Dual magnetic resonance imaging (MRI)-fluorescent imaging (FI) agents : ultrasmall mixed lanthanide oxide nanoparticles

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Introduction

There is a continuing interest in lanthanide oxide nanoparticles because they have a variety of possible applications to molecular imaging as highly sensitive single or dual imaging agents. This arises from diverse magnetic and fluorescent properties of 4f-electrons in lanthanide ions. From combination of several precursor lanthanide ions in synthesis, a variety of single-phase mixed lanthanide oxide nanoparticles with both magnetic resonance imaging (MRI) and fluorescent imaging (FI) properties can be synthesized. In particular, magnetic and fluorescent properties of lanthanide oxide nanoparticles do not much depend on surface coating because 4f-electron properties are not much affected by chemical bonding due to compactness of 4f-orbitals close to nucleus. Lanthanide oxide nanoparticles can be also made ultrasmall because their magnetic properties do not sharply decrease with particle diameter and because fluorescent intensities become rather enhanced. This makes them extremely useful for in vivo applications because only ultrasmall particle diameters could be excreted through renal system.

Materials and Methods

4 mmol of $Dy(NO_3)_3.xH_2O$ (or $Ho(NO_3)_3.5H_2O$) and 1 mmol of $Eu(NO_3)_3.5H_2O$ were added to 40 mL of triethylene glycol in a 100 mL three neck flask. The mixture solution was magnetically stirred at 40 °C under atmospheric condition until the precursors were completely dissolved in triethylene glycol. In a separate flask, NaOH solution was prepared by dissolving 15 mmol of NaOH in 5 mL of methanol. Then NaOH solution was slowly added to the above precursor solution by using a syringe. The reaction temperature was raised up to 240 °C and maintained at that temperature for 24 hours. The product nanoparticle solution was cooled to 80 °C for surface coating. In case of mixed Ho/Tb oxide nanoparticles, 2.5 mmol of $Ho(NO_3)_3.5H_2O$ and 2.5 mmol of $Tb(NO_3)_3.5H_2O$ were added to 40 mL of triethylene glycol. The mixture solution was magnetically stirred until the precursors were completely dissolved in triethylene glycol. Then, NaOH solution separately prepared as mentioned above was slowly added to the above precursor solution. The reaction mixture was magnetically stirred at 80°C for 2 hours. Then, 7.5 mL of 50% H_2O_2 aqueous solution was slowly added to the reaction solution. After addition of H_2O_2, the reaction continued for additional 2 hours. For surface coating, 5 mmol of D-glucuronic acid was added to the above nanoparticle solution which was then magnetically stirred at 80 °C under atmospheric condition for 24 hours. The product solution was cooled to room temperature. It was transferred into a 1 L beaker containing 500 mL of triply distilled water (or ethanol) and magnetically stirred for 30 minutes. After nanoparticles were settled down into the beaker bottom, top transparent solution was decanted and the remaining nanoparticles were again washed with triply distilled water (or ethanol) for three times. When ethanol was used as a washing solvent, nanoparticles were more quickly settled down into the beaker bottom than when triply distilled water was used. In this case, nanoparticles were finally washed again with triply distilled water to remove ethanol. The first half volumes of washed nanoparticles were diluted with triply distilled water to prepare aqueous sample solutions whereas the remaining half volumes were subject to powder samples by drying them in air for various characterizations.

Results and Discussion

From high resolution transmission electron microscope (HRTEM) and high voltage electron microscope (HVEM) images, the average core particle diameters (d_{avg}s) of D-glucuronic acid coated ultrasmall $Dy_{1.5}Eu_{0.5}O_3$, $Ho_{1.6}Eu_{0.4}O_3$, and $Ho_{1.1}Tb_{0.9}O_3$ nanoparticles were estimated to be 2.3±0.1, 2.1±0.1, and 2.5±0.1 nm, respectively. From dynamic light scattering (DLS) patterns, the average hydrodynamic diameters (a_{avg}s) of D-glucuronic acid coated ultrasmall $Dy_{1.5}Eu_{0.5}O_3$, $Ho_{1.6}Eu_{0.4}O_3$, and $Ho_{1.1}Tb_{0.9}O_3$ nanoparticles were estimated to be 6.7±0.1, 6.4±0.1, and 7.6±0.1 nm, respectively. The surface coating was investigated by recording Fourier transform-infrared (FT-IR) absorption spectra of powder samples of D-glucuronic acid coated ultrasmall mixed lanthanide oxide nanoparticles in pallet forms in KBr. Stretching frequencies characteristic of C–H at 2910 cm^{-1}, C=O at 1610 cm^{-1}, and C–O at 1070 cm^{-1} of D-glucuronic acid in powder samples confirmed the surface coating. Furthermore, the C=O stretch was red-shifted by ~100 cm^{-1} from that (= 1710 cm^{-1}) of a free D-glucuronic acid, confirming that its –COOH group was bonded to a nanoparticle. The toxicities of three aqueous sample solutions of D-glucuronic acid coated ultrasmall mixed lanthanide oxide nanoparticles were investigated by measuring cytotoxicity up to 400 (or 500) μM combined concentrations using both DU145 and NCTC1469 cells. They were non-toxic and thus, used for in vivo MR experiments. To measure in vivo 3 tesla T_2 MR images, aqueous sample solutions of D-glucuronic acid coated ultrasmall mixed lanthanide oxide nanoparticles were injected into a mouse tail vein. Decent negative contrast enhancements in both liver and kidneys were observed one hour after intravenous injection. Therefore, D-glucuronic acid coated ultrasmall mixed lanthanide oxide nanoparticles clearly functioned as T_2 MRI contrast agents. Stronger negative contrast enhancements will be observed at higher MR fields because r_2 is proportional to the square of applied MR field, implying that these nanoparticles are potential T_2 MRI contrast agents at high MR fields. To measure in vitro fluorescent confocal cellular images, aqueous sample solutions were treated to DU145 cells. Control DU145 cells to which no sample solution was treated were also prepared in the same condition. The cell nuclei tinted pale blue at λ_{ex} = 405 nm because they were stained with 4',6 diamidino-2-phenylindole (DAPI). No fluorescence was observed in control cells at λ_{ex} = 488 nm. However, red fluorescence with treatment with $Dy_{1.5}Eu_{0.5}O_3$ nanoparticles and green fluorescence with treatment with $Ho_{1.1}Tb_{0.9}O_3$ nanoparticles were observed at λ_{ex} = 488 nm. These results demonstrated that ultrasmall mixed lanthanide oxide nanoparticles functioned sensitively as FI agents.

Conclusions

We demonstrated that mixed lanthanide oxide nanoparticles should be potential dual T_2 MRI-FI agents by using three systems of ultrasmall mixed lanthanide (Dy/Eu, Ho/Eu, and Ho/Tb) oxide nanoparticles. Appreciable r_2 values at 1.5 tesla MR fields and appreciable fluorescence in visible region were observed in all samples. T_2 MRI-FI dual capability was investigated by measuring 3 tesla in vivo T_2 MR images in a mouse and fluorescent confocal images in DU145 cells. We clearly observed appreciable negative contrast enhancements in 3 tesla T_2 MR images in both liver and kidney in a rat and fluorescences in confocal images in DU145 cells, proving T_2 MRI-FI dual functionality of ultrasmall mixed lanthanide oxide nanoparticles.

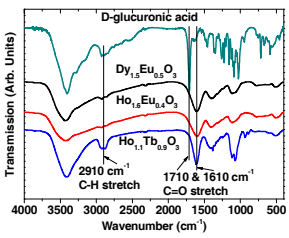

Figure 1. FT-IR absorption spectra of a free D-glucuronic acid and three powder samples of D-glucuronic acid coated ultrasmall mixed lanthanide oxide nanoparticles.

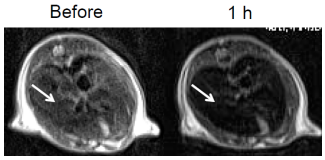

Figure 2. 3 tesla T_2 MR images in a mouse.

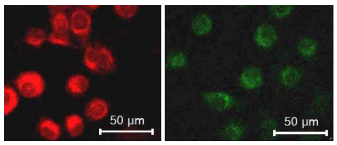

Figure 3. Fluorescent confocal cellular images with 488 nm for DU145 cells treated with aqueous sample solutions of D-glucuronic acid coated ultrasmall $Dy_{1.5}Eu_{0.5}O_3$ and $Ho_{1.1}Tb_{0.9}O_3$ nanoparticles.