# Imaging developing neural structures in chick embryo using novel $Gd_2O_3$ contrast agent

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**Purpose:** Labeling cells in developing chick embryos 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 produces 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 applied them in imaging the development of chick embryos 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.

**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.

Figure 1 – 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 $Mg^{2+}$.

**Chick Embryo electroporation:** *In ovo* electroporation was performed ad described (Thaler et al., 2002). Briefly, HH13 (E2) chicks were injected with 2ul of either vehicle or 7.6mM PAMPS nanoparticles. The embryos were then electroporated with a BTX Electro Square Porator. After electroporation embryos were incubated until E5 when they were isolated and prepared in 1% agarose for imaging.

**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 5.1 software (Bruker BioSpin, Billerica, MA). Obtained images were analyzed using Paravision 5.1 software. Graphs and statistics from MRI data and cell labeling were generated using Prism (GraphPad Software, San Diego, CA). Embryos were imaged with a T1 weighted RARE scan TR: 600ms TE: 8.5ms RARE FACTOR: 4 FOV: 2.2x1.5x1.5cm Matrix: 128x128x128 Time: 2h2m52s. Images were masked and analyzed in Amria 5.1. (FEI, Hillsboro, OR).

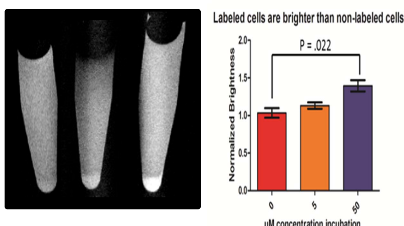


Figure 2– Brightness of pelleted labeled Raw 264.7 monocytes/ macrophages. Cells labeled in medium+10% FBS containing 0, 5, or 50uM PAMPS-LA for 2 hours, wash cells 3X, and image pelleted cells. All measurements were taken in triplicate. Brightness of cell pellet normalized to corresponding supernatant.

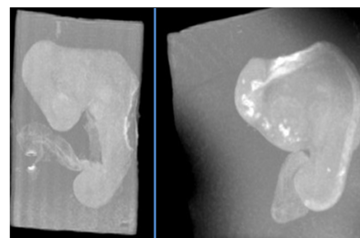

Figure 3– Dorsal view of vehicle (left) and PAMPS (Right) injected and electroporated E5 chick embryos.

**Results:** Gadolinium oxide nanoparticles 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. 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. Raw 264.7 cells are viable when labeled in 100uM PAMPS-LA. Cells labeled in 50uM for 2hr are significantly brighter than non-labeled cells (figure 2). Figure 3 shows *ex vivo* images of sham injected (left) and PAMPS injected (right) embryos. The PAMPS particles have clearly been retained by the developing spinal column of the embryos and the PAMPS particles have also ended up in the embryo's developing brain and eyes.

**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*. In developing embryos we have demonstrated a proof of concept imaging strategy to image developing neural system structures using PAMPS through electroporation into E2 chick embryos.

References: 1.Shapiro, E.M. et al., In vivo detection of single cells my MRI. Mag, Reson Med (2006); 2.Bridot, J.L. et al., Hybrid Gadolinium Oxide Nanoparticles: Multimodal Contrast Agents for In Vivo Imaging J. Am. Chem. Soc (2007); 3.Ray, A. et al., Isolation of Mouse Peritoneal Cavity Cells JOVE (2010)