Supporting Information

Elemental bio-imaging of PEGylated $NaYF_4$: Yb/Tm/Gd upconversion nanoparticles in mouse by laser ablation inductively coupled plasma mass spectrometry to study toxic side effects on spleen, liver and kidney

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Synthesis of $NaYF_4$:Yb/Tm/Gd

Spherical $NaYF_4$:Yb (20%), Tm (2%), and Gd (15%) were prepared following a thermal decomposition method. In a typical procedure, $YCl_3 \cdot 6H_2O$ (458.13 mg, 1.51 mmol), $YbCl_3 \cdot 6H_2O$ (96.88 mg, 0.25 mmol), $TmCl_3$ (8.26 mg, 0.03 mmol), and $GdCl_3 \cdot 6H_2O$ (78.08 mg, 0.3 mmol) were dissolved in a 100-mL flask containing 2 mL deionized water. Oleic acid (15 mL) and 30 mL 1-octadecene were then added to the flask and mixed for 1 h at room temperature. The mixture was then slowly heated to remove water under an argon atmosphere and maintained at 160°C for another 1 h. The mixture was cooled down to room temperature, and a homogeneous transparent yellow solution was obtained. Subsequently, 15 mL of a methanolic solution containing NaOH (288 mg, 7.2 mmol) and NH_4F (260 mg, 7.0 mmol) was added, and the mixture was stirred at room temperature for a further 2 h. After evaporation of methanol, the mixture was slowly heated to 280°C and maintained at this temperature for a further 1 h, before cooling to room temperature. The product was washed several times with ethanol and cyclohexane and the final product, containing $NaYF_4$:Yb/Tm/Gd nanoparticles, was re-dispersed in 20 mL of cyclohexane.

Synthesis of PEG-UCNPs

PEG-UCNPs with good biocompatibility were synthesized by strong thiol-metal interactions. Briefly, the as-synthesized OA-free UCNPs were dispersed in 15 mL PEG_{5k}-SH (200 mg, 0.1 mmol). The solution was stirred for 24 h at room temperature with intervals of ultrasonic treatment. Excess PEG was removed by centrifugation (13000 rpm, 10 min), and the collected product was washed five times with ethanol and water. The purified nanoparticles were obtained and are referred to as PEG-UCNPs.

Characterizations of PEG-UCNPs

As shown in the SEM and TEM images (Figure S1 a, b, c), PEG-UCNPs and UCNPs were uniform spherical nanoparticles with a well-defined size distribution having an average particle size of 20 nm. The mass percentage of each rare earth element in the PEG-UCNPs was determined to be 55.6% (Y), 14.6% (Gd), 1.0% (Tm), and 28.8% (Yb) using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The variations associated with the characteristic functional groups in fourier transform infrared spectroscopy (FT-IR) verified the successful elimination of OA and subsequent surface PEG modification (Figure S2).[1] Specifically, the C-H stretching vibration of CH_2 at 2927 cm^{-1} and 2856 cm^{-1} and the C=O at 1466 cm^{-1} and 1562 cm^{-1} missing in OA-UCNPs, were found to be present in PEG-UCNPs, demonstrating the successful elimination of OA and subsequent PEG coating.

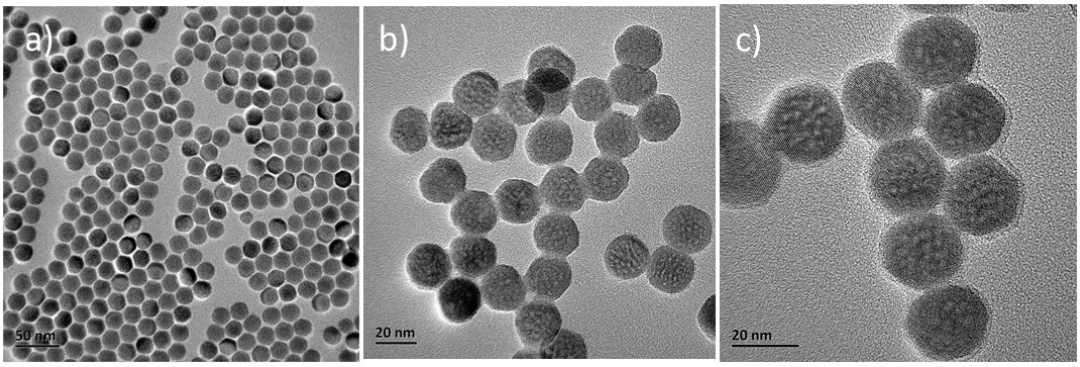

Figure S1. (a) SEM image of PEG-UCNPs core ($NaYF_4$: Tm, Yb, Gd); (b, c) TEM image of PEG-UCNPs.

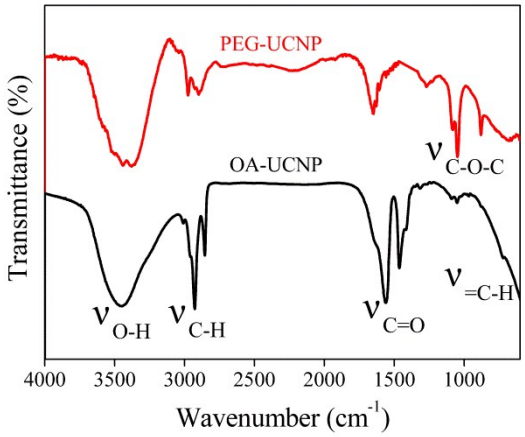

Figure S2 The FT-IR spectrum of OA-UCNPs and PEG-UCNPs.

Toxicity Studies in vivo

The in vivo toxicity of PEG-UCNPs has been investigated by H&E staining analysis. The tissue

sections of heart, liver, spleen, lung and kidney collected after single intravenous injection of PEG-UCNPs for 1, 6, 24 hours and 7, 30 days were stained with H&E. As presented in Figure S3, there is no visible tissue damage or any other side effect as compared with the control group, exhibiting the good biocompatibility of PEG-UCNPs.

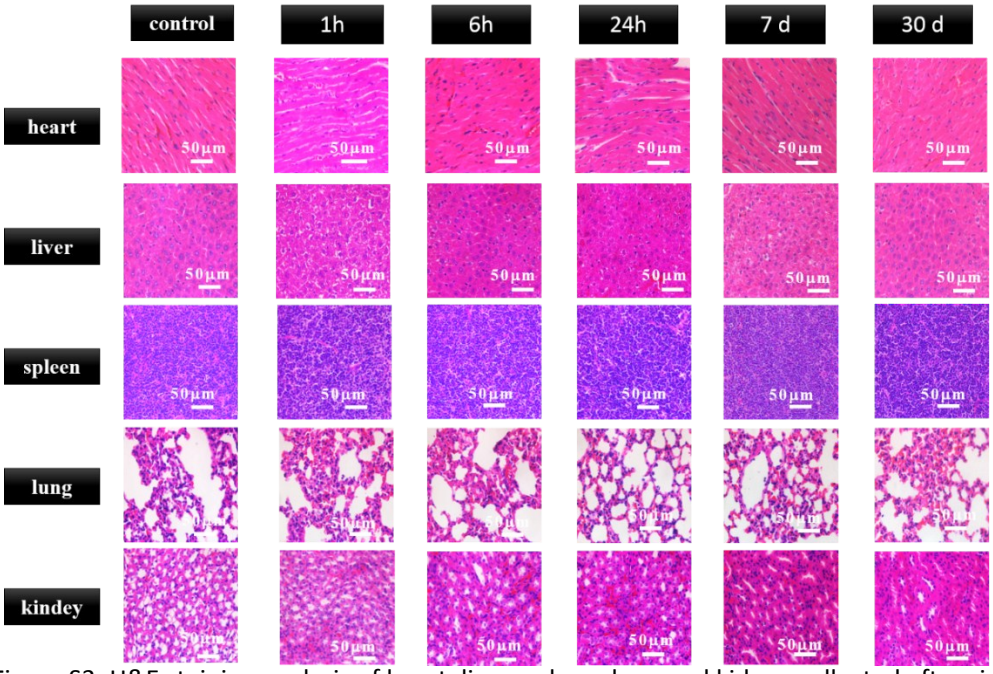

Figure S3. H&E staining analysis of heart, liver, spleen, lung and kidney collected after single intravenous injection of PEG-UCNPs for 1, 6, 24 hours and 7, 30 days

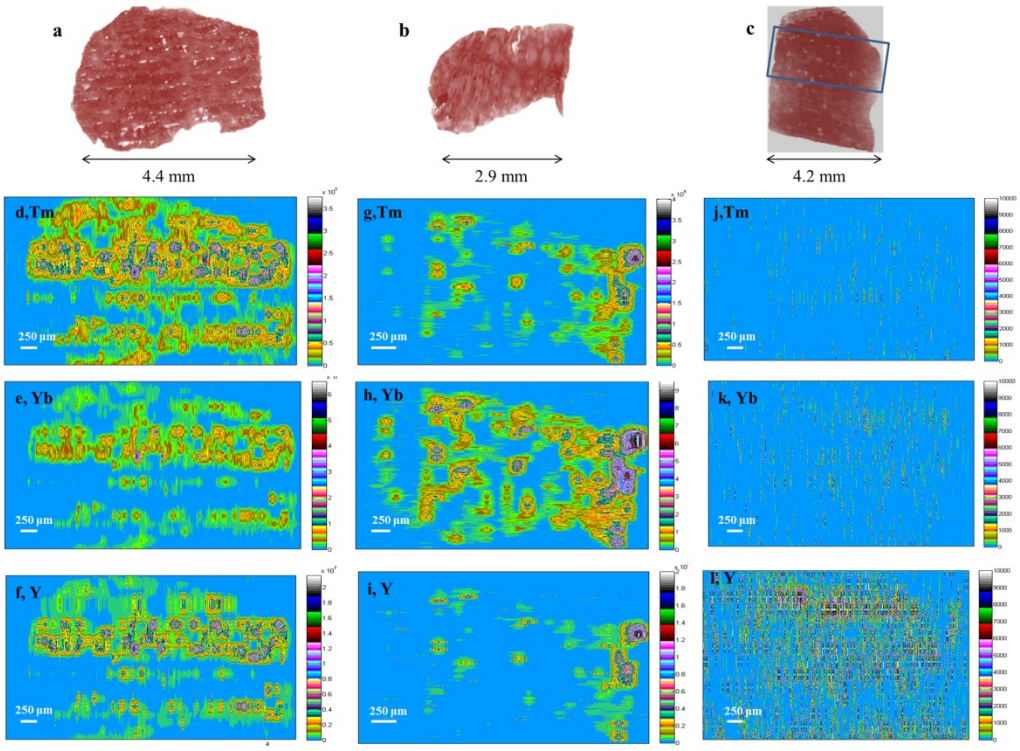

Figure S4. Distribution of PEG-UCNPs in mouse spleens. (a-c) Photographic images of amouse spleen 1 h (a) or 6 h (b) following PEG-UCNP injection and the control spleen (c). (d-i) Images for the simultaneous monitoring of Tm (d, g), Yb (e, h), Y (f, i) on spleen sections 1 h (d, e, and f) or 6 h (g, h, and i) after intravenous injection; corresponding images from the section shown as a blue rectangle in the control spleen (c) are shown in the right hand panel (j, k, and l).

Optimized LA-ICP-MS conditions

A laser ablation system (LSX-213, Cetac, USA) and an ICP-MS (X Series II, Thermo Fisher, USA) were combined for LA-ICP-MS analysis. The operating parameters are summarized in **Table S1**.

Table S1. LA and ICP-MS operation parameters

LA parameter	Value	ICP-MS parameter	Value
Laser wavelength (nm)	213	RF power (W)	1300
Laser energy (%)	40	Sampling depth	150
Laser frequency (Hz)	20	Cooling gas (L/ min)	13
Ablation spot size (μm)	100	Signal acquisition mode	Time resolved analysis
Scan rate (μm/s)	10	Isotopes	^{56}Fe, ^{65}Cu, ^{66}Zn, ^{89}Y, ^{157}Gd, ^{169}Tm, ^{172}Yb
Carrier gas (He)	0.7 L/ min	Carrier gas (Ar)	0.7 L/ min

Reference

1. D. Ni, J. Zhang, W. Bu, H. Xing, F. Han, Q. Xiao, Z. Yao, F. Chen, Q. He, J. Liu, S. Zhang, W. Fan, L. Zhou, W. Peng and J. Shi, Dual-targeting upconversion nanoprobes across the blood-brain barrier for magnetic resonance/fluorescence imaging of intracranial glioblastoma, *ACS Nano*, 2014, **8**, 1231-1242.