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Lanthanide-based nanocrystals as dual-modal probes for SPECT and X-ray CT imaging

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ABSTRACT

Applications of lanthanide-based nanoparticles for bioimaging have attracted increasing attention. Herein, small size PEG-EuOF:^{153}Sm nanocrystals (~5 nm) (PEG = poly(ethylene glycol)bis(carboxymethyl)ether) combined with the radioactive and X-ray absorption properties were synthesized. The distribution of the PEG-EuOF nanocrystals in living animals was studied by *ex vivo* radioassay, inductively coupled plasma-atomic emission spectrum (ICP-AES) analysis and *in vivo* SPECT imaging, which indicated that the small size PEG-EuOF:^{153}Sm had long blood retention time (blood half-life ($t_{1/2}$) reach to 4.65 h) and were eliminated significantly through biliary/gastrointestinal pathway *in vivo*. Meanwhile, benefiting from the high attenuation ability of Eu, the small size PEG-EuOF was successfully applied for lymph node CT imaging, extending the bio-applications of these small nanocrystals. The results of cytotoxicity and *in vivo* toxicity also showed that the PEG-EuOF nanocrystals have relatively low toxicity, which suggest their safety for *in vivo* imaging. The studies provide preliminary validation for the use of PEG-EuOF nanocrystals for *in vivo* bioimaging applications.

1. Introduction

Due to their unique 4f electron structure, lanthanide ions provide rich optical, electronic, and magnetic properties [1–3]. Then, the lanthanide-based nanoparticles have been explored as imaging agents for luminescence imaging [4–11], magnetic resonance imaging (MRI) [12–16], X-ray CT imaging [17,18], positron emission tomography (PET) [19–21] and single photon emission computed tomography (SPECT) imaging [22–24]. Although many multimodal imaging agents has been explored, before *in vivo* applications such as tumor targeting, the time in blood circulation should be extended. Until now most of the lanthanide-based nanoparticles were quickly captured by mononuclear phagocyte system (MPS) and barely interacted with the target tissues. For example, Liu's group have investigated the *in vivo* biodistribution of host lanthanide-based $NaYF_4$ coated with either polyethylene glycol (PEG) or polyacrylic acid (PAA), which shown that those nanoparticles were mainly captured by the liver and spleen of mice [25]. Our group have investigated the *in vivo* biodistribution of lanthanide-based NPs with different host of $NaYF_4$, $NaLuF_4$ and $Gd(OH)_3$ [22,23,26–28]. Another problem was the long term deposition of lanthanide nanomaterials in organs such as liver and spleen and thus present potentials of toxicity. To realize fast excretion, many nanomaterials decrease their sizes to less than 5 nm to be clearable. For example, some small nanomaterials such as Au NPs and QDs were investigated for their excretion pathway [29,30].

Different molecular imaging methods provide different spatial resolution, imaging depth, and areas of application [31,32]. For example, nuclear imaging of PET and SPECT show the highest sensitivity, and are widely used for quantitative *in vivo* monitoring of living subjects. ^{153}Sm (E_γ = 103 keV), a rare-earth metal radioisotope, has a physical half-life of 46.3 h (1.93 days) and also emits both medium energy beta particles and gamma photons, making it suitable for long-term SPECT imaging and biodistribution quantification studies in small animals. Meanwhile, X-ray computed tomography (CT) imaging is another powerful imaging technology, which gives more anatomic detail in living animals than other *in vivo* imaging tools based on differential X-ray absorption [33,34]. And thus researchers combined SPECT imaging with X-ray CT imaging to achieve high spatial resolution, hypersensitivity and quantification imaging ability [35,36].

In this study, to realize long term circulation in blood stream, the size of the synthesized particle should be less than 10 nm [37].

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Moreover, polyethylene glycol (PEG = poly(ethylene glycol)bis(-carboxymethyl)ether, $M_n \sim 600$) was capped onto the nanocrystals. Herein, a kind of lanthanide-based nanocrystals, PEG-EuOF:^{153}Sm nanocrystals with a small size (~5 nm), was synthesized through $^{153}Sm^{3+}$ doped into the crystal lattice of EuOF. Accordingly, the biodistribution of small PEG-EuOF:^{153}Sm nanocrystals in mice were studied by *ex vivo* analysis and *in vivo* SPECT imaging. Meanwhile, based on X-ray absorption of high atomic number Eu [38], the small size PEG-EuOF was successfully applied for lymph node CT imaging. Last, the assessment of cytotoxicity and *in vivo* toxicity of the PEG-EuOF nanocrystals was conducted.

2. Experimental section

2.1. Materials

Rare-earth oxides Eu_2O_3 (99.99%) was purchased from Beijing Lansu Co., China. Oleic acid (OA, >90%), Oleylamine (OM, >80%) and Poly(ethylene glycol)bis(carboxymethyl)ether (PEG, $M_n \sim 600$) were purchased from Sigma–Aldrich. Trifluoroacetic acid (99%), ethanol, cyclohexane, hydrochloric solution were purchased from Sinopharm Chemical Reagent Co., China. The precursors of $Eu(CF_3COO)_3$ was prepared from the corresponding lanthanide oxides and trichloroacetic acid with the literature method [39]. Aqueous solution of $^{153}SmCl_3$ was purchase from HTA Co., Ltd., China. All other chemical reagents were of analytical grade and were used directly without further purification. Deionized water was used throughout.

2.2. Characterization and instruments

Powder X-ray diffraction (XRD) measurements were measured with a Bruker D4 X-ray diffractometer (Cu Kα radiation, $\lambda = 0.15406$ nm). Fourier-transform infrared (FT-IR) spectra were measured using an IR PRESTIGE-21 spectrometer (Shimadzu) from samples in KBr pellets. The size and morphologies of OA-EuOF and PEG-EuOF were determined at 200 kV using a JEOL JEM-2010F low- to high-resolution transmission electron microscope (HR-TEM). Samples were prepared by placing a drop of dilute dispersions in cyclohexane and water on the surface of a copper grid respectively. Energy-dispersive X-ray analysis (EDXA) of the samples was also performed during HR-TEM measurements. Dynamic light scattering (DLS) experiments were carried out on an ALV-5000 spectrometer–goniometer equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a JDS Uniphase He–Ne laser (632.8 nm) with an output power of 22 mW. The solution of the OA-EuOF was filtered through two membrane filters with 0.45 mm nominal pore size connected in series. Luminescence spectrum was measured with an Edinburgh Instruments Spectrometer (LFS-920), using an excitation source of the Xenon source in the spectrophotometer. Inductively Coupled Plasma-Atomic Emission Spectrum (ICP-AES) analysis was performed by Hitachi P-4010 (Hitachi Limited). The SPECT imaging of the mouse were recorded on a NanoSPECT/CT puls scanner (Bioscan). CT imaging was acquired using a dual-source CT system (SIEMENS Inveon MMCT micro-CT instrument).

2.3. Synthesis of ^{153}Sm-doped-EuOF nanoparticles (OA-EuOF:^{153}Sm)

The synthesis of OA-EuOF was based on the method reported by published literature [40]. In a typical procedure, 0.25 mmol $Eu(CF_3COO)_3 \cdot 2H_2O$ was added into a mixture of OA (2.5 mmol) and OM (2.5 mmol) in a three-necked flask (5 mL) at room temperature. Then, 0.6 mL of $^{153}SmCl_3$ (aq, 1591 MBq) was also added into the reaction system. The as-obtained slurry was heated to 110 °C to remove water and oxygen with vigorous magnetic stirring under vacuum in a temperature-controlled electromantle, and thus to form an optically transparent solution. The solution was heated to 310 °C at a heating rate of 15 °C/min and kept for 1 h at this temperature under an Ar atmosphere. After cooling to room temperature, the products were precipitated by adding excess ethanol into the reacted solution, followed by washing with ethanol. The as-formed nanocrystals were easily redispersed in various nonpolar organic solvents.

To investigate the biodistribution of EuOF nanocrystals *in vivo* by radioassay, the nanocrystals were labeled with radioactive $^{153}Sm^{3+}$ ($E_\gamma = 103$ keV) by doping $^{153}Sm^{3+}$ ions into the EuOF nanocrystals. As $^{153}Sm^{3+}$ has a similar atomic radius and chemical characteristics as for Eu^{3+}, it can be easily incorporated into the EuOF during the synthesis process.

2.4. Converting hydrophobic OA-EuOF:^{153}Sm into hydrophilic PEG-EuOF:^{153}Sm

Hydrophilic PEG-EuOF:^{153}Sm particles were prepared according to a simple and versatile procedure previously reported literature [41]. Firstly, the 20 mg OA-EuOF:^{153}Sm was washed with CH_2Cl_2 saturated $NOBF_4$ and then shaking 5 min to precipitate the EuOF nanoparticles. Then EuOF:^{153}Sm nanocrystals were dispersed in the 1 mg mL^{-1} PEG (poly(ethylene glycol)bis(carboxymethyl)ether) solution ultrasound for 10 min and PEG-EuOF:^{153}Sm were obtained. Finally, the PEG-EuOF:^{153}Sm nanocrystals were washed three times with distilled water.

Herein, for characterization, toxicity evaluation (*ex vitro* and *in vivo*) and application in CT imaging, the PEG-EuOF:^{153}Sm samples were stored for at least 30 days to decrease the radioactivity to a safe level until it cannot be determined by the γ-counter. Herein, the stored sample with very weak radioactivity was called PEG-EuOF.

2.5. Ex vivo distribution studies

For *ex vivo* radioassay, PEG-EuOF:^{153}Sm nanocrystals were used to inject to Kunming mouse. Radioactivity in the organs was measured using a γ-counter (WIZARD 1470, Perkin Elmer Wallac, USA) and calibrated against a known aliquot of the injection. The percentage of added dose per gram (%ID/g) for each organ was calculated by comparing its activity with appropriate standard of added dose (ID), the values expressed as mean ± standard deviation (SD) for each time. Animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee.

2.6. ICP-AES (inductively coupled plasma-atomic emission spectrum) analysis of Eu(III) in blood

For distribution of PEG-EuOF nanocrystals in blood of mouse *via* the tail vein injection with 1 mg mL^{-1}, determination of Eu element content in blood was performed by ICP-AES analysis (Hitachi P-4010). After injection with PEG-EuOF at different time, blood was dissolved in deionized water (5 mL) and HNO_3 (5 mL), and diluted with deionized water to 1:25 (v/v). Data presented are from the average of three experimental animals.

2.7. Cytotoxicity of PEG-EuOF

In vitro cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the HeLa cells. Cells were seeded into a 96-well cell culture plate at 1×10^4/well, and were cultured at 37 °C and 5% CO_2 for 24 h; Different mass concentrations of PEG-EuOF nanocrystals (0, 50, 100, 200, 300, and 400 μg mL^{-1}, diluted in RPMI 1640) were then added to the wells. The cells were subsequently incubated for 24 h at 37 °C under 5% CO_2. Thereafter, MTT (20 μL, 5 mg mL^{-1}) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. The assay plate was allowed to stand at room temperature for 15 min. The optical density OD570 value (*Abs.*) of each well, with background subtraction at 690 nm, was measured by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader. The following formula was used to calculate the inhibition of cell growth: Cell viability (%) = (mean of *Abs.* value of treatment group/mean of *Abs.* value of control) × 100%.

2.8. In vivo SPECT imaging

For SPECT imaging, PEG-EuOF:^{153}Sm nanocrystals (total radioactivity 1.2 mCi) were injected into Kunming mouse through tail vein injection. SPECT images of the mouse were imaged on a Bioscan NanoSPECT/CT scanner at 1 h, 2 h, 4 h, 24 h, and 96 h after injection. The experimental results were analyzed by HiSPECT software.

2.9. X-ray attenuation measurement in vitro and CT imaging in vivo

Various concentrations of PEG-EuOF (0, 1.0, 2.0, 4.0, and 8.0 mg mL^{-1}) dispersed in deionized water were prepared in a series of 1.5 mL tubes for phantom test. CT images were acquired using a dual-source CT system (SIEMENS Inveon MMCT micro-CT instrument). Imaging parameters were as follows: effective pixel size, 104.51 μm; 80 KVp, 500 mA; field of view, 53.10 mm-146.50 mm; rotation steps, 180; binning, 4; exposure time 150 ms/rotation. HU values were measured by the SIEMENS Inveon MMCT micro-CT software. For *in vivo* lymphatic CT images, 150 μL of hydrophilic PEG-EuOF nanocrystals (4 mg/mL) were injected into the pawl of the mouse. CT images were acquired 20 min after the injection. Imaging parameters were as follows: effective pixel size, 50.71 μm; 80 KVp, 500 mA; field of view, 53.10 mm-83.15 mm; rotation steps, 180; binning, 2; exposure time 600 ms/rotation.

2.10. In vivo toxicity studies

3 mg mL^{-1} of PEG-EuOF (0.3 mL) were injected into four-to five-week-old Kunming mice through tail vein injection ($n = 3$); this group of mice constituted the test group. Kunming mice ($n = 3$) without injection were selected as the control group. Histology and hematology studies: blood samples and tissues were harvested from test and control group after 7 days. Blood was collected from the orbital sinus. Upon completion of the blood collection, mice were sacrificed. The heart, liver, spleen, lung, kidney, and intestine were removed, and fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

3. Results and discussion

3.1. Synthesis and characterization of OA-EuOF:^{153}Sm

Oleic acid-capped EuOF (OA-EuOF) nanocrystals were synthesized as reported in the literature [40]. As shown in Figs. 1a and b,