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The Use of Silica Coated MnO Nanoparticles to Control MRI Relaxivity in Response to Specific Physiological Changes

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ABSTRACT

MnO nanoparticles have been tested to engineer a delayed increase in MRI T_1 relaxivity caused by cellular uptake via endocytosis into acidic compartments. Various coatings on core–shell structured MnO nanoparticles were tested for those that had the lowest T_1 relaxivity at pH 7.4, a pH where MnO does not dissolve into Mn^{2+} ions. The rate of dissolution and release of Mn^{2+} of the different coated MnO particles as well as changes in T_1 relaxivity were measured at pH 5, a pH routinely obtained in the endosomal-lysosomal pathway. Of a number of coatings, silica coated MnO (MnO@SiO_2) had the lowest relaxivity at pH 7.4 (0.29 mM^{-1} sec^{-1}). About one third of the MnO dissolved within 20 min and the T_1 relaxivity increased to that of free Mn^{2+} (6.10 mM^{-1} sec^{-1}) after three days at pH 5. MRI of MnO@SiO_2 particles injected into the rat brain showed time-dependent signal changes consistent with the *in vitro* rates. Thalamocortical tract-tracing could be observed due to the released Mn^{2+}. Intravenous infusion of MnO@SiO_2 particles showed little enhancement in any tissue except gallbladder. The gallbladder enhancement was interpreted to be due to endocytosis by liver cells and excretion of Mn^{2+} ions into the gallbladder. The MnO@SiO_2 core–shell nanoparticles show the best potential for delaying the release of MRI contrast until endocytosis into low pH compartments activate MRI contrast. The delayed enhancement may have benefits for targeting MRI contrast to specific cells and surface receptors that are known to be recycled by endocytosis.

1. Introduction

Magnetic resonance imaging has been widely used for providing high resolution anatomical imaging of animals and humans but is less sensitive than radiotracer imaging modalities for molecular imaging applications. Nonetheless, various contrast agents have been developed for cellular and molecular imaging using MRI [1,2]. With most of these agents, MRI relaxivity do not change and often there are problems distinguishing non-specific effects from specific effects. New contrast agents, with the capability of altering MRI relaxivity in response to specific physiological changes, such as tissue/cellular variations in pH, redox state, oxygenation, or metabolite levels, are gaining more attention. For example, Gd^{3+}-based MRI contrast agents where water access is blocked and unblocked either with binding of small molecules or via enzymatic cleavage have been developed [3,4]. New paramagnetic chelates have been designed that are very sensitive to altered water exchange that can be altered by binding of molecules such as glucose [5]. Advanced protein engineering techniques are also being applied to make MRI based biosensors [6]. Small iron oxide particulates [7] have also been developed that serve as a platform for magnetic resonance T_2 relaxation switches [8–10]. None of these switchable agents have found widespread use due to the relatively small dynamic range of relaxivity changes and the problem of delivering these agents even in animal models.

Manganese enhanced MRI (MEMRI) has found widespread use in MRI [11]. Mn^{2+} ions can visualize anatomical structures of the brain, neuronal activity, and connections between brain regions [12,13]. MEMRI has also been shown to be useful in imaging cardiac function and pancreatic function [14,15]. There has been interest in engineering Mn nanoparticles for MRI [16]. Recently, it has been shown that MRI contrast from certain Mn particles that have very low MRI T_1 relaxivity can be increased dramatically due to dissolution of the particles and release of Mn^{2+} ions in the low pH environment found in the mammalian endosomal-lysosomal

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pathway [17,18]. Mn^{2+} can leave the endosomal-lysosomal pathway to fill the entire cell leading to a much larger volume distribution of the contrast agent [17,19]. This opens the possibility of getting a large, specific, increase in MRI contrast upon cellular uptake. A limitation is that the particles have not been optimized for low relaxivity at normal pH and optimal dissolution rate.

It is well-known that transition metal oxides, such as MnO, dissolve in acidic solutions leading to the release of Mn^{2+} ions. Therefore, MnO nanoparticles were tested with different coating materials, creating core–shell morphologies, to test their effect on controlling MRI T_1 relaxivity and dissolution rate at pH 5. The goal was to have low relaxivity at pH 7.4 and a slow dissolution rate at pH 5. The slow dissolution rate opens the possibility of imaging only those particles that have been taken up into the endosomal–lysosomal pathway, enabling other particles to be cleared from blood and non-specific extracellular binding sites. This in turn is expected to lead to more specific imaging of cellular uptake processes occurring *in vivo*. In the present work, five different coatings on MnO nanocrystals were tested *in vitro* to study the release rate of the Mn^{2+} ions and change in relaxivity at pH 7.4 compared to pH 5. Silica coated MnO was selected and applied for *in vivo* experiments. Particles were injected directly into the cortex of the brain and intravenously into rodents. The system of neuronal thalamocortical connectivity in a rodent model is well established [13]. Particles injected into the thalamus were expected to increase MRI T_1 contrast on a time scale similar to the dissolution rate measured *in vitro*. Furthermore, Mn^{2+} tracing to thalamus should also be detected after injection of particles into the brain cortex. The latter part, intravenous infusion of the MnO@SiO_2 nanoparticles into mice, was performed to identify biodistribution of nanoparticles and MRI contrast enhancement after cellular uptake process. In both *in vivo* studies, silica coated iron oxide particles were utilized as control to investigate whether MRI contrast enhancement resulted from transport of particles or Mn^{2+}. The enhancement in specific tissues indicates that particles will be excellent for visualizing particle uptake by endocytic cells such as macrophages. The lack of contrast in other tissues indicates that these particles may be excellent to be used as molecular targeting agents.

2. Materials and methods

2.1. Synthesis of MnO nanocrystals

To develop an MRI contrast agent that is activated by cell uptake into low pH compartments, the first requirement is for the contrast agent to exhibit a low relaxation rate in neutral pH (in normal body compartments and/or fluid) and a higher relaxation rate in lower pH. A second design feature for the present studies was to have delayed activation when transferred to a low pH environment to help potentially increase the specificity for MRI detection. Inorganic manganese oxides are known to be insoluble at neutral pH but their dissolution can be accelerated by the presence of excess protons, i.e. acidic solutions. Using MnO nanoparticles as the Mn^{2+} source and coating them with five different materials, to create core-shells, we tested the dissolution rate of Mn^{2+} from the nanoparticles. Coatings were chosen to see how efficiently different types of materials (small molecules, polymers, and inorganic materials) would affect the relaxivity change. Mercaptosuccinic acid (MSA), a representative small molecule; Pluronic PF127, a widely used polymeric molecule in drug delivery applications [20]; PMAO and PMAO-PEG, both proven to provide high quantum yield of quantum dots after phase transferring, and SiO_2, a popular inorganic coating material were used. All of them were tested for their ability to retard the particles from dissolving at pH 5 and slowly release Mn^{2+} ions over time.

Highly monodisperse, single-phase, MnO nanocrystals (NCs), ~10 nm in diameter, were prepared by chemical routes and their structure and magnetic properties were extensively characterized [21,22]. Monodispersed MnO nanoparticles were synthesized by thermal decomposition of a Mn-oleate complex in an organic solvent [23]. The Mn-oleate complex was prepared by mixing 0.2 g of $Mn_2(CO)_{10}$ in 2 mL of oleylamine at 100 °C. The complex solution was cooled to room temperature and then 10 mL of trioctylphosphine was added. For the growth of nanocrystals, the solution mixture was heated up to 280 °C under argon atmosphere and kept under vigorous stirring for 1 h. The reactants were then cooled to room temperature and the nanoparticles were obtained by adding ethanol, followed by centrifugation. They were then re-dispersed in non-polar solvents such as hexane or toluene.

2.2. Transferring MnO particles into aqueous solution with five different coatings

Nanoparticles synthesized in non-polar solvents have narrow size distributions but are not biocompatible. Hence, the MnO nanocrystals were coated with amphiphilic molecules to help disperse them in aqueous solutions for biomedical applications. Mercaptosuccinic acid (MSA), poly(maleic anhydride-alt-1-octadecene) (PMAO), Pluronic F-127 (PF127), PMAO-PEG and SiO_2 were then used, respectively, to transfer native hydrophobic particles to aqueous solutions. The method of producing MnO nanoparticles with five different coatings are as follows:

a) MnO@MSA: MnO nanoparticles (20 mg) in 4 mL chloroform and mercaptosuccinic acid (MSA) (30 mg) in 4 mL dimethyl sulfoxide (DMSO) were loaded in a small vial. The mixture was stirred for 20 h. The particles were washed with ethanol and collected by centrifugation. These particles were re-dispersed in buffer solutions and were ready to be used for *in vivo* experiments.
b) MnO@PF127: MnO nanoparticles (20 mg) and pluronic F127 (80 mg) were mixed with 4 mL chloroform in a small vial. The mixture was stirred for 20 h. Then all the solvents were evaporated and particles were re-dispersed in buffer solutions.
c) MnO@PMAO: A solution of 100 mg poly(maleic anhydride-alt-1-octadecene) in 2 mL $CHCl_3$ and a solution of 15 mg MnO nanoparticles in 3 mL $CHCl_3$ were mixed and stirred for 2 h at room temperature. 10 mg 1,10-diaminodecane in 1 mL $CHCl_3$ was then added to crosslink the polymer shell that had formed around each particle. The mixed solution was sonicated for 10 min and stirred for 20 h. Followed by evaporating the solvent, a brown solid was obtained and re-dispersed in TAE buffer solutions.
d) MnO@PMAO-PEG: A solution of 85 mg PMAO-PEG in 2 mL $CHCl_3$ and a solution of 15 mg MnO nanoparticles in 3 mL $CHCl_3$ were mixed and stirred for 2 h at room temperature. The solvent was then evaporated and the solid obtained was re-dispersed in TAE buffer solutions. PMAO-PEG was obtained as follows. 2 g poly(maleic anhydride-alt-1-octadecene), 0.6 g poly ethylene glycol methyl ether and 0.3 mL H_2SO_4 were mixed in 20 mL Acetone solution and refluxing at 57 OC for 24 h. Followed by adding de-ionized water, white PMAO-PEG was obtained and ready for the above reaction.
e) MnO@SiO_2: Igepal CO-520 (0.54 mmol) dispersed in 4.5 mL cyclohexane by sonication and MnO nanoparticles (0.4 mg) in 0.4 mL cyclohexane were mixed in a small vial. Then ammonium hydroxide (0.04 mL) was added to the mixture and stirred for 2 min. Subsequently, tetraethyl orthosilicate (TEOS) (0.03 mL) was added and the reaction was continued for 48 h. The particles were precipitated with excess hexane and collected by centrifugation. The total size of particles was ~20 nm in diameter with 10 nm core and 5 nm shell thickness as determined by transmission electron microscopy (TEM).

2.3. MRI parameters for in vitro relaxivity measurements

T_1 relaxation times of the various-coated MnO core–shell nanoparticles, dispersed in water and prepared in 1 mL syringes with varying concentrations, were measured on an 11.7T/31 cm horizontal bore magnet (Magnex Scientific Ltd., Abingdon, UK), which was equipped with a 12 cm gradient/shim set (Resonance Research Inc., Billerica, MA, USA) and interfaced to a Bruker Avance console (Bruker BioSpin, Billerica, MA, USA). A 90 mm volume coil was used for transmit and receive. A T_1 saturation recovery sequence (TR = 200, 642.6, 1211.7, 2010.4, 3360.2, 10,000 ms; TE = 8.63 ms) was used to measure T_1. The images were fitted into a 3-parameter function to calculate T_1 values using Bruker TopSpin built-in analysis program. The specific relaxivities (r_1) of the MnO nanoparticles were measured as follows. Each sample was prepared in five different concentrations, and T_1 values were measured for each concentration, which were then used for r_1 calculations, respectively. Relaxivity was determined from the slope of concentration-dependent T_1 changes. The Mn concentrations were based on the molar concentration of manganese atom measured by ICPMS.

2.3.1. In vivo observation of increasing contrast enhancement in the rat brain with injection of MnO@SiO_2 core–shell nanoparticles

MnO@SiO_2 core–shell nanoparticles were injected into the thalamus to observe the local contrast enhancement with time. It was also injected into the cortex (S1) to test their capability of being neuronal tract-tracing agents. Silica coated Fe_3O_4 was used as a control for independently verifying the location of particles.

2.4. Synthesis of SiO_2 coated Fe_3O_4 (Fe_3O_4@SiO_2) core–shell nanoparticles

As a control for the properties of MnO@SiO_2, silica coated iron oxide particles (Fe_3O_4@SiO_2) were synthesized. Igepal CO-520 (10 mL) dispersed in 170 mL cyclohexane by sonication and Fe_3O_4 nanoparticles [24] (52 mg) in 10 mL cyclohexane were mixed in a flask. Then ammonium hydroxide (1.3 mL) was added to the mixture and stirred for 2 min. Subsequently, tetraethyl orthosilicate (TEOS) (0.7 mL) was added and the reaction was continued for 48 h. The particles were precipitated with excess hexane and collected by centrifugation.

2.5. Animal procedure for brain injections of nanoparticles

All animal work was performed according to the guidelines of the Animal Care and Use Committee and the Animal Health and Care Section of the National Institute