Biodegradable MPIO and SPIO using FDA approved polymers

M. K. Nkansah[1], D. Thakral[1], and E. M. Shapiro[1,2]
[1]Department of Biomedical Engineering, Yale University, New Haven, CT, United States, [2]Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT, United States

Introduction: Magnetic cell labeling for the purpose of MRI based cell tracking has primarily been accomplished using dextran-coated iron oxide nanoparticles. These have been preferred mostly due to formulations being clinically approved in North America and Europe. However, despite this perceived benefit, a serious drawback to their use is the low iron content per particle. This is coupled with the cessation of commercial production. As an alternative, micron-sized iron oxide particles (MPIOs) have been used, the benefits of which are that they contain as much as 30% iron. The perceived downside with MPIOs is that these particles are composed of inert, non-degradable and not FDA-approved polymer matrices. This potentially limits any clinical utility of MPIOs. An ideal particle for MRI-based cell tracking would therefore combine the biocompatibility of dextran-coated nanoparticles with the high iron content of MPIOs. Here we demonstrate the fabrication of fluorescent, biodegradable MPIOs, composed of poly(lactide-co-glycolide) (PLGA) and cellulose, two FDA-approved polymers commonly employed for drug delivery. These particles have high relaxivity and are capable of labeling cells for MRI-based cell tracking.

Methods: For all particles, magnetic cores were composed of 10 nm magnetite nanocrystals. Magnetic PLGA and cellulose particles were fabricated using an oil-in-water single emulsion method. Briefly, iron oxide nanocrystals were dispersed in a solution of PLGA or acetylated cellulose in methylene chloride to form a stable organic phase. The organic phase was then added to an aqueous phase containing a surfactant and further dispersed via ultrasonics to form nanodroplets of iron oxide-containing polymer. The nanodroplets are allowed to harden into nanoparticles as the organic solvent evaporates. Pure cellulose particles were generated by incubation of acetylated cellulose particles in dilute sodium hydroxide. PLGA microparticles were made employing homogenization rather than sonication. Fluorescence was achieved by adding coumarin-6 to the organic phase during particle fabrication. Iron content of particles was measured using ICP spectroscopy. r_2 and r_2* relaxivity measurements were made at 4.0T. Particle degradation studies were carried out by incubation of particles in PBS and citrate buffer at 37°C for 21 days. To assay cell labeling, mesenchymal stem cells and MCF-7 human cancer cells were labeled overnight with magnetic PLGA and cellulose particles, as well as with dextran coated particles, at a basis [Fe] of 1mM. Cellular iron content was assayed with a ferrozine based spectrophotometric assay. MTT assays were used to investigate toxicity.

Results and Discussion: PLGA micro- and nano -particles were fabricated at sizes of 2208 ± 648 and 130 ± 35 nm, respectively. Cellulose particles were sized at 416 ± 170 nm. Iron oxide nanocrystals were optimally loaded at an encapsulation efficiency of 50%. TEM shows distribution of magnetite cores throughout the particles. Table 1 lists the r_2 and r_2* molar relaxivity of the three particle types, as well as inert MPIOs and dextran coated nanoparticles. As expected for micron sized magnetic particles, PLGA microparticles had very low r_2, but large r_2*, indicative of static dephasing regime. PLGA nanoparticles had more characteristic nanoparticle r_2 and r_2* behavior. Interestingly, the cellulose acetate and especially the pure cellulose particles had remarkably high r_2 and r_2* relaxivity, on par with inert MPIOs. This is likely due to the very thin coating over the magnetite cores, as well as the high iron density of these particles, as seen in Fig 1C.

In cell labeling experiments, the magnetic PLGA nanoparticles were 500% more effective at labeling human mammary tumor cells than dextran coated nanoparticles. Rather than a function of polymer, this could simply be due to the increased mass of the PLGA and cellulose particles with respect to dextran coated iron oxide nanoparticles. MTT assays showed that magnetic PLGA particles were non-toxic. Lastly, an *in vitro* degradation study showed that both PLGA micro- and nanoparticles were completely degraded by seven days after incubation in PBS and citrate buffer, a common mimic for endosomal environment, while cellulose particles remained partially intact. This trend matches data from published drug release studies using both polymers. In summary, the use of biodegradable, FDA approved polymers to encapsulate high levels of magnetite should accelerate the use of MPIOs towards clinical investigation of MRI-based cell tracking.

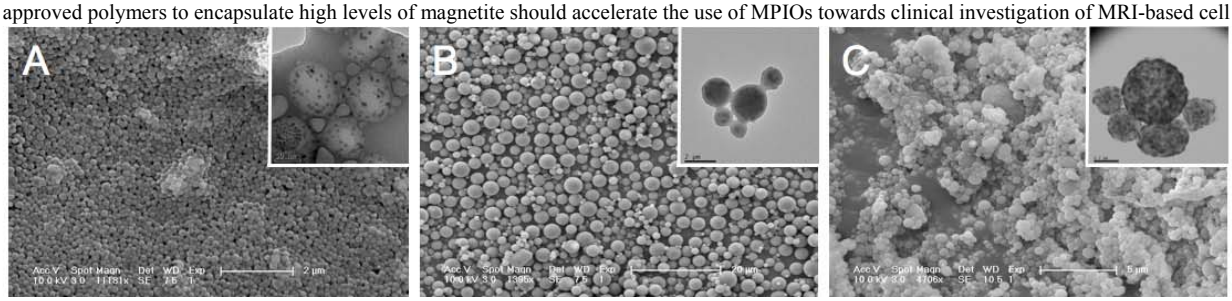

Figure 1: Scanning electron microscopy (SEM) of nanoparticles with TEM inset showing distribution of magnetite cores. A) PLGA nanoparticles; B) PLGA microparticles; C) Cellulose nanoparticles. Particles are uniform in size, spherical and have high amounts of encapsulated magnetite, especially cellulose.

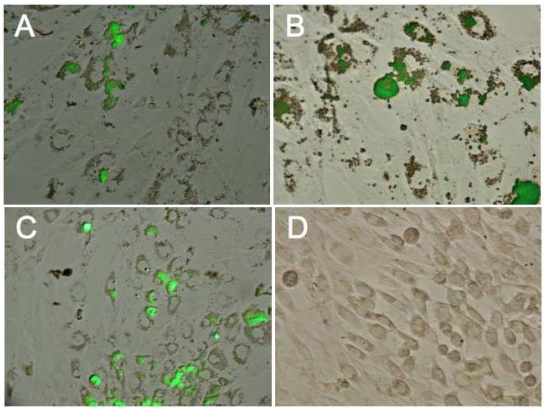

Figure 2: Fluorescence microscopy of mesenchymal stem cells labeled with magnetic A) PLGA nanoparticles, B) PLGA microparticles, C) Cellulose nanoparticles, D) dextran coated particles. A-C are fluorescent due to the encapsulated coumarin-6 dye.

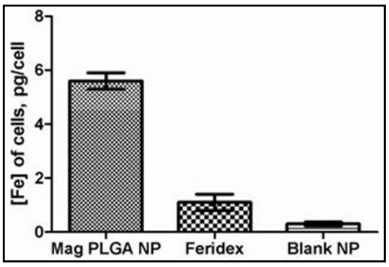

Figure 3: Quantification of cell labeling in MCF-7 human cancer cell line using magneic PLGA nanoparticles, dextran coated particles and empty PLGA nanoparticles..

	PLGA micro	PLGA nano	Cellulose acetate	Cellulose	Dextran coated	Inert MPIO
r_2	13	102	170	451	120	54
r_2*	386	209	345	491	330	454

Figure 4: r_2 and r_2* molar relaxivity measurements for various magnetic particles at 4.0T. All numbers are $mM^{-1}sec^{-1}$. PLGA microparticles have low r_2 and high r_2*, placing relaxation properites in the static dephasing regime. PLGA nanoparticles exhibited relaxation properties associated with diffusion limited regime, similar to dextran coated particles. Cellulose particles had both high r_2 and r_2*. This interesting phenomenon could be due to the extremely thin coating of these particles as shown above.