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Polyphosphoric acid capping radioactive/upconverting $NaLuF_4$:Yb,Tm,^{153}Sm nanoparticles for blood pool imaging *in vivo*

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ABSTRACT

Nanoparticles that circulate in the bloodstream for a prolonged period of time have important biomedicine applications. However, no example of lanthanide-based nanoparticles having a long-term circulation bloodstream has been reported to date. Herein, we report on difunctional radioactive and upconversion nanoparticles (UCNP) coated with polyphosphoric acid ligand, that is ethylenediamine tetramethylenephosphonic acid (EDTMP), for an application in single-photon emission computed tomography (SPECT) blood pool imaging. The structure, size and zeta-potential of the EDTMP-coated nanoparticles (EDTMP-UCNP) are verified using transmission electron microscopy and dynamic light scattering. Injection of radioisotope samarium-153-labeled EDTMP-UCNP (EDTMP-UCNP:^{153}Sm) into mice reveal superior circulation time compared to control nanoparticles coated with citric acid (cit-UCNP:^{153}Sm) and ^{153}Sm complex of EDTMP (EDTMP-^{153}Sm). The mechanism for the extended circulation time may be attributed to the adhesion of EDTMP-UCNP on the membrane of red blood cells (RBCs). *In vivo* toxicity results show no toxicity of EDTMP-UCNP at the dose of 100 mg/kg, validating its safety as an agent for blood pool imaging. Our results provide a new strategy of nanoprobe for a long-term circulation bloodstream by introducing polyphosphoric acid as surface ligand.

1. Introduction

Lanthanide elements with unique 4f electron structures show rich optical and radioactive properties. Due to the similar ion radius, different lanthanide ions with physicochemical properties can be doped into one nanoparticle to fabricate a multifunctional system [1–5]. In particular, some nanoparticles doped with a sensitizer of Yb^{3+} and an activator of Er^{3+} (Tm^{3+}, Ho^{3+}, *etc.*) show a unique upconversion luminescence (UCL) process where low-energy light is converted to higher-energy one through sequent multiple photon absorptions or energy transfers [6–12], with special optical characteristics, such as sharp emission lines, large anti-Stokes shifts of several hundred nanometers, and the absence of autofluorescence of biosamples [13–16]. As a result, lanthanide-based upconversion nanoparticles (UCNP) have been successfully used in bioimaging *in vivo* [17–19]. To overcome the shortcoming of UCNP as luminescent probes for *in vivo* imaging, that is, a deficiency of penetration depth in biological samples, UCNP have recently been developed into multifunctional nanoparticles with UCL, magnetic, and radioactive properties that could be used for multi-modal imaging, including magnetic resonance imaging (MRI), X-ray computed tomography (CT) and nuclear imaging [20,21].

Extending the circulation half-life of nanoparticles in blood is crucial for bio-applications, especially for drug delivery, tumor targeting and blood pool imaging [22–25]. For UCNP, only two examples have been reported to display an extended circulation half-life in blood [26,27]. Liu et, al. reported the Gd_2O_3:Yb^{3+}, Er^{3+} upconversion nanoprobes as high-performance contrast agents for multi-modality imaging. Cao et, al. reported $^{153}Sm^{3+}$-doping $NaYF_4$:20%Yb, 1%Er upconversion nanoparticles with a polyethylene glycol (PEG) coating exhibited a prolonged blood retention time. It should be noted that the enhanced colloidal stability of UCNPs in the aforementioned two cases is all based on the surface modification of PEG chains, the sole strategy of introducing surface ligand containing PEG moiety limits the further application of UCNPs in blood pool imaging.

In the present study, we have developed ethylenediamine tetramethylenephosphonic acid (EDTMP) capped, Yb^{3+}, Tm^{3+}, and $^{153}Sm^{3+}$ co-doped $NaLuF_4$ nanoparticles to integrate UCL properties and radioactivity into one nanoparticle. Our design strategy is

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introducing a polyphosphonic acid as surface ligand to provide a large negative charge and doping radioactive isotope ^{153}Sm with a relatively long halftime of 1.99 days to achieve high sensitive nuclear imaging. Considering the relative slow metabolization process of nanoparticles, the long-term observation of radioactive nanoparticles within animals is beneficial. Therefore, ^{153}Sm-labeling becomes a good choice. Furthermore, $^{153}Sm^{3+}$ has a similar atomic radius and chemical character to the other lanthanide ions Ln^{3+}, and hence it can be doped into $NaLuF_4$ nanocrystals easily. Meanwhile, polyphosphoric acid EDTMP [28] can also chelate with lanthanide cations [29,30]. Moreover, the ^{153}Sm complex with an EDTMP ligand is a well-known commercial agent for single-photon emission computed tomography (SPECT) imaging. Therefore, it is reasoned that EDTMP can coordinate on the surface of UCNP and improve its water-solubility. Herein, the as-prepared EDTMP capped, ^{153}Sm-dopped UCNP EDTMP-$NaLuF_4$:Yb,Tm,^{153}Sm (denoted as EDTMP-UCNP:^{153}Sm) were characterized by transmission electron microscopy (TEM), X-ray powder diffraction (XRD), dynamic light scattering (DLS) and spectroscopy techniques. Furthermore, the EDTMP-UCNP:^{153}Sm was developed for UCL and SPECT dual-modality blood pool application (Scheme 1). In addition, the toxicity of UCNP as blood pool imaging agent was also tested.

2. Materials and methods

2.1. Materials and instrumentation

All the starting materials were obtained from commercial supplies and used as received. Rare-earth oxides Lu_2O_3 (99.999%), Yb_2O_3 (99.999%) and Tm_2O_3 (99.98%) were purchased from Shanghai Yuelong New Materials Co., Ltd., China. Oleic acid (OA) (>90%) was purchased from Alfa Aesar Co., Ltd., China. 1-octadecence (ODE) (>90%) was purchased from Aladdin Reagent Co., Ltd., China. NaOH, NH_4F, ethanol, cyclohexane, hydrochloric solution were purchased from Sinopharm Chemical Reagent Co., China. EDTMP (>90%) was purchased from TCI Chemical Reagent Shanghai Co., Ltd., China. Rare-earth chlorides ($LnCl_3$, Ln: Lu, Yb and Tm) were prepared by dissolving the corresponding metal oxide in 10% hydrochloric solution at elevated temperature and then evaporating the water completely. All other chemical reagents were of analytical grade and were used directly without further purification. Deionized water was used throughout the experiments.

XRD measurements were measured with a Bruker D4X-ray diffractometer (Cu Kα radiation, λ 0.15406 nm). The morphologies of OA-$NaLuF_4$:Yb,Tm and EDTMP-$NaLuF_4$:Yb,Tm were determined at 200 kV using a JEOL JEM-2010F low- to high-resolution transmission electron microscope (HRTEM). 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 HRTEM measurements to obtain the elements of samples. Fourier-transform infrared (FTIR) spectroscopy was measured using an IR Prestige-21 spectrometer (Shimadzu) from samples in KBr pellets. Upconversion luminescence (UCL) spectra were measured on an Edinburgh LFS-920 spectrometer, where an external 0–3 W adjustable CW laser at 980 nm (Connet Fiber Optics, China) replaced the Xenon lamp as the excitation source. Dynamic light scattering (DLS) and zeta-potential 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. Confocal luminescence imaging of tissues of mouse was performed with on a modified OLYMPUS FV1000 laser scanning confocal fluorescence microscope equipped with a continuous-wave NIR laser operating at 980 nm (Connet Fiber Optics, China) [31]. The upconversion luminescence photographs were acquired digitally with a Nikon multiple CCD camera under CW excitation at 980 nm (excitation power ~3 W).

2.2. Synthesis of OA-$NaLuF_4$:Yb,Tm,^{153}Sm and OA-$NaLuF_4$:Yb,Er nanocrystals

$NaLuF_4$:Yb,Tm,^{153}Sm nanocrystals had been synthesized according to the previously reported method [32]. In a typical experiment, 1 mmol $LnCl_3$ (Ln: Lu, Yb, Tm) with the molar ratio of 79:20:1 and $^{153}SmCl_3$ (398.86 MBq) were added to a 100 mL flask containing 12 mL OA and 15 mL ODE. The mixture was heated to 140 °C for 30 min to obtain a clear solution and then cooled down to room temperature. NH_4F (4 mmol) and NaOH (2.5 mmol) solids were added into the bottle. Kept on being stirred at 80 °C for 30 min, and then the solution was heated to 300 °C under argon for 1 h. Cooled down to room temperature, nanoparticles were precipitated by 30 mL ethanol and cyclohexane (V/V = 1:2), and collected by centrifugation. After washing with ethanol and cyclohexane for several times, $NaLuF_4$:Yb,Tm,^{153}Sm nanoparticles were finally redispersed in cyclohexane.

For laser scanning upconversion luminescence microscopy, OA-$NaLuF_4$:Yb,Er was synthesized, a molar ratio for Lu, Yb, Er of 78:20:2 was chosen. The other experimental details were the same as the synthesis of OA-$NaLuF_4$:Yb,Tm,^{153}Sm nanocrystals.

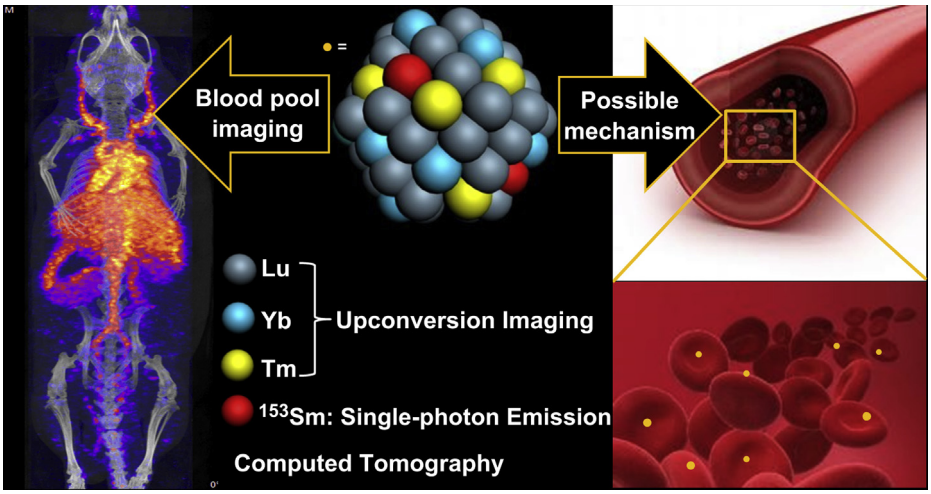
2.3. Synthesis of water-soluble EDTMP-UCNP:^{153}Sm and cit-UCNP:^{153}Sm nanocrystals

The water-soluble $NaLuF_4$:Yb,Tm,^{153}Sm nanocrystals were synthesized according to the previous ligand-free method reported by Capobianco and coworkers [33]. Firstly, the 10 mg OA-$NaLuF_4$:Yb,Tm,^{153}Sm was washed with 10 mL pH = 4 hydrochloric acid and obtain water-dispersible, ligand-free, brightly upconverting UCNP. Then ligand-free UCNP were dispersed in 5 mL 10 mg mL^{-1} EDTMP solution ultrasound for 30 min and EDTMP-UCNP were obtained. Finally, the EDTMP-UCNP:^{153}Sm were washed three times with 5 mL distilled water by sonication and separated by centrifugation. The final pH value of the dispersion is measured to be 7.

For the synthesis of cit-UCNP:^{153}Sm nanocrystal, the ligand-free UCNP:^{153}Sm were dispersed 5 mL sodium citrate solution (5 mg mL^{-1}), and other experimental details are the same as EDTMP-UCNP:^{153}Sm.

2.4. Cytotoxicity assay

After sufficient disintegration of the radioisotopes, the EDTMP-UCNP was used for the cytotoxicity study. A human nasopharyngeal epidermal carcinoma cell line (KB cell) was provided by Shanghai Institutes for Biological Sciences (SIBS), Chinese



Scheme 1. Polyphosphoric acid-capped $NaLuF_4$ nanoparticles co-doped with Yb^{3+}, Tm^{3+}, and $^{153}Sm^{3+}$ (EDTMP-UCNP) combine UCL and radioactivity into one integrate for blood pool imaging.