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Manipulating the surface coating of ultra-small Gd_2O_3 nanoparticles for improved T_1-weighted MR imaging

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ABSTRACT

In this report, monodispersed ultra-small Gd_2O_3 nanoparticles capped with hydrophobic oleic acid (OA) were synthesized with average particle size of 2.9 nm. Two methods were introduced to modify the surface coating to hydrophilic for bio-applications. With a hydrophilic coating, the polyvinyl pyrrolidone (PVP) coated Gd_2O_3 nanoparticles (Gd_2O_3-PVP) showed a reduced longitudinal T_1 relaxation time compared with OA and cetyltrimethylammonium bromide (CTAB) co-coated Gd_2O_3 (Gd_2O_3-OA-CTAB) in the relaxation study. The Gd_2O_3-PVP was thus chosen for its further application study in MRI with an improved longitudinal relaxivity r_1 of 12.1 mM^{-1} s^{-1} at 7 T, which is around 3 times as that of commercial contrast agent Magnevist®. In vitro cell viability in HK-2 cell indicated negligible cytotoxicity of Gd_2O_3-PVP within preclinical dosage. In vivo MR imaging study of Gd_2O_3-PVP nanoparticles demonstrated considerable signal enhancement in the liver and kidney with a long blood circulation time. Notably, the OA capping agent was replaced by PVP through ligand exchange on the Gd_2O_3 nanoparticle surface. The hydrophilic PVP grants the Gd_2O_3 nanoparticles with a polar surface for bio-application, and the obtained Gd_2O_3-PVP could be used as an in vivo indicator of reticuloendothelial activity.

1. Introduction

Magnetic resonance imaging (MRI) is a promising imaging technique for early stage disease diagnosis. It is a complementary modality for molecular imaging as it provides the images with high spatial resolution and anatomical details without exposure to radioisotopes such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) [1–4]. Contrast agents are introduced to differentiate the hydrogen nuclei situated at diverse environment and highlight the part of interest by means of contrast difference resulted from the alternation of the signal intensity [5–7].

Gadolinium possesses seven unpaired electrons, and the spin of which perturbs the proton relaxation in water results in an efficient shortening of longitudinal relaxation time and increase the magnetic resonance signal intensity. Therefore, gadolinium based agents performing as high efficiency T_1 contrast agents, which provide with positive signals, have been attracting wide research interest [8–10]. And they are the most commonly used contrast agents in MRI, especially for fine vasculature in MR angiography and brain tumor enhancement associated with the degradation of the blood–brain barrier [11]. But the unbounded gadolinium ion is known to inhibit the calcium channels and has considerable cardiovascular and neurologic toxicity [12,13]. The toxicity can be enormously reduced when the gadolinium ions are chelated. It is reported that the LD_{50} increases 100-fold after chelation [14]. However, the concern of the toxicity of gadolinium chelates still continues because they suffer stability problem and release small amount of free gadolinium ions. In 2009, the World Health Organization (WHO) issued a restriction on use of several gadolinium chelates contrast agents in patients with severe kidney problems, or with who are scheduled for or have recently received a liver transplant.

Inorganic crystalline gadolinium based compound nanoparticles provide a rigid crystal environment that is expected to effectively prevent nanoparticles from releasing free gadolinium

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ions, so that they are considered as a new generation of T_1 contrast agents [8,15]. It is generally recognized that only the gadolinium close to the surface contributes to the contrast effect. Therefore, the preparation of monodispersed ultra-small nanoparticles with size uniformity and large surface area is of specific importance. Among the gadolinium based inorganic nanoparticles, GdF_3 or $NaGdF_4$ nanoparticles and Gd_2O_3 nanoparticles have been mostly investigated [15–21]. The former could be doped with other lanthanides to integrate optical and MR contrast effect into dual modality probes [16,17,19]. The later demonstrates promising T_1 contrast agent with improved T_1-weighted MR contrast enhancement [15,20–22]. Bridot et al. [23] and Johnson et al. [19] found that with the decrease in the particle size, the r_1 value was enormously enhanced. The r_1 values of 9.9 and 9.4 $mM^{-1}\ s^{-1}$ have been achieved when the Gd_2O_3 particle size reduced to 1.0 nm and 1.1 nm, respectively [20,21].

Several methods have been developed to synthesize small sized Gd_2O_3 nanoparticles for the application in MRI contrast agents. Preparation from gadolinium precursors in the presence of stabilizers such as polyethylene glycol or its derivatives results in stable Gd_2O_3 colloidal solutions with particle size ranging from 2 to 15 nm [22–24]. Decomposition of gadolinium acetate encapsulated in single wall carbon nanotubes produces Gd_2O_3 nanoparticles with a small particle size of 2.3 nm [25]. For the preparation of ultra-small metal oxides, the organic synthesis route has been proved to be able to produce very good particle quality with a controlled size and narrow distribution. This method includes the decomposition of metal precursors in a non-polar organic solvent with the presence of OA or trioctylphosphine oxide as the capping agent [26–28]. Efforts have been made to transfer these nanoparticles from hydrophobic into hydrophilic for bio-applications [29]. However, most works still maintain OA on the surface that keeps the water molecules away from the Gd_2O_3 core, and thus seriously affects the magnetic influence of Gd(III) to the relaxation of protons.

In this report, we adopted the organic synthesis route with an improved procedure to synthesize monodispersed ultra-small Gd_2O_3 nanoparticles. Then the OA capped Gd_2O_3 nanoparticles were phase transferred into hydrophilic through ligand exchange and bi-layer coating methods. The influence of different surface coating on Gd_2O_3 nanoparticles was then investigated based on their performance in reducing T_1 relaxation time. The in vitro cell viability and in vivo experiment were carried out to evaluate their cytotoxicity and application to T_1-weighted MR imaging.

2. Experimental section

2.1. General methods

Gadolinium acetate, oleic acid (OA), oleylamine, cetyltrimethylammonium bromide (CTAB), polyvinyl pyrrolidone (PVP), gadolinium oxide, dimethylformamide (DMF), dichloromethane (DCM), ethyl ether, chloroform and toluene were purchased from Sigma–Aldrich and used without further purification. XRD patterns were collected by Bruker D8 advance. TEM images and SAED were obtained on JEOL FE-TEM 2010. FT-IR spectra were achieved on Agilent Cary 600 FT-IR. Dynamic light scattering (DLS) measurements were carried out on Malvern Zetasizer Nano-ZS. Thermal gravimetric analysis (TGA) was performed on SDT Q600. Inductively coupled plasma (ICP) analysis was conducted with Dual-view Optima 5300 DV ICP-OES system.

2.2. Synthesis and sample preparations

2.2.1. Synthesis of Gd_2O_3-OA nanoparticles

Gadolinium acetate (1.0 mmol, 334.0 mg) was mixed with oleic acid (18.0 mmol, 5.1 g) and oleylamine (60.0 mmol, 16.1 g). The mixture was heated at 120 °C in a three neck flask for 1 h under mild N_2 flow to remove water. Then a reflux condenser was mounted to the flask and the temperature was raised to 280 °C immediately and maintained for another 6 h. After cooling down, the mixture was treated with ethanol for the product to crash out. The product was collected with centrifugation and washed with toluene and ethanol three times. The final product was suspended and stored in 20 ml toluene or chloroform.

2.2.2. Ligand exchange to prepare Gd_2O_3-PVP

PVP (0.02 mmol, 1.0 g), Gd_2O_3-OA (0.25 mmol, 5.0 ml) stock solution in toluene and 25.0 ml DMF/DCM (volume ratio of 1:1) were mixed and refluxed at 80 °C for 12 h. The reaction solution was cooled down and added to 130 ml ethyl ether dropwise under vigorous stirring. Precipitate appeared immediately and was collected by centrifugation. The product was washed with toluene and ethyl ether three times. And the final product was dispersed and kept in DI water.

2.2.3. Phase transfer to prepare Gd_2O_3-OA-CTAB

Gd_2O_3-OA (0.25 mmol, 1.0 ml) stock solution in chloroform was then mixed with CTAB (1.0 mmol, 365.0 mg) in 5.0 ml DI water, followed by vortex mixing for 40 min to form micro-emulsion. Then the mixture in unsealed flask was heated at 80 °C for 1 h. The product was collected by immediate centrifugation.

2.2.4. Dialysis

Gd_2O_3-PVP and Gd_2O_3-OA-CTAB colloidal solutions were sealed in dialysis tubing cellulose membrane (MWCO = 2000) and dialyze against 200 ml DI water in a beaker with mild stirring for 3 days. Then the dialysis tubes were taken out, and the remaining liquid was concentrated to about 20 ml by evaporation. The Gd(III) concentration in the remaining liquid was then measured by inductively coupled plasma (ICP) analysis to determine the release of Gd(III) from the nanoparticles.

2.3. In vitro cell viability studies

HK-2 proximal tubule epithelial cells (ATCC, CRL-2190™) were cultured in keratinocyte serum free media (K-SFM, Gibco® Invitrogen™) supplemented with prequalified human recombinant epidermal growth factor, bovine pituitary extract and 1% penicillin-streptomycin solution at 37 °C in a humidified incubator with 5% CO_2. For the cell viability assay the cell passage number was kept within 4–6. In general, 5000 viable cells/100 μl media were added in each well of a 96-well plate. The cells were allowed to attach for 24 h. Gd_2O_3-PVP was prepared at different concentrations using sterile 1× PBS. Gd_2O_3 (Sigma–Aldrich®) was used for comparison study. The prepared solution was further sterilized under UV for 45–60 min. After 24 h cell attachment, 10 μl of the contrast agent at different concentration was added (6 wells for one concentration). To another 6 well, 10 μL of sterile 1× PBS was added, these wells were used as positive control. Each concentration of the contrast agent was added to one well containing no cell but 100 μL of the culture media alone. The cells were incubated with the contrast agent at different time points. 4 h before each time point 10 μL of CCK-8 (Cell Counting Kit-8, Sigma–Aldrich®) was added into each of the well. After 4 h incubation, the absorbance of each well was read at 450 nm (reference wavelength of 650 nm) using a well plate reader (SpectraMax 340PC, Molecular Devices LLC). The cell viability was calculated after correction for absorbance with the control wells. The data is represented as concentration Vs. % Cell viability ± SE and % Cell viability = [OD of sample well/OD of positive control] × 100.

2.4. Relaxivity measurements

Longitudinal (r_1) and transverse (r_2) relaxivities were measured using a 7T Bruker ClinScan® with a Siemens interface and 72 mm volume coil. T_1 was measured using an inversion-recovery sequence; TR/TE = 5000/7; Average = 3; flip angle = 90° and inversion-times (TIs) = 31, 150, 300, 700, 1200, 1800, 2400, 3000, 3600, 4000, 4500 and 4900 ms. T_1 was calculated from the inversion-recovery images, by fitting for the decay curve. T_2 was measured using a multiple spin echo sequence TR = 5000 ms; TE = 12.9; NE = 14. The sample was maintained at 36 °C using a circulated water bath. T_2 maps were obtained along with the inline acquisition sequence program. The r_1 and r_2 values were reported for 1.0 mM concentration of the contrast agent (slope of the linear fit between $1/T_1$ or $1/T_2$ Vs. concentration of contrast agent in mM). The relaxivity is represented as $mM^{-1}\ s^{-1} \pm SD$ ($n = 3$).

2.5. Xenograft tumor model and in vivo MRI

All animal work was carried out under IACUC ethical policies and guidelines #100591. HT1080 fibrosarcoma xenograft was grown on SCID mice (6 week old, female). In brief, the cells grown in DMEM with 10% FBS and 1% penicllin-streptomycin were prepared at concentrations of 1×10^6 to 3×10^6 viable cells in volumes of approximately 100–150 μl 1× PBS (with 5% v/v Matrigel to get a slightly viscous solution). Using a 30G needle, the cells were injected slowly under the skin on the right flank of the animal. Tumors were measured periodically and animals were scanned when the tumor size reached 150–200 mm^3.

The first phase tracer kinetics was studied using a FLASH sequence with a temporal resolution of 10 s in the coronal plane. TR/TE = 10.62/1.32 ms, flip angle = 20°. T_1-weighted image was obtained using a gradient echo sequence in the axial plane with TR/TE = 400/2.6 ms and flip angle = 90°, the image was acquired when animal was gated with 80 beats per min respiration rate in 1.5% (v/v) isoflurane. T_1 maps were obtained using a turbo-spin echo inversion-recovery sequence with respiration gating. With TR/TE = 5000/7.7 ms and flip angle = 180°, with fat saturation and turbo factor of 3. Inversion times = 41, 500, 1000, 1500, 2000, 3000, 4000 ms. T_1 maps were obtained with the in-house Matlab program.