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Synthesis of bifunctional $Gd_2O_3:Eu^{3+}$ nanocrystals and their applications in biomedical imaging

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Abstract: Ultrafine $Gd_2O_3:Eu^{3+}$ nanocrystals were successfully prepared by a simple reverse microemulsion method and subsequent calcination. Their structural, optical and magnetic properties were investigated using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR), photoluminescence (PL), and magnetic property measurement system (MPMS). The amorphous $Gd_2(CO_3)_3:Eu^{3+}$ colloidal spheres were proved as an intermediate product, and gradually transformed into crystallized $Gd_2O_3:Eu^{3+}$ with average diameter less than 100 nm. The paramagnetic property of the synthesized $Gd_2O_3:Eu^{3+}$ nanocrystals were confirmed with its linear hysteresis plot (*M-H*). And $Gd_2O_3:Eu^{3+}$ nanocrystals showed high contrast T_1-enhancing modality due to the presence of the Gd^{3+} ions onto the particle surface. In addition, the application of the $Gd_2O_3:Eu^{3+}$ nanocrystals as biotag for cell labeling was reported, red fluorescence from Eu^{3+} ions observed by fluorescence microscopy showed that the nanocrystals could permeate the cell membrane. Cytotoxicity studies of the $Gd_2O_3:Eu^{3+}$ nanocrystals showed no adverse effect on cell viability, evidencing their high biological compatibility. Therefore, the nanoprobe formed from $Gd_2O_3:Eu^{3+}$ nanocrystals provided the dual modality of optical and magnetic resonance imaging.

Keywords: $Gd_2O_3:Eu^{3+}$ nanocrystal; dual-modality; optical and MR imaging; rare earths

In the past decades, more and more attention has been paid to nanostructures with multiple functions such as imaging, targeting, drug delivery and so on, in the biomedical field[1–3]. These nanostructures provide multiple modalities and necessary information[4–8]. For example, the most popular nanostructured multimodal imaging probe is a combination of magnetic resonance (MR) and optical imaging modalities[9–12]. Nanoparticles combining an optical imaging modality and MRI modality offer the advantages of optical imaging, i.e., high sensitivity, along with the high spatial resolution anatomic imaging capability of MRI. However, the traditional multifunctional composites often have two or more cores, which makes the introduction of two or more kinds of magnetic/fluorescent nanoparticles into such a hybrid system complex and difficult. Thus, the single-phase multifunctional nanoparticle without adding any other moieties is the trend in biomedical development.

Gadolinium (III) nanomaterials, such as $GdPO_4$, $Gd_2O(CO_3)_2·H_2O$, $NaGdF_4$, and so on, have been widely used in optical and magnetic probes, dual probe for both and other biological applications[13–15]. The seven unpaired electrons of Gd^{3+} ion provide a strong paramagnet, which could be used as the contrast agent for T_1-enhanced MR imaging[15–18]. Luminescent imaging agent with controllable emission wavelengths can be obtained by doping different light-emitting Ln^{3+} ions into the Gd^{3+}-containing host matrix. In addition, the Ln^{3+}-doped nanophosphors are non-toxic and highly resistant to photobleaching, blinking and photochemical degradation[19,20]. Ln^{3+}-doped nanophosphors are the promising alternatives to the conventional organic dyes and semiconductor quantum dots luminescent labels for biological assays and medical imaging.

Among various Gd^{3+}-based inorganic nanoparticles, Gd_2O_3 nanoparticles are most widely used[21,22]. Gd_2O_3 is a rare-earth oxide with high chemical and thermal stability and a large band gap of 5.4 eV. It can provide the high spatial resolution for MRI. Meanwhile, it is also an excellent luminescent host matrix for a variety of optically active Ln^{3+} ions. Many efforts have been made to develop methods for preparing $Gd_2O_3:Ln^{3+}$ of different shapes and sizes[23–25]. In comparison, the synthesis of particles in reverse microemulsion is more attractive than many other methods, in terms of being able to have homogeneous morphology and being nanosized.

In the present work, ultrafine bifunctional $Gd_2O_3:Eu^{3+}$ nanocrystals were synthesized by calcining $Gd_2(CO_3)_3$:

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Eu^{3+} colloids which were obtained via a reverse microemulsion for the first time. Their magnetic and luminescent properties were characterized in detail using various techniques. The results indicated that $Gd_2O_3:Eu^{3+}$ nanocrystals were a very promising dual-mode imaging nanoprobe for fluorescence and MRI analysis.

1 Experimental

1.1 Materials

$RECl_3$ (RE=Gd or Eu) salts were freshly prepared by the reaction of RE_2O_3 (99.99%) with dilute hydrochloric acid. All other chemicals were of analytical grade and used directly without further purification.

1.2 Synthesis of $Gd_2O_3:Eu^{3+}$ nanocrystals

The $Gd_2O_3:Eu^{3+}$ nanocrystals were prepared by a simple reverse microemulsion method. In a typical procedure, heptane (as oil phase, 73.1 wt.%), CTAB (as surfactant, 7.4 wt.%), butanol (as cosurfactant, 12.2 wt.%) and 0.1 mol/L $LnCl_3$ or 0.15 mol/L $Na_2(CO_3)_3$ (as aqueous phase, 7.4 wt.%) were mixed and stirred until the mixture became transparent. Mixture I with 0.1 mol/L $LnCl_3$ as aqueous phase was poured into mixture II with 0.15 mol/L $Na_2(CO_3)_3$ as aqueous phase. The mixture was magnetically stirred for 12 h at room temperature and centrifuged. The $Gd_2(CO_3)_3:Eu^{3+}$ pellet was collected and washed with water and ethanol for three times. The resultant $Gd_2(CO_3)_3:Eu^{3+}$ pellet was calcinated at 600 ºC for 2 h to obtain $Gd_2O_3:Eu^{3+}$ nanocrystals.

1.3 Characterization

The X-ray diffraction (XRD) pattern of the $Gd_2O_3:Eu^{3+}$ nanocrystals was performed on a diffractometer (Bruker D8 Advance). The morphology of the samples was determined with a scanning electron microscopy (SEM) (FEI Quanta200F) and a transmission electron microscope (TEM) (Tecnai G20). The samples were pelletized with KBr and their Fourier transform infrared (FTIR) spectra were recorded on a Nicollet 5700 FTIR spectrometer. The photoluminescence (PL) spectra of $Gd_2O_3:Eu^{3+}$ nanocrystals was examined with an F4500 fluorescent spectroscope. Its magnetic property was characterized with a magnetic property measurement system (MPMS XL-7, –20 to 20 kOe).

1.4 *In vitro* MRI and calculation of longitudinal relaxivity (r_1)

In vitro T_1-weighted MRI and longitudinal relaxivity value (r_1, unit of s^{-1} mM^{-1}) of $Gd_2O_3:Eu^{3+}$ was evaluated by a small animal 7.0 T MR scanner (Varian, America). The r_1 was calculated according to the equation $r_1=\Delta R_1/$[Gd^{3+} concentration, where R_1 was the longitudinal relaxation rate ($R_1=1/T_1$, unit of s^{-1}). In this study, R_1 values of Gd^{3+} with different concentrations (0–0.4 mmol/L) were measured with inversion recovery pulse sequences with TR=1000 ms and TI=20–2560 ms.

1.5 Cell imaging

Lung cancer NCI-H460 cells were plated on 14 mm glass coverslips and allowed to adhere for 24 h. The cells were then washed with phosphate buffer solution (PBS) and incubated in a serum-free medium containing 100 μg/mL $Gd_2O_3:Eu^{3+}$ nanoparticles for 3 h at 37 ºC under 5% CO_2. The excess nanoparticles were removed by washing the cells with PBS sufficiently. Fluorescence images of the cells were collected on a confocal microscopy (ZEISS 710) under excitation at 405 nm.

1.6 Cell cytotoxicity of $Gd_2O_3:Eu^{3+}$ nanocrystals

Cells of rat kidney were incubated in a culture medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ºC under 5% CO_2 for 96 h. The culture medium was then replaced with a culture medium containing $Gd_2O_3:Eu^{3+}$ nanocrystals with different concentrations and the cells were incubated for another 24 h. The cells were then washed with the culture medium without $Gd_2O_3:Eu^{3+}$ nanocrystals twice. A 200 μL culture medium containing 10% MTT was introduced on the cells, followed by a 4 h incubation under the same conditions to allow the formation of formazan dye. After the culture medium was removed, the purple formazan product was allowed to dissolve in DMSO for 10 min. The amount of formazan formed by the cells was measured with an enzyme-linked immunosorbent assay reader at 490 nm. The following formula was used to evaluate the inhibition of cell growth by $Gd_2O_3:Eu^{3+}$ nanocrystals.

$$\text{Cell viability}(\%)=(\text{mean Abs of treatment group}/\text{mean Abs of control})\times 100\ \% \quad (1)$$

2 Results and discussion

2.1 Phase structure, morphology, and formation process

The synthesis process is presented in Scheme 1. $Gd_2(CO_3)_3:Eu^{3+}$ precursor was prepared first via the reverse microemulsion method, then Gd_2O_3:Eu nanocrystals were obtained after calcination. It can be seen from their SEM image (Fig. 1(a)) that the CTAB-capped $Gd_2(CO_3)_3:Eu^{3+}$ colloidal particles are monodispersed quasi-spherical spheres. The diameter of the colloidal spheres was measured as 8–12 nm from their TEM image (Fig. 1(b)). The unclear nanosphere TEM image is ascribed to the poor crystallinity of the $Gd_2(CO_3)_3:Eu^{3+}$ precipitates. $Gd_2O_3:Eu^{3+}$ nanocrystals were prepared by calcining the $Gd_2(CO_3)_3:Eu^{3+}$ precipitate precursors at 600 ºC for 2 h. As shown in its TEM image (Fig. 1(c), (d)),