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Simple synthesis of carboxyl-functionalized upconversion nanoparticles for biosensing and bioimaging applications

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ABSTRACT

We report a simple one-step hydrothermal method for the synthesis of hydrophilic luminescent upconversion nanoparticles (UCNPs) using malonic acid as the stabilizer and functional agent. Using this method, two UCNPs with different colors of upconversion luminescence were synthesized. The surface of the as-prepared UCNPs was capped with carboxyl groups, which not only resulted in the UCNPs having good dispersity in water, but also allowed further conjugation with other functional molecules, thus indicating the potential applications in biosensing and bioimaging. To demonstrate this, amino-labeled single-stranded DNA (ssDNA) was conjugated on the surface of the UCNPs. Based on the different absorption and luminescence quenching abilities of graphene oxide (GO) to ssDNA-modified UCNPs before and after exonuclease I (Exo I)-triggered hydrolysis of ssDNA, a detection platform was developed for the detection of Exo I activity with a detection limit of 0.02 U mL^{-1}. The prepared hydrophilic UCNPs were also used successfully for in vivo upconversion luminescence imaging of nude mice.

1. Introduction

The sensitive detection of disease biomarkers and reliable determination of disease location within the human body have significantly contributed to the diagnosis and treatment of some diseases [1–4]. To achieve this, the most important step is to design sensing and imaging materials that make molecular processes quantifiable, visible, and traceable over time, thus allowing the noninvasive study of biological processes in vivo at the cellular and molecular level [5–7].

As a new type of luminescent nanomaterial, lanthanide-doped upconversion nanoparticles (UCNPs) have received considerable attention due to their potential applications in sensing and imaging. Compared to some traditional luminescent agents, such as quantum dots and organic dyes, whose application in the biological domain are greatly restricted by their high toxicity and low chemical stability [8–13], UCNPs offer a number of unique advantages, such as low toxicity, high chemical stability, deep penetration depth in living tissues, large signal-to-noise ratio, sharp absorption and emission under NIR excitation (980 nm), and resistance to photobleaching [14]. In particular, their unique anti-Stokes luminescent mechanism can effectively exclude the interference due to autofluorescence of biological samples and biological tissues, thus making them good candidate probes for bioimaging [15,16] and biosensing [17–19].

Although great advances have been achieved in UCNP studies [20–23], a simple method to make UCNPs hydrophilic and functionalizable is still urgently needed [24]. In classic methods, hydrophobic UCNPs were synthesized first, and then surface modification was necessary to ensure the hydrophobic UCNPs transferred into hydrophilic ones. Commonly used surface modification methods include surface silanization [25,26], ligand oxidation [27,28], and surface ligand competing exchange [29,30]. Compared to the relatively complicated two-step strategies, simple one-step strategies for the direct synthesis of hydrophilic UCNPs are ideal [31]. Herein, a new and simple method was developed for the one-step hydrothermal synthesis of hydrophilic UCNPs. In this method, malonic acid was used as a stabilizer and functional reagent. Under relatively mild reaction conditions, different colors of UCNPs, whose surface was capped with carboxyl groups, were prepared. The carboxyl-rich surface not only increased the hydrophilicity of the prepared UCNPs, but also allowed further functionalization with biomolecules. As a proof-of-concept, the feasibility of the prepared UCNPs in biosensing and bioimaging applications was demonstrated.

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2. Materials and methods

2.1. Materials and reagents

$Lu(NO_3)_3 \cdot 6H_2O$ (99.99%), $Yb(NO_3)_3 \cdot 5H_2O$ (99.99%), $Gd(NO_3)_3 \cdot 6H_2O$ (99.99%), $Er(NO_3)_3 \cdot 6H_2O$ (99.99%), $Tm(NO_3)_3 \cdot 6H_2O$ (99.99%) were obtained from Alfa Aesar (Tianjin, China). NaOH, NaF, malonic acid were purchased from Concord Chemical Reagent Co. Ltd. (Tianjin, China). 2-(N-morpholino) ethanesulfonic acid (MES, 99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC · HCl, 98%), N-hydroxysuccinimide (NHS, 98%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), tri (hydroxymethyl) aminomethane (Tris) were purchased from Sigma-Aldrich (Shanghai, China). Exonuclease I (Exo I), restriction endonuclease EcoR I and bovine serum albumin (BSA) were obtained from New England Biolabs (Beijing, China). Amino-functioned single-stranded DNA (NH_2-ssDNA: NH_2-GGAAGTGTTGATAAGATA-3′) was synthesized and purified by Sangon Biotech Co., Ltd (Shanghai, China). Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers' method [32]. All glassware was cleaned with aqua regia (HCl/HNO_3=3:1, v/v) and thoroughly rinsed with ultrapure water before use. Deionized and sterilized water (resistance > 18 MΩ cm^{-1}) was used in all experiments. All chemicals were of analytical grade and used without further purification.

2.2. Apparatus and characterization

Transmission electron microscopy (TEM) was carried out on a Philips Tecnai G20 at 200 kV. All samples were first dispersed in ethanol and then collected using copper grids covered with carbon film for measurements. X-ray diffraction (XRD) patterns were recorded on a Bruker D8 diffractometer with CuKα radiation (λ=1.5418 Å). Fourier Transform Infrared (FT-IR) spectra in the range of 4000–400 cm^{-1} were measured on a Bruker VECTOR 22 spectrometer with KBr pellet technique. Upconversion luminescent spectra were collected on RF-5301 PC spectrofluorometer (SHIMADZU, Japan) with anexternal 0–2 W adjustable continuous-wave laser (980 nm, Tianjin Hi-Tech Optoelectronic Co., China) as the excitation source.

2.3. Synthesis of carboxyl-functionalized UCNPs

Two carboxyl-functionalized UCNPs ($NaLuF_4$:Yb/Er and $NaLuF_4$:Gd/Yb/Tm) were synthesized. To obtain water dispersible UCNPs, a simple hydrothermal method was designed. Malonic acid was used in this one-step method, and played two important roles as a stabilizer and functional reagent. That is, it linked lanthanide ions to form luminescent UCNPs, and provided the prepared UCNPs with a high density of carboxyl functionalized groups on the surface. As for the synthesis of $NaLuF_4$:Yb/Er, the rare-earth stearate ($(C_{17}H_{35}COO)_3RE$ (RE=$Lu_{0.78}Yb_{0.20}Er_{0.02}$) was used as the synthesis precursor, it was synthesized according to literature [33]. The details are available in *Supporting information*. One-step hydrothermal method was designed for the synthesis of hydrophilic UCNPs. Typically, deionized water (10 mL), ethanol (20 mL) and malonic acid (0.2 g) was mixed under stirring to form a homogeneous solution, to which NaF (0.05 g) and the prepared rare-earth stearate precursor (0.1916 g) was added at room temperature under stirring. The mixture was sonicated for 30 min so that the precursor can be decomposed into small particles. Then, the mixture was transferred into a 50 mL Teflonlined autoclave and heated at 150 °C for 24 h. After the autoclave was cooled down to room temperature naturally, the precipitate was collected using ethanol, then purified and washed with deionized water for several times. Finally, white powder of carboxyl-functionalized UCNPs ($NaLuF_4$:Yb/Er), was obtained by drying at 60 °C for 12 h. $NaLuF_4$:Gd/Yb/Tm could be prepared in the similar way but changing the synthesis precursor as ($(C_{17}H_{35}COO)_3RE$ (RE=$Lu_{0.55}Gd_{0.24}Yb_{0.20}Tm_{0.01}$). The details of the synthesis of this precursor can also be found in *Supporting information*. When malonic acid was replaced with maleic anhydride, highly water dispersible UCNPs capped with carboxyl groups were also obtained using a similar one-step hydrothermal synthesis method.

2.4. Conjugation of $NaLuF_4$:Yb/Er with DNA

The conjunction of UCNPs with amino-functioned DNA (NH_2-ssDNA) was operated according to literature [34]. The carboxyl group on the surface of UCNPs was linked with NH_2-ssDNA through the formation of amide bond. Typically, the prepared carboxyl-functionalized UCNPs (5 mg, containing 20 μmol Ln^{3+}) was dispersed in 100 mM MES buffer (pH=5.0) with the aid of ultrasound. Then, EDC · HCl (3 mg) and NHS (2 mg) were added, and mixed on the vortex for 2 h. After centrifugation, the supernate was discarded. The obtained UCNPs was redispersed in HEPES buffer (100 mM, pH=6.5), to which 100 μM NH_2-ssDNA (200 μL) was added. After mixing for 3 h, the product was centrifugated and washed three times with deionized water. Then, the obtained ssDNA-UCNPs was redispered in 10 mM Tris–HCl buffer (pH=7.2) and stored at 4 °C for further use.

2.5. Detection of Exo I activity

10 μL ssDNA-UCNPs (5 mg mL^{-1}) was dispersed in 70 μL deionized water. After being maintained at 95 °C for 5 min and 25 °C for 1 h, 10 μL 10 × Exo I buffer (67 mM Glycine–KOH, pH 9.5 25 °C, 6.7 mM $MgCl_2$, 10 mM β-mercaptoethanol), and different concentrations of Exo I were added. After incubating the mixture at 37 °C for 2 h, GO (0.45 mg mL^{-1}, 10 μL) was added to a total volume of 100 μL. Under 980 nm excitation, the luminescent spectra were collected, and the luminescent intensity at 540 nm was used for the detection of Exo I activity.

2.6. In vivo upconversion luminescence (UCL) imaging

BALB/c nude mice (9–10 weeks old) were bought from Laboratory Animal Center of the Academy of Military Medical Science (Beijing, China). All animal procedures were in agreement with the guidelines of the Institutional Animal Care Committee of Nankai University. BALB/c nude mice were anesthetized with 4% chloral hydrate and the carboxyl-functinalized ($NaLuF_4$:Gd/Yb/Tm, 5 mg mL^{-1}, 300 μL) was injected into the mice via the tail vein. In vivo UCL imaging was performed on a NightOWL LB 983 animal in vivo imaging system (Berthold Technologies, Germany) equipped with IndiGO software. An external adjustable continuous wave (CW) infrared laser (980 nm, BWT Beijing Ltd., Beijing, China) was used as the excitation source. UCL images were collected by putting a filter (800 ± 20 nm) in front of the charge-coupled device camera.

3. Results and discussion

3.1. Synthesis and characterization of carboxyl-functionalized UCNPs

Two types of UCNPs ($NaLuF_4$:Yb/Er and $NaLuF_4$:Gd/Yb/Tm) were synthesized using the proposed one-step synthesis strategy using malonic acid as the stabilizer and functional reagent. The use of malonic acid provided the prepared UCNPs with a high density of carboxyl functionalized groups on the surface. The presence of carboxyl groups not only increased the water dispersity of the prepared UCNPs, but also allowed further conjugation of the