Labeling of Macrophages with novel Gadolinium Oxide Nanoparticles for In vivo Imaging of Inflammation

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Purpose: To label macrophage with novel T_1 gadolinium oxide agent for *in vivo* tracking with MRI.

Background: Cell labeling with MR contrast agents has been a major focus of *in vivo* imaging. Labeling of cells with T2 agents has allowed the visualization of cell populations *in vivo* but precise concentration and location measurements remains challenging.[1] T_1 labeling of cells is oftentimes preferable to T2 labeling because T_1 contrast exudes positive contrast. Quality labeling of cells with T_1 agents has been difficult due to toxicity, poor stability at physiological pH, and no standardized assay to easily determine targeted T_1 agent efficacy in vivo.[2] We have synthesized and characterized novel PAMPS-LA coated Gd_2O_3 nanoparticles and imaged labeled macrophages at 9.4T.

Methods: Synthesis of gadolinium oxide nanoparticle: Particles were synthesized from $Gd(NO)_3{\cdot}6H_2O$ oleic acid and 1-octadecene, purified by centrifugation, and dispersed in hexane solution. To make the lauryl acrylate-poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS-LA) we dissolved 2-acrylamido-2-methylpropane sulfonic acid in DMF then lauryl acrylate (LA) monomers were added with photo initiator D1173 followed by photo-polymerization in a UV radiator. The particles were coated (PAMPS-LA) by introducing the coating into the Gd_2O_3 nanoparticle/ethyl ether solution followed by stirring and evaporation. The resultant was purified by ultracentrifugation and filtration.

Imaging Protocol: All images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 35mm resonator. Phantoms were imaged using a Rapid Acquisition with Refocused Echoes protocol with Variable Acquisition Time (RAREVTR) protocol to measure T_1-times. Imaging parameters used for RAREVTR: TE=10ms, TR=200 - 9000ms, FOV=20mm, matrix size=128x128, taking 10mins, 33s and 600ms using Paravision 4.0 software (Bruker BioSpin, Billerica, MA). Obtained images were analyzed using Paravision 4.0 software. Graphs and statistics from MRI data and cell labeling were generated using Prism (GraphPad Software, San Diego, CA).

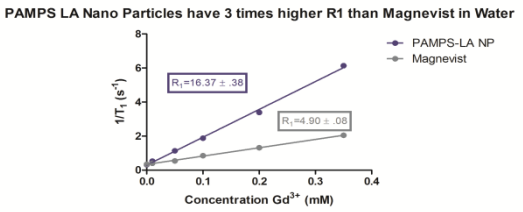

Figure 1 –T_1 relaxivity (R1) as measuredat 9.4T by RAREVTR at 250, 200, 100, 50, 10, and 0mM $Gd^{3+}$for both Magnevist and PAMPS-LA

Cell Culture: The monocyte/macrophage raw 264.7 mouse leukemia cell line was used for labeling and viability tests of PAMPS-LA nanoparticles. Cells were incubated with DMEM plus 10% FBS with various concentrations of the agents for 24 hours (viability) or 2 hours (labeling). Viability was determined using a in triplicate and labeling was measured by comparing brightness of labeled cell pellets.

Results: Gadolinium oxide nanoshells coated with PAMPS-LA are water soluble and are stable in a pH range from 3-10. These T_1 agents have an R1 of 16.37 versus Magnevist's 4.9 in our 9.4T MRI (figure 1). These nanoparticles' relaxivity approaches that of Magnevist when imaged in PBS . Since these particles are being used for targeting the limiting factor for their usefulness is contrast per particle rather than the contrast per gadolinium ion demonstrated by relaxivity. Relative relaxivity is defined as contrast per particle and comparison between PAMPS-LA particles and Magnveist demonstrates that one PAMPS-LA nanoparticle has over 2000 times more contrast than one Magnevist molecule (figure 2).Raw 264.7 cells are viable when labeled in 100uM PAMPS-LA or less(Data not shown due to space). Cells labeled in 50uM for 2hr are significantly brighter than non-labeled cells (figure 3).

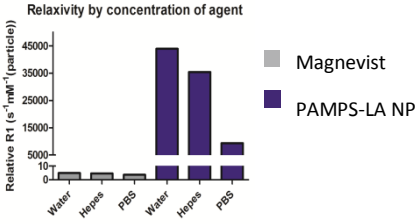

Figure 2 – Relative T_1 relaxivity of Magnevist and PAMPS-LA nano particles in different solvents. Hepes is 20mM pH 7.2. PBS is DPBS without Ca^{2+} or Mg^{2+}.

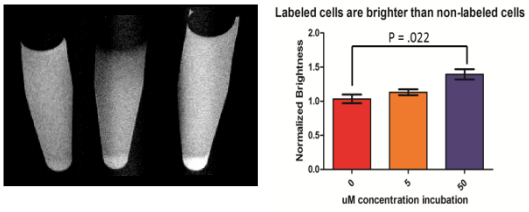

Figure 3– Brightness of pelleted labeled Raw 264.7 monocytes/ macrophages. Cells labeled in medium+10% FBS containing 0, 5, or 50uM PAMPS-LA for 2 hours, wash cells 3X, and image pelleted cells. All measurements were taken in triplicate. Brightness of cell pellet normalized to corresponding supernatant.

Conclusions: These agents provide high T_1 relaxivity and their relaxivity per agent makes them excellent candidates for targeted imaging. With cells labeled with 50μM being significantly brighter than non-labeled cells, it is likely that we will be able to quantify the labeled cells *in vivo*.

References:
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3.Ray, A. et al., Isolation of Mouse Peritoneal Cavity Cells JOVE (2010)