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Growth of lanthanide-doped $LiGdF_4$ nanoparticles induced by $LiLuF_4$ core as tri-modal imaging bioprobes

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ABSTRACT

Multimodal imaging can compensate for the deficiencies and incorporate the advantages of individual imaging modalities. In this paper, we demonstrated the synthesis of core–shell nanocomposites $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm constituted of tetragonal $LiLuF_4$ nanoparticles as core and Yb,Er/Tm-codoped $LiGdF_4$ as shell. $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm nanoparticles display brighter upconversion luminescence (UCL) than $NaGdF_4$:Yb,Er/Tm nanoparticles with the same size under continuous-wave excitation at 980 nm. The active shell layer of $LiGdF_4$:Yb,Er/Tm not only provide the UCL center, but also serve as magnetic resonance (MR) imaging contrast agent. To further improve the UCL intensity, the inert $LiGdF_4$ shell was coated on the $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm nanoparticles. Furthermore, $LiLuF_4$@$LiGdF_4$:Yb,Tm@$LiGdF_4$ nanoparticles have been successfully applied to UCL/X-ray computed tomography (CT)/MR tri-modal imaging on the modal of tumor-bearing mice.

1. Introduction

Lanthanide (Ln^{3+})-doped upconversion nanoparticles (UCNPs), which can convert near-infrared (NIR) radiation into NIR or visible or ultraviolet (UV) light, are receiving a great deal of attention for potential application in bioimaging and photodynamic therapy (PDT) [1–6]. The UCNPs have many advantages over conventional biomarkers, such as deep penetration of NIR excitation light into biological tissue, low toxicity, and high signal-to-noise ratio as well as high resistance to photobleaching, blinking, and reduced photodamage [7–14]. To date, Ln^{3+}-doped fluorides have been extensively investigated and proved to be the ideal host candidates for producing high upconversion luminescence efficiency [11,13].

Although photoluminescence imaging based on UCNPs is suitable for multiscale imaging from cellular level to whole-body animals, it has one shortcoming of the low penetration depth of less than several centimeters [15–17]. Due to the intrinsic restrictions of every imaging modality, single-modality imaging could not provide enough information for the accurate diagnosis. For more accurate imaging and diagnosis, multimodal imaging probes combining different imaging modalities have been developed. Magnetic resonance imaging (MRI) is widely used to discriminate the infinitesimal change in soft tissues [18–20], and X-ray computed tomography (CT) displays a high degree of spatial resolution of the hard-tissues (<50 μm) [21–23]. All lanthanide elements (from lanthanum to lutetium) have potential X-ray attenuation properties owing to the large atomic number and high X-ray absorption coefficient [24,25]. Gadolinium ions (Gd^{3+}) possess seven unpaired electrons which can efficiently alter the relaxation time of surrounding water protons, and have been widely used in MRI diagnosis of routine clinical disease [26]. Up to now, the studies on the corresponding fluoride UCNPs are mostly focused on $BaGdF_5$ [27,28], GdF_3 [29–31], $NaGdF_4$ [32–35], and $KGdF_4$ [36,37] hosts, and few attention has been paid to $LiGdF_4$ host matrix. As known, $LiREF_4$ (RE = Y, Er–Lu) host material is an outstanding host matrix for both upconversion (UC) and down-conversion (DC) luminescence [38–42]. Mahalingam et al. reported that their synthesized Yb and Tm codoped $LiYF_4$ UCNPs not only exhibit intense UC emission under excitation at 980 nm, but also generate additional emission lines in the deep-UV (294 nm) and near-infrared regions

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(1.46 μm), which can be used for anti-counterfeiting and telecommunication applications, respectively [38]. Huang et al. reported that the total upconversion quantum yield of $LiLuF_4$:Yb,Tm/Er core–shell nanoparticles was almost 5 times higher than that of $NaYF_4$:Yb,Tm/Er core–shell nanoparticles which, for a long time, was considered to be one of the most efficient host lattices [41]. They also found an enhanced efficacy of $LiYF_4$:Yb,Er UCNPs-based PDT agent for the inhibition of tumor growth both *in vitro* and *in vivo* due to high energy transfer efficiency [42]. Although $LiREF_4$ (RE = Y, Er–Lu) UCNPs have been widely reported, a systematic survey on the optical and magnetic properties of $LiGdF_4$ nanoparticles is still lacking because the syntheses of tetragonal-phase $LiGdF_4$ UCNPs via wet chemical method is challenging [31,43,44] and instead, orthorhombic-phase GdF_3 is apt to be formed (Fig. S1).

Herein, our strategy is based on using small $LiLuF_4$ core to induce the growth of tetragonal-phase $LiGdF_4$ shells. $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm nanoparticles show intense UCL than β-$NaGdF_4$:Yb,Er/Tm UCNPs. We also constructed the optically inert $LiGdF_4$ shell layer around $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm nanoparticles to enhance the UCL intensity. Furthermore, the feasibility of $LiLuF_4$@$LiGdF_4$:Yb,Tm@$LiGdF_4$ core-shell-shell nanoparticles to be applied as UCL/CT/T_1-weighted MR tri-modal imaging bioprobes was investigated.

2. Materials and methods

2.1. Chemicals and materials

$LnCl_3$ (Ln = Lu, Gd, Yb, Er, and Tm, 99.99%) compounds were purchased from Shanghai Shabo Chemical Technology Company. Oleic acid (OA) and 1-octadecene (ODE) were obtained from Alfa Aesar. Trifluoroacetic lithium (CF_3COOLi) was purchased from Aladdin. Ammonium fluoride (NH_4F) and lithium hydroxide (LiOH) with the purity of A.R. was bought from Beijing Fine Chemical Company, China.

2.2. Synthesis of tri-modal imaging probes

2.2.1. Synthesis of $LiLuF_4$ or GdF_3 nanoparticles

The typical procedure is as follows: 1 mmol of $LnCl_3$ (Ln = Lu or Gd) was added to a 100 mL four-necked flask containing 10 mL of OA and 10 mL of ODE. The solution was stirred and heated to 110 °C for 1 h under vacuum and then cooled to room temperature (RT) with the gentle flow of Ar gas. A solution of 4 mmol of NH_4F and 2.5 mmol of LiOH in 10 mL of methanol was added, and then the solution was kept at 50 °C for 30 min. After methanol was evaporated, the reaction was then heated to 310 °C rapidly under Ar atmosphere, kept for 1 h and then cooled to RT. The nanoparticles were precipitated with ethanol, collected by centrifugation, and redispersed in cyclohexane.

2.2.2. Synthesis of $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm core–shell nanoparticles

The shell stock solution was obtained by mixing 1 mmol of $LnCl_3$ (Ln = Gd, Yb, and Er or Tm) in a 100 mL four-necked flask containing 10 mL of OA and 10 mL of ODE. The solution was stirred and heated to 110 °C for 1 h under vacuum and then cooled to RT with the gentle flow of Ar gas. The as-prepared $LiLuF_4$ core and 2.5 mmol of CF_3COOLi in cyclohexane were then mixed together into the above solution. The mixture solution was degassed at 120 °C for 30 min under Ar flow to remove the remaining water, cyclohexane and oxygen. Then, the resulting mixture was heated to 305 °C rapidly under Ar flow with stirring for 1 h. Then, the solution was cooled down to RT. The solid products were precipitated by addition of ethanol, collected by centrifugation, and redispersed in cyclohexane.

2.2.3. Synthesis of $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm@$LiGdF_4$ core-shell-shell nanoparticles

The synthesis procedures for shell coating of $LiGdF_4$ on $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm nanoparticles were similar to those for shell coating of $LiGdF_4$:Yb,Tm/Er on $LiLuF_4$ nanoparticles except that 1 mmol $GdCl_3$ was used to prepare the shell stock solution. The prepared $LiLuF_4$@$LiGdF_4$:Yb,Er/Tm@$LiGdF_4$ nanoparticles were dispersed in cyclohexane.

2.2.4. Surface modification of OA-capped UCNPs

Water-soluble UCNPs were prepared according to the reported method [16]. The as-prepared oleic acid-capped nanoparticles in cyclohexane solution were dispersed in 10 mL HCl solution (pH = 2) and ultrasonicated for 15 min to remove the surface ligands. After the reaction, the nanoparticles were collected via centrifugation at 10,000 rpm for 15 min, and further purified by adding acidic ethanol solution. The resulting products were washed with ethanol and deionized water for several times, redispersed in 20 mg/mL sodium citrate solution, and washed with deionized water for three times.

2.3. Characterization

The phase identification was performed by X-ray diffraction (XRD) (Model Rigaku Ru-200b), using a nickel-filtered Cu Kα radiation (λ = 1.5406 Å) in the range of $10° \leq 2\theta \leq 70°$. Transmission electron microscopy (TEM), high-resolution TEM (HRTEM) imaging, and electron diffraction pattern were implemented by a JEM-2100F electron microscope at 200 kV. FT-IR spectra were recorded on a BioRad Fourier transform infrared spectrometer with the KBr method. Under the excitation at 980 nm, UCL spectra were recorded using a SPEX1000M spectrometer (1.5 mm for slit width). The temporal properties were studied by using a 953.6 nm Raman shifter pulsed laser with a pulse width of 10 ns and a repetition rate of 10 Hz, a monochromator, and a digital oscilloscope. All the measurements were performed at RT.

2.4. Cytotoxicity assay

The *in vitro* cytotoxicity of the core-shell-shell nanoparticles was tested by using the typical methyl thiazolyl tetrazolium (MTT) reduction assay on Hela cells. Hela cells were cultivated in a 96-well cell-culture plate at a density of 10^4 per well at 37 °C under 5% CO_2 for 24 h. Successively, different concentrations of $LiLuF_4$@$LiGdF_4$:Yb,Tm@$LiGdF_4$ (0, 15.6, 31.25, 62.5, 125, 250, 500, 1000 μg/mL, diluted in RPMI 1640 culture medium) were added to the wells. After incubation for 24 h, 20 μL of MTT solution (5 mg/mL) was added to each well and the plate was incubated for an additional 4 h. After formation of formazan dye, the medium was removed from the wells. Then, the formazan dye was extracted into DMSO. The optical density at 490 nm (OD_{490}) of each well was measured on a microplate reader. The cell viability could be calculated by the following formula: Cell viability (%) = (mean of absorbance value of treatment group/mean of absorbance value of control) × 100%.

2.5. Animal experiments

Animal care and handing procedures were in agreement with the guidelines of the Regional Ethics Committee for Animal Experiments. The H22 cells were cultured and injected subcutaneously in the left axilla of each Balb/c mouse. The mice were used for experiments when the tumors had grown to reach the size of