine the form of the local ferritin. An example stability measurement is shown in Fig.2 which shows 1.5 T spin echo images of an array of spheres with different concentrations of 7 nm iron oxide particles in 20 % PVP. Clear texturing of the particle distribution occurs after 42 hrs in the MRI. This type of texturing is not necessarily undesirable since it mimics many biogenic-iron deposits; however it needs to be controlled and reproducible. Developing a stable reference standard with fully characterized biomimetic iron remains an important but challenging goal.

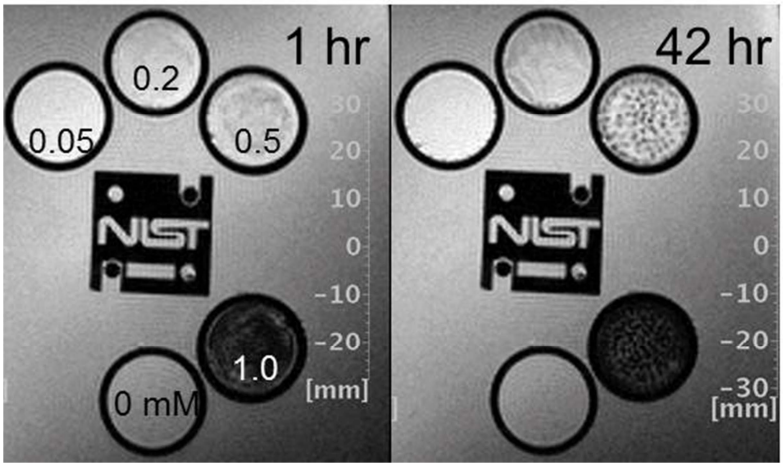

Figure 1 Spin echo (TE = 30ms) images of 7 nm iron oxide particles suspended in a PVP matrix, 1 hr after insertion into the MRI and 42 hrs after insertion into the MRI. The Fe concentration, in mM, is shown in the left image.

References:

[1] Kirschvink JL, Kobayashi-Kirschvink A, Woodford BJ. (1992) Magnetite biomineralization in the human brain. PNAS, 89:7683-7687.

[2] C. Quintana, J. M. Cowley and C. Marhic, (2004).Electron nanodiffraction and high-resolution electron microscopy studies of the structure and composition of physiological and pathological ferritin. Journal of structural biology 147 (2), 166-178.