In vivo biodegradation of PLGA encapsulated magnetite nanoparticles

Erik M Shapiro[1] and Michael K Nkansah[2]
[1]Department of Radiology, Michigan State University, East Lansing, Michigan, United States, [2]Department of Biomedical Engineering, Yale University, New Haven, Connecticut, United States

INTRODUCTION: Previously, we described the fabrication of PLGA encapsulated iron oxide nano- and microparticles (1). These particles are important advancements for molecular and cellular MRI. Firstly, they incorporate large amounts of iron, especially relative to dextran coated (U)SPIO. Secondly, PLGA is an FDA approved material with a long history of use in drug delivery, enabling a clear trajectory towards potential clinical use. In vitro experiments demonstrate that PLGA encapsulated magnetic particles have favorable degradation characteristics, with particles degrading within a few weeks, and iron dissolution complete at 100 days. Here we describe the first *in vivo* examination of the biodegradation of a specifically designed magnetic particle for MRI-based cell tracking using PLGA encapsulated iron oxide particles.

MATERIALS AND METHODS:

Magnetic particle fabrication: Magnetic PLGA nanoparticles (NPs) were fabricated and characterized as described in (1) with 2:1 weight ratio of magnetite: PLGA. Particles were characterized for size by scanning electron microscopy and iron content of particles was measured by ICP-OES.

In vivo biodegradation: *In vivo* biodegradation of PLGA NPs was investigated using a procedure originally designed by Briley-Saebo, et al (2). PLGA 2:1 NPs were formulated at 1 mg Fe/ml in 0.9% saline and injected via tail vein into 8 week old CD-1 female mice at a dose of 2 mg Fe/kg (n=5). Mice were on average 30 g, and so received 60 µl injections. Mice underwent serial MRI for 12 weeks (time points were 1 day, 2 weeks, 4 weeks, 7 weeks, 9 weeks and 12 weeks post injection), after which they were sacrificed. Sacrifice was carried out by intracardiac perfusion of saline. MPIOs and Feridex were similarly prepared and injected into mice (n=5 for each) and were scanned serially for all 12 weeks, after which they were sacrificed. A set of control animals receiving no injections was also scanned (n=5). The MRI procedure consisted of a respiratory gated, multislice T_2* mapping protocol, acquired on a 4.0T Bruker Biospec. A separate transmit volume and surface receive coil were used to enhance sensitivity and restrict the field of view to the liver. Mice were anesthetized with 1% isoflurane delivered via nosecone in 100% oxygen. Image resolution was 200 x 200 microns; 8 TE were acquired, spaced 3 ms apart. Particle remnant was calculated as fractional R_2* relative to R_2* of liver at Day 0. Data were also baseline corrected for the increase in R_2* of normal mouse liver over the course of the twelve week long experiment. Iron content of mouse liver was measured following chemical digestion using ICP-MS.

RESULTS and DISCUSSION: By scanning electron microscopy (SEM), NPs average diameters of 95-105 nm (Figure 1) with low polydispersity. Using 2:1 weight ratio of magnetite:PLGA achieved 83.7% magnetite weight percent. Magnetite volume fractions of particles of 57.0 vol% NPs were at over four times higher than that of Bangs beads (13 vol%), and compared favorably with the random close packing limit of 63.4 vol% for monodisperse spheres in a unit-volume.

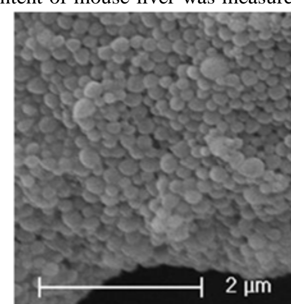

Figure 1: SEM of magnetic PLGA particles.

2:1 NPs, as well as inert MPIOs and Feridex, were injected intravenously into mice. Due to their size, all particle types are removed from the blood within 24 hours by Kupfer cells in the liver. Serial MRI of the liver over 12 weeks was used to investigate the biodegradation of the three particle types (Figure 2). T_2* of mouse liver at baseline was 21.8 ± 1.3 ms. 1 Day following injection of Feridex, MPIO and 2:1 NPs, T_2* fell to 6.0 ± 1.1 ms, 5.7 ± 2.6 ms and 7.5 ± 3.5 ms, respectively. At two weeks, T_2* measurements were 17.3 ± 1.3 ms, 5.3 ± 0.5 ms and 8.6 ± 3.0 ms, respectively. At 12 weeks, T_2* values were 15.7 ± 1.2 ms, 5.8 ± 0.3 ms and 11.2 ± 0.7 ms, respectively. Naïve mouse liver had a T_2* of 17.7 ± 1.3 ms, significantly lower than naïve mice 12 weeks prior. Converting the T_2* at the 12 week time point to R_2*, which is linear to concentration indicates that 2:1 NPs exhibited 80% biodegradation over this time course.

Comparison of this *in vivo* biodegradation data to the previously performed *in vitro* study indicates that during the first two weeks, particle degradation is most closely aligned with particle degradation, that is, the decomposition of the integrity of the PLAG:magnetite complex. This is reflected by the initial steep drop in particle remnance. The gradual decrease in particle remnance after week 2 is likely due to the slow dissolution of the iron oxide nanocryslats in the acidic environment of the endosomes/lysosomes and parallels *in vitro* studies. ICP analysis of mouse livers showed that while relaxivity of the particles diminish, iron clearance from the liver was even slower. Whether this rate of biodegradation will be suitable and safe for humans remains unanswered. Indeed, we may be the first to specifically address this question. But having the ability to tailor magnetic particle properties, both in the size of the magnetic nanocrystals and in the composition, and by performing these types of *in vivo* MRI experiments, biodegradation rate can be altered and reliably measured, with the ultimate goal of a safe, well characterized product for clinical use.

NIH grants: P30 NS052519 and DP2 OD004362 **Refs**: **1)** Nkansah, et al, MRM 2011; **2)** Briley-Saebo, et al, Cell Tissue Res, 2004.

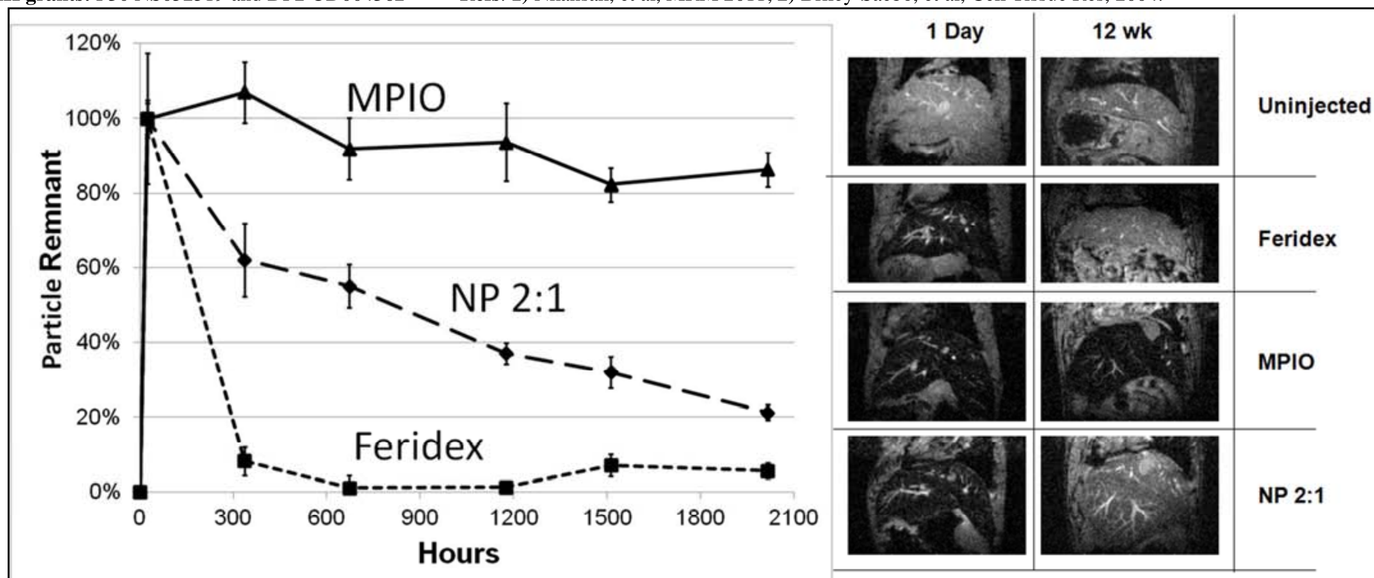

Figure 2: In vivo biodegradation over 12 weeks following intravenous injection of Feridex, inert MPIOs and NP 2:1. Data are mean ±- SEM. MRI of mice livers are at TE = 6 ms for mice injected as indicated, both at 1 day and 12 weeks following injection.