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Electronic Supplementary Information

One-pot Synthesis of Ln^{3+}-doped Porous BiF_3@PAA Nanospheres for pH-Responsive Drug Delivery and CT Imaging

Shuang Zhao,[a,b] Rongrong Tian,[a,b] Baiqi Shao,[a] Yang Feng,[a,b] Senwen Yuan,[a] Langping Dong,[a,b] Liang Zhang,[a,b] Zhenxin Wang,[a,b] and Hongpeng You*[a,b]*

[a]State Key Laboratory of Rare Earth Resource Utilization and State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China

[b]University of Science and Technology of China, Hefei 230026, P. R. China

1 Experimental Section

1.1 Materials

$Ln(NO_3)_3$ were obtained by dissolving respective Ln_2O_3 in dilute HNO_3 under continuous heating and stirring. $Bi(NO_3)_3 \cdot 5H_2O$, NH_4F, PAA (Mw=3000) were purchased from Aladdin (Shanghai, China). Ethylene glycol (EG) were received from Beijing Chemical Reagent Co. (Beijing, China). All reagents were of analytical grade and used as obtained.

1.2 Characterization

The morphology was observed by transmission electron microscope (JEOL-2010, Japan) and a field emission scanning electron microscope (FE-SEM, S-4800) with an energy-dispersive spectrometer (EDS) (Hitachi, Japan). The phase structure and purity were characterized using XRD measurements with a D8 Focus diffractometer (Bruker, Germany). Hydrodynamic sizes of the products were inspected on a Zetasizer Nano ZS (Malvern Instruments, UK). UV-visible-NIR spectra were recorded by Shimadzu UV-3600 spectrophotometer (Shimadzu, Japan). The TG curve was obtained by thermogravimetric (TGA) analyses (TA Instruments, USA). Fourier transform infrared spectroscopy (FTIR) analysis was carried out on a PerkinElmer 580B IR spectrophotometer (PerkinElmer, USA). The fluorescence spectra were obtained on an Edinburgh FLS980 fluorescence spectrometer (Hitachi, Japan). CT images were performed on a 64-detector row CT unit (General Electric, USA).

1.3 Synthesis of BiF_3 and BiF_3: Ln^{3+} particles

BiF_3 sub-microspheres were synthesized by a solvothermal method. Typically, 0.5 mmol $Bi(NO_3)\cdot 5H_2O$, 0.2 g PAA were dissolved in 30 mL EG through ultrasonication to form transparent solution A. At the same time, solution B was prepared by dissolving 2 mmol NH_4F in 8 mL EG. After 10 min continuous stirring, solution B was added into solution A and white precipitation was formed immediately. Then, the mixture was transferred into a 50 mL Teflon-lined autoclave. After heating at 160 ^{o}C for 20 h and cooling to room temperature, the products were collected by centrifugation, washed by ethanol and dried at 60 ^{o}C. The BiF_3: Ln^{3+} samples were obtained by the same method except the adding of appropriate Ln^{3+} into the solution A accompanied with Bi^{3+}.

1.4 In vitro DOX storage and release

The DOX loading capacity was estimated by mixing DOX with BiF_3: 20％Yb, 2％Er in PBS buffer with different pH and agitating overnight at room temperature. The DOX-loaded samples (denoted as BiF_3:Yb,Er@DOX) were obtained by centrifugation at 8000 rpm for 6 min and washed three times with 10 mL PBS buffer at pH 7.4. The DOX loading content (LC) and loading efficiency (LE) were determined by

the following equations:[1]

LC (wt %) = (mass of initial DOX - mass of DOX in supernatant solutions) / mass of DOX@BiF_3

LE (%) = (mass of initial DOX - mass of DOX in supernatant solutions) / mass of initial DOX

The in vitro pH-responsive drug release behavior was verified by incubating the DOX-loaded samples at PBS buffers with different pH values (pH 5.0 and pH 7.4) at 37 ^{o}C. At different time intervals, the supernatant solution was collected and replaced by the same amount of fresh PBS. The amount of released DOX was calculated by UV–Vis spectral analysis.

1.5 In vitro cytotoxicity and cellular Uptake

A549 cells (1×10^4 cells per well) were seeded and then incubated with different concentrations of the synthesized BiF_3:Yb,Er, pure DOX and BiF_3:Yb,Er@DOX in 96-well plates for 24 h. The cytotoxicity was estimated by the standard MTT assay. For the intracellular uptake, A549 cells seeded in 48-well plates were cultured with free DOX and BiF_3:Yb,Er @DOX (DOX: 5 μg mL^{-1}). At various time intervals, the cells were stained by Hoechst 33324 and then imaged by a fluorescence microscope.

1.6 In vivo antitumor efficacy and toxicity

Balb/c mice (4-7 weeks) were obtained from Vital River Company (Liaoning, China). All animal experiments were performed according to guidance of Institutional Animal Care and Use of Laboratory Animals. The A549 tumor-bearing nude mice model was used to detect the antitumor efficacy of BiF_3:Yb,Er@DOX. The mice were randomly divided into three groups when the tumor reached 100 mm^3 and then intravenously injected with saline (100 μL), pure DOX (100 μL, DOX: 5 mg kg^{-1}), and BiF_3:Yb,Er@DOX (100 μL, DOX: 5 mg kg^{-1}) three times every second day, respectively. The body weight and tumor size were recorded every other day for 14 days. The tumor volumes were calculated as V= length × $width^2/2$. Besides, histology and blood hematology analysis were performed on healthy mice to investigate the toxicity of the synthesized BiF_3:Yb,Er, DOX and BiF_3:Yb,Er@DOX. Complete blood panel was recorded at 24 h and the 10th day after intravenously injected with saline, BiF_3:Yb,Er (10 mg kg^{-1}), pure DOX (5 mg kg^{-1}), and BiF_3:Yb,Er@DOX (DOX: 5 mg kg^{-1}). At the 30th day, the heart, liver, spleen,

lung, and kidney were collected and stained with H&E for histology measurement, respectively.

1.7 In vitro and in vivo CT imaging

To evaluate the CT imaging capacity of the synthesized BiF_3, the HU values and the corresponding images of various concentrations of BiF_3:Yb,Er and iobitridol (0, 1.875, 3.75, 7.5, 15, 30, 60, 120 mM) were recorded. Besides, the CT images of A549 cells incubated with BiF_3:Yb,Er (500 μg mL^{-1}) for different times (0, 6, 12 and 24 h) were obtained at the same time. For in vivo CT imaging, the images of A549 tumor-bearing mice were collected before and after intratumoral injection with 50 μL BiF_3:Yb,Er (10 mg mL^{-1}) and the parameters were identical with the in vitro measure.

2. Additional Fig. S1-S6 and Table S1-S2

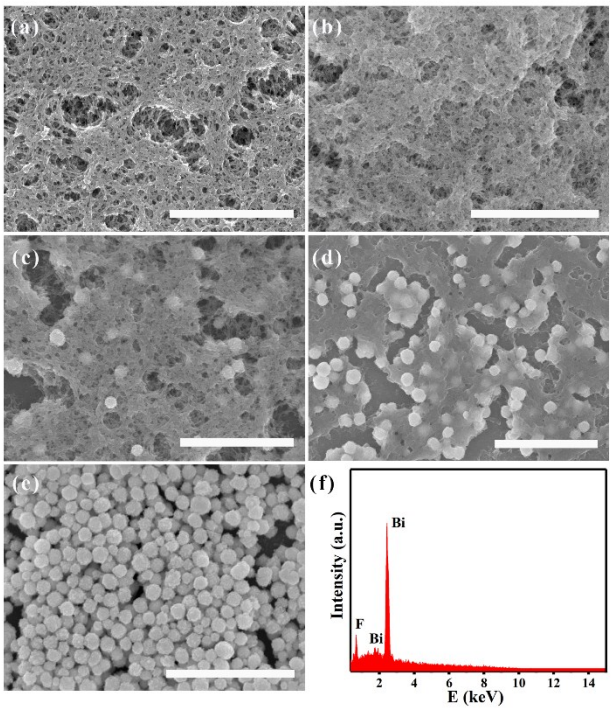

Fig. S1 SEM images of the samples at different reaction times, (a) 0 h, (b) 4 h, (c) 8h, (d) 12 h and (e) 20 h; (f) The corresponding EDX spectrum of the samples obtained at 20 h.

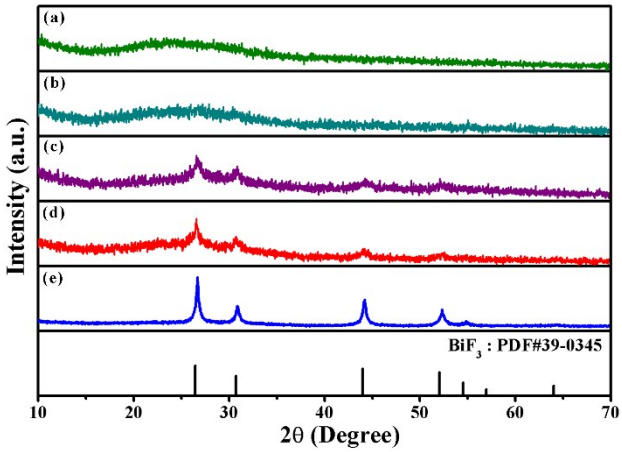

Fig. S2 XRD patterns of the samples at various reaction times, (a) 0 h, (b) 4 h, (c) 8h, (d) 12 h and (e) 20 h.

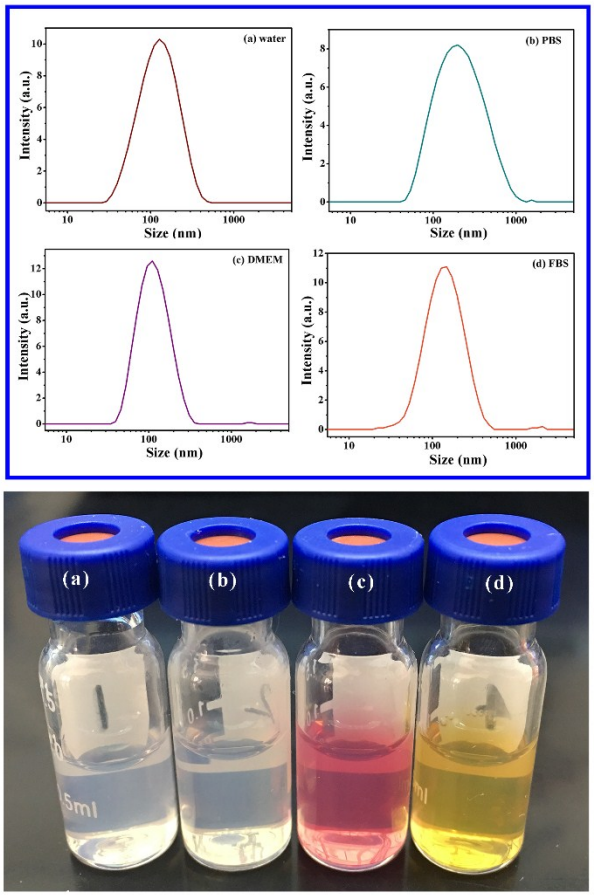

Fig. S3 Hydrodynamic sizes and corresponding digital photographs of BiF_3:Yb,Er dispersed in (a) DI water, (b) PBS buffer, (c) DMEM, and (d) fetal bovine serum (FBS) for two days, respectively.

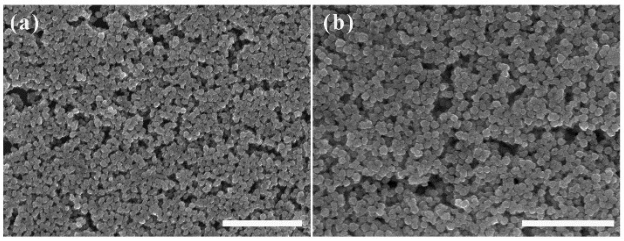

Fig. S4 SEM images of (a) BiF_3:20％Yb,2％Ho and (b) BiF_3:20％Yb,2％Tm samples. The scale bars are 1 μm.

Calculation of the energy gap (Δ E) and sensing sensitivity (S)

The fluorescence intensity ratio (FIR) resulting from the $^2H_{11/2}$ and $^4S_{3/2}$ thermally coupled levels follows the equation:[2]

$FIR = I_{522}/I_{543} = A + B \exp(- \Delta E / k_B T)$

where I_{522}, I_{543} represent the UC intensities, A and B are constants, Δ E is the energy gap between the thermally coupled levels of $^2H_{11/2}$ and $^4S_{3/2}$, k_B is the Boltzmann constant, and T is the absolute temperature. As shown in Fig. 4(b), a good linear relationship is obtained between Ln(FIR) and 1/T, which can be fitted well with a straight line with a slope of -989.249. And the energy gap Δ E is calculated to be 687.973 cm^{-1}.

In addition, sensing sensitivity (S) is investigated, which can be defined as follows:[3]

$S = dR/dT = FIR \times (\Delta E/k_B T^2)$

As exhibited in Fig. 4(d), the sensitivity values increase gradually with temperature, reaching maximum value of 4.478×10^{-3} K^{-1} at 493 K.

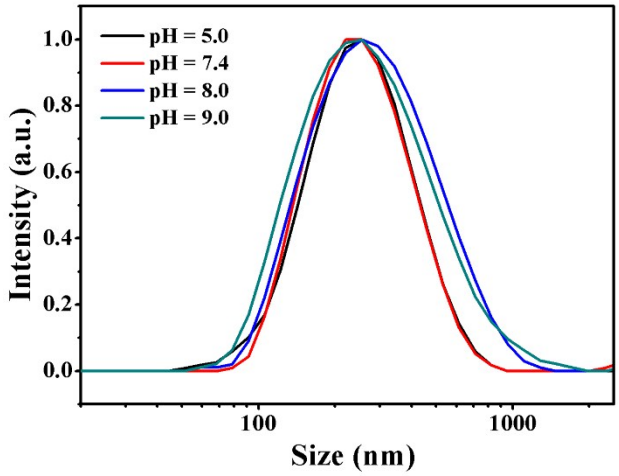

Fig. S5 Hydrodynamic sizes of BiF_3:Yb,Er samples in PBS buffer with different pH.

Table S1 DOX loading content and efficiency at different pH and feeding ratios.

Mass ratio of DOX to BiF_3:Yb,Er	pH of PBS buffer	LC (wt%)	LE (%)
1:4	7.4	16.26	77.68
1:2	7.4	26.90	73.60
1:1	7.4	38.99	63.91
1:1	8.0	43.55	77.14
1:1	9.0	44.88	81.41
1:2	8.0	28.49	79.70
1:2	9.0	31.76	93.09

Table S2 Hematology data of the mice treated with saline (control), BiF_3:Yb,Er, pure DOX, and BiF_3:Yb,Er@DOX, respectively.

Time		24 h				10 days			
Biochemistry Parameters	Units	Control	BiF_3:Yb,Er	DOX	BiF_3:Yb,Er @DOX	Control	BiF_3:Yb,Er	DOX	BiF_3:Yb,Er @DOX
WBC	10^9 L^{-1}	1.66	1.39	0.39	1.25	1.65	1.09	1.89	1.84
RBC	10^{12} L^{-1}	9.09	7.71	9.65	10.08	8.33	8.34	7.66	8.83
HGB	g L^{-1}	144.00	132.00	153.00	161.00	134.00	124.00	121.00	135.00
HCT	%	45.90	40.40	46.50	46.90	44.00	44.10	39.90	46.60
MCV	Fl	50.50	52.40	48.20	46.50	52.80	52.90	52.10	52.80
MCH	Pg	15.80	17.10	15.90	16.00	16.10	14.90	15.80	15.30
MCHC	g L^{-1}	314.00	327.00	329.00	343.00	305.00	281.00	303.00	290.00

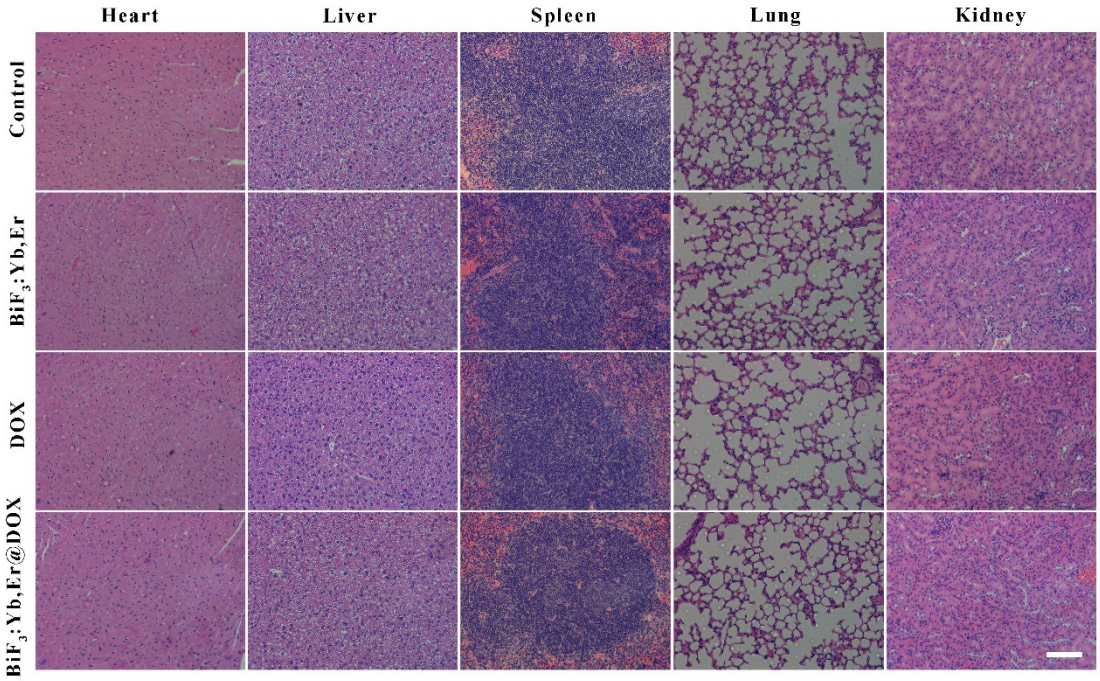

Fig. S6 Histology analysis major organs for the groups of saline (control), BiF_3:Yb,Er, pure DOX, and BiF_3:Yb,Er@DOX.

Reference

1. H. Li, Y. Cui, J. Sui, S. Bian, Y. Sun, J. Liang, Y. Fan and X. Zhang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 15855-15865.
2. B. Dong, B. Cao, Y. He, Z. Liu, Z. Li and Z. Feng, *Adv. Mater.*, 2012, **24**, 1987-1993.
3. M. Lin, L. Xie, Z. Wang, B. S. Richards, G. Gao and J. Zhong, *J. Mater. Chem. C*, 2019, **7**, 2971-2977.