Supplementary Information

A Novel Deep Photodynamic Therapy Modality Combining with CT Imaging Established via X-Ray Stimulated Silica-Modified Lanthanide Scintillating Nanoparticles

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Materials

Lanthanum (III) chloride heptahydrate (LaCl₃·7H₂O , 99.99%), Terbium (III) chloride hexahydrate (TbCl₃·6H₂O, 99%), Ammonium fluoride (NH₄F, 98%), Polyvinylpyrrolidone (PVP-10, 90%), tetraethyl orthosilicate (TEOS, 99%), Aminopropyl tryethoxysilane (APTES, 99%), Rose Bengal (RB, 95%), 6-bromohexanoic acid (95.5%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 99%), N-Hydroxysuccinimide (NHS, 98%) and 1,3-Diphenylisobenzofuran (DBPF, 97%) were purchased from Aldrich. Ammonia aqueous solution (28 wt %), ethanol and dimethylformamide (DMF) were supplied by Sinopharm Chemical Reagent Co., Ltd. All chemicals were used without further purification and ultrapure water with a conductivity of 18.2 m Ω .cm was used throughout the experiments.

Synthesis of LaF₃:Tb scintillating nanoparticles (ScNPs)

Tb-doped lanthanide NPs were prepared by hydrothermal process as reported before with little modification.¹ Briefly, 1.6 mmol of LaCl₃ and 0.4 mmol of TbCl₃ were dissolved in 25 mL distilled H₂O. Then, 11.5 mL of aqueous solution containing 5.25 mmol NH₄F was injected with rate of 1.5 mL/min to the above mixture under stirring at room temperature for further 1 hour. The resultant mixture washed with water twice by centrifugation at 12000 rpm for 10 min. Afterward, the final centrifuged precipitate was dispersed with 8 mL of distilled H₂O and then transferred into a Teflon bottle held in a stainless steel autoclave, sealed and heated in the oven at 200 °C for 3 hours. After the autoclave was allowed to cool down to the room temperature, the ScNPs were separated by centrifugation and washed with distilled water and absolute ethanol four times and finally dispersed in 1 ml absolute ethanol to form clear transparent colloidal solution.

Silica coating of ScNPs

 LaF_3 :Tb@SiO₂ ScNPs were prepared by using PVP-10 as a coupling agent² prior to coating ScNPs with homogenous silica layer by Stober method³ with minor modifications. Typically, add 50 mL absolute ethanol to 1 mmol of as synthesized LaF₃:Tb ScNPs with gentle mixing for 5 min. Then, add 16.25 mL of aqueous solution of PVP (25 mg/ml) to the above solution and let it to be stirred for 24 h under room temperature. The resultant PVP adsorbed ScNPs from the final reaction were washed with ethanol for four times. 1 mmol of yielded PVP adsorbed ScNPs was directly redispersed in 24.6 mL of ammonia solution in ethanol (4.2 vol%). Then, add 120 µL of TEOS (10 vol% in ethanol) in two steps over 6 h reaction under vigorous stirring at room temperature. The resultant silica coated ScNPs were washed with ethanol for at least four times. For introducing NH₂ terminals to the surface of silica coated ScNPs, additional 120 µL of APTES was added to the SiO₂ coated NPs before the washing step.

CT imaging

In vitro CT imaging was obtained by a medical CT scanner (Discovery CT750 HD system, GE). The contrast enhancement was measured in Hounsfield units (HU) with different concentration of ScNPs and Ultravist[®] 300 in 2 mL centrifuge tube. X-ray imaging ability of the ScNPs was further confirmed on tumorbearing Balb/c mice by intratumoral injection of 50 µL aqueous solution of ScNPs. Then, in vivo X-ray imaging was tested by an IVIS Lumina XR system (Caliper Life Sciences, Hopkinton, USA). All animal procedures comply with the institutional animal use and care regulations.

Conjugation of Rose Bengal

Hexanoic acid-modified RB activated by NHS ester was efficiently synthesized according to the reported method.⁴ For the conjugation of RB onto ScNPs, 1 mmol of amino modified ScNPs were dispersed in 8 mL DMF and then mixed with 800 μ L of EDC (10 mg/ml in DMF). The final mixture was divided equally into four samples. Next, 8.7 nmol, 11.1 nmol and 15.6 nmol of RB-NHS (17 mg/ml in DMF) were respectively added to the first three samples and the fourth one was control without adding RB. The four samples were stirred in the dark overnight. Then, the samples were washed for 3 times with DMF to remove the free RB.

Singlet oxygen measurements

Generation of ${}^{1}O_{2}$ was detected chemically by DBPF. Once reacted with singlet oxygen, DBPF is converted to its endoperoxide form leading to obvious photobleaching, which can be monitored by measuring the reduction of DBPF absorption at 411 nm. To this purpose, 100 µL of DBPF (210 µg/mL in DMF) was added to 2 mL of DMF mixed with certain amount of ScNPs-RB nanocomposites. The UV-Vis absorption spectrum of DBPF was measured every 2 minutes. The reduction in absorption was measured as a function of time after irradiating samples with 375 nm light. All measurements were conducted at the room temperature.

Characterization

Transmission electron microscopy (TEM) images were acquired on JEM-2100F (JEOL) transmission electron microscope. UV-Vis absorption spectra were measured by TU-1901 UV spectrometer. Dynamic light scattering (DLS) measurements were done using Nanozs90 (Malvern) instrument at a single scattering angle (90°). Fluorescence spectra were acquired on F-4500 (Hitachi) fluorescence spectrometer. Wide-angle X-ray diffraction (XRD) measurements were carried out on a D8 Advance (Bruker) X-ray diffractometer. The X-ray excited luminescence spectra were measured on a home-made, named SicOmni-X, X-ray excited luminescence spectrometer, which is equipped with a F50-100 II tube (tungsten target, 75kV, 20mA) and Hamamatsu R92828 PMT (Shanghai Institute of Ceramics, CAS). All measurements were carried out at room temperature. Fluorescent lifetime measurements were performed with excitation at 375 nm on a FLS 920 fluorescence

system (Edinburgh Instruments, UK). FTIR measurements were conducted on the VERTEX 70/70v FT-IR spectrometer.

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Fig. S1 TEM images of PVP adsorbed ScNPs of different bar scales.



Fig. S2 DLS measurements of size and zeta potential (a), and Fluorescence spectra (b) of naked ScNPs, PVP adsorbed ScNPs, silica coated ScNPs and amino modified silica coated ScNPs excited at 375 nm.



Fig. S3 TEM images of different silica coating shell size at bar scale 50 nm. Figs. a, b, c, d and e of silica shell size 3, 11, 15, 33 and 45 nm respectively. Fig f. the added vol. of TEOS(10 vol%) corresponding to silica shell size thickness.



Fig. S4 Photostability experiment of naked ScNP and silica coated ScNPs that excited at 375 nm with emission of 542 nm wavelength for 30 min.



Fig. S5 Lifetime decay curves of naked ScNP and amino modified silica coated ScNPs.



Fig. S6 FTIR measurements of naked ScNPs, PVP adsorbed ScNPs, silica coated ScNPs and amino modified silica coated ScNPs.

The FTIR spectrum of the nanocrystals was studied as shown in (Fig. S5). It shows obvious peak at 1649 cm⁻¹ which is attributed to the C=O stretching vibration of PVP indicating the adsorption of PVP on the nanocrystal's surface. The peak of 1070 cm⁻¹ assigned to the oxidized Si-O-Si vibrational modes, which usually interpreted assuming a continuous layer of silica. The peaks at 2850 and 2920 cm⁻¹ can be assigned to CH₂ asymmetric and symmetric stretching modes (Propyl chains of APTES) that confirms the presence of the amino terminal groups of the APTES molecules after grafting. ⁵