Manganese - Gold Nanospheres as Positive Contrast Agents for Magnetic Resonance Imaging (MRI)

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Introduction

Gold nanoparticles have become of particular interest to medical researchers for their tunable physicochemical properties [1], their ability to bind to cell markers and treatment drugs [2], and to absorb near-IR light and radiate the energy as heat [3]. Pure gold nanoparticles can be modified by sorbing Mn^{2+} and these Mn-Au nanoparticles were evaluated as positive MRI contrast agents. This nanomaterial could be used as a tunable contrast agent where the sorbed Mn^{2+} can be control-released to reduce the toxicity of the agent by limiting the amount of free manganese in the body. It has been found previously that gold nanoparticles will resonate in the near-IR range causing them to release the drugs contained in them [3]. By using MR imaging the location of the Mn-Au nanoparticles can be monitored for more accurate and precise dosing. In this study we are trying to attenuate the Mn^{2+} releasing rate, therefore the complex can act as a better cell labeling agent and a minimally toxic contrast agent in MRI.

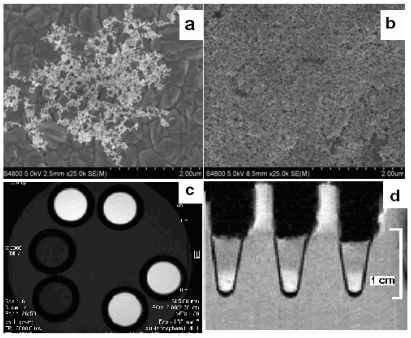

Figure 1: SEM image of (a) 0.1 mM and (b) 1.0 mM Mn-Au nanosphere clusters at 200 µm. T_1 weighted image of (c) Mn-Au nanosphere phantom and (d) labeled MSCs

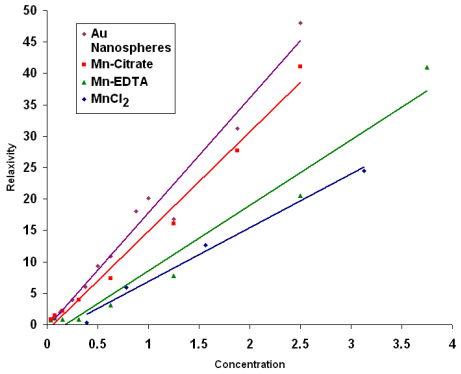

Figure 2: Relaxivity of manganese phantoms

Methods

The Mn-Au nanospheres were synthesized in two steps: (1) synthesis of Au nanospheres and (2) formation of Mn complexes on Au nanosurface. First, an aqueous solution of $HAuCl_4$ was heated to boiling. To this was added an aqueous solution of 1% citrate; and the boiling was continued until the solution turned ruby red, indicating the formation of gold nanoparticles. This method of Au reduction by citrate is known to produce nanoparticles of well-defined size, shape and surface charge [6-9]. The Mn-Au nanospheres were synthesized by adding manganese (II) chloride ($MnCl_2$) to a solution of suspended citrate capped nanospheres. The concentration of Mn in the Mn-Au nanospheres ranged from 62.5 µM to 3.75 mM for the phantom. Bone marrow derived mesenchymal stem cells (MSCs) were used for cell labeling. The MSCs were harvested from 18 month old C57Bl/6 mice. When the cells reached 70-80% confluence, they were lifted with trypsin/EDTA, washed with PBS and suspended at a density of 1×10^6 cells/ml in DMEM containing 20% FBS and 10% DMSO. The cells were cryopreserved in liquid nitrogen. Vials of MSCs were thawed and placed in expansion media (DMEM, 10% FBS and 1% antibiotic/ antimicotic). The media was changed at 24 hours post-expansion, then every 48 hours and when passaged. The cells were cultured at 37°C in an incubator with 5% CO_2. Mn-Au nanoparticles were added to each dish, and the labeled cells were cultured for three days, two days and one day. At each of the prescribed contact durations, cells were then harvested for imaging. The concentration of manganese in the nanospheres ranged from 0.1 mM to 1 mM for cell labeling. The cells were centrifuged to concentrate the cells at the bottom of the container, then imaged in a pre-cast mold made with agarose gel. Images were acquired on a 7.0-T, 20-cm horizontal bore BioSpec MRI spectrometer (Bruker Instruments, Billerica, MA) equipped with a micro imaging gradient insert (950 mT/m). A 35 mm inner diameter volume coil was used to transmit and receive at ^1H frequency. T_1-map short axis phantom images were acquired with a Look-Locker MRI pulse sequence. The T_1-mapping parameters were as follows for the imaging: matrix = 128 x 128; Inter-TE/TR = 2.5 ms/2.6 sec; slice thickness = 5.0 mm (phantom) and 1.0mm (labeled cells); FOV = 3.0 x 3.0 cm; NA = 2; inversion time/interval = 9/50 ms; echo images = 50; flip angle = 10. The total imaging time per T_1-map was approximately 11 minutes. The T_1 value of each pixel was calculated in two steps using a custom written C^{++} program [4]. Regions of Interest (ROIs) analysis on the calculated T_1 maps were performed using AMIDE [5].

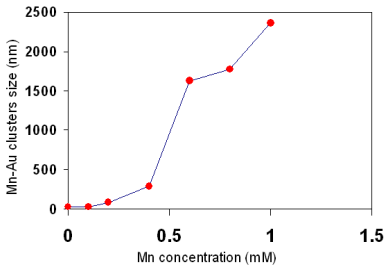

Figure 3: Zeta sizer data

Results

The complex phantom (purple circles) resulted in a relaxivity of 18.3 mM^{-1} sec^{-1} for the entire concentration range and a relaxivity of 20.8 mM^{-1} sec^{-1} for the range used for cell labeling. The relaxivity of the Mn^{2+} phantom was compared to that of other known manganese contrast agents including $MnCl_2$, Mn-citrate, and Mn-EDTA. The relaxivity of the nanoparticles is much higher compared to other Mn^{2+} contrast agents (Figure 2); $MnCl_2$ (blue diamonds) at 8.6 mM^{-1} sec^{-1}, Mn-citrate (red squares) at 15.8 mM^{-1} sec^{-1}, and Mn-EDTA (green triangles) at 10.4 mM^{-1} sec^{-1}, respectively. As the concentration of the Mn in the complexes increases, there is aggregation of the particles as the mean size of the Mn-Au nanospheres increase from 26 nm with no added Mn to 2400 nm at 1 mM Mn. This may lead to a different relaxation rate in the phantom and also has implication for the uptake of nanoparticles in the cells. Preliminary results of the labeled cells (Figure 3) indicate an uptake of manganese at concentrations ranging from 62.5 µM to 1 mM, but show increased uptake at lower concentrations. The ideal retention can be observed at 0.4 mM to 0.5 mM. The Mn-Au nanospheres in the cells must be small enough to enter the cells, but large enough to be retained by the cells to be carried to various organs. The ramifications of the size distribution of the Mn-Au nanoparticles will warrant further investigation. The ΔR_1 values of the average R_1 for the control cells and the average R_1 values for the cells with the Mn-Au nanospheres are shown to the left. SEM images show that the clusters at low Mn concentrations are relatively small, whereas at higher concentrations the clusters aggregate to form larger micro-scale particles (Figure 4).

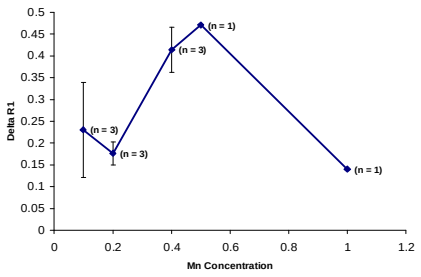

Figure 4: ΔR_1 values for manganese in the cells

Conclusions

The high relaxivity of the Mn-Au nanoparticles makes it ideal for a low toxicity Mn^{2+} MRI contrast agent. This study demonstrates that cells can be labeled with Mn-Au nanospheres. The cells appear to uptake and retain more manganese at concentrations from 0.4 mM to 0.5 mM Mn in the Mn-Au nanosphere clusters. This uptake results in a T_1 enhancement of the cells *ex vivo*. The possibility of attaching drug agents to the nanoparticles combined with the tunability of the Mn^{2+} release from the nanoparticles shows promise for its use as a contrast agent that can be paired with drug treatment for accurate non-invasive MRI monitoring and drug administration.

Reference

1. Hirsch, L.R. et al, PNAS 2003; 100 (23): 13549-13554. **2.** Lowery, A.R. et al., International Journal of Nanomedicine; 2006: I (2) 149-154. **3.** Park, H. et al., Small 2008; 4(2): 192-196. **4.** Chuang, K.H., Koretsky A., Magn Reson Med 2006; 55(3): 604-611. **5.** Loening, A.M., Gambhir, S.S., Mol Imaging 2003; 2(3): 131-137. **6.** Frens, G., Nat-Phys Sci. 1973, 241, 20-22. **7.** Murphy, C.J., et al, Hunyadi, SE, Chem. Comm., 2008, 4, 554-557. **8.** Murphy, C.J., et al, Hunyadi, S.E. J. Phys. Chem. B 2005, 109, 13857-13870. **9**. Murphy, C.J., et al, Hunyadi, SE, Inorg. Chem 2006, 45, 7544-7554.