Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI) for

Novel Fe_3O_4@YPO_4:Re (Re=Tb, Eu) Multifunctional Magnetic-fluorescent Hybrid Spheres for Biomedical Applications

Wei Wang,[a] Min Zou[a,b] and Kezheng Chen*[a]

[a] *College of Materials Science and Engineering, Qingdao University of Science and Technology, Qingdao, 266042 (China)*

[b] *Yebio Bioengineering Co. Ltd, Qingdao, 266032 (China)*

**To whom correspondence should be addressed. E-mail: kchen@qust.edu.cn*

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1. Materials and Methods

(1) Materials

Iron (II) sulfate heptahydrate ($FeSO_4•7H_2O$), ferric chloride ($FeCl_3•6H_2O$), $(NH_4)_2HPO_4$, poly (ethylene glycol) (PEG, MW 1500), and ammonia ($NH_3•H_2O$) were obtained from Shanghai Chemical Corporation (Shanghai, China). Yttrium oxide (Y_2O_3, 99.99%), terbium oxide (Tb_4O_7, 99.99%) and europium oxide (Eu_2O_3, 99.99%) were obtained from Shanghai Sanpu Chemical Corporation (Shanghai, China), and are of SpecPure grade.

(2) Methods

Synthesis of bare YPO_4:Re spheres

Bare YPO_4:Re spheres were prepared by a solvothermal technique as follows: stoichiometric amounts of high purity Y_2O_3 and Re_xO_y were dissolved in concentrated nitric acid and H_2O_2 by heating to form Y(III) and Re(III) solutions. Appropriate volume of $(NH_4)_2HPO_4$ solution was dripped into the former solution. After that EG (40 ml) containing PEG-1500 (0.1 g) was added under vigorous stirring. And then the mixture was transferred into a Teflon-lined stainless steel autoclave with a filling capacity of 40%. The solvothermal reaction lasted for 18 h at 180 °C. The resulting products were washed with ethanol and de-ionized water for several times, and centrifuged at 8000 rpm. The obtained precipitates were redispersed in de-ionized water (10 ml) to form YPO_4:Re colloid.

Synthesis of Fe_3O_4@YPO_4:Re hybrid spheres

Loading of Fe_3O_4 nanoparticles on YPO_4:Re bare spheres was based on the co-precipitation method with Fe^{2+} and Fe^{3+}. Typically, YPO_4:Re colloid (1 ml) containing YPO_4:Re (0.1 mmol) was added into Fe^{3+} solution (50 ml, 2 mM) and stirred overnight. Appropriate amount of Fe^{2+} (Fe^{3+}:Fe^{2+}=2:1 mol ratio) was added and the mixture was transferred into a flask, then degassed with N_2 for 30 min. Ammonium hydroxide (2 ml) was added quickly under rapid mechanical stirring. The suspension was kept under N_2 for 15 min. The resultant brown magnetic hybrid spheres were separated magnetically and washed with de-ionized water and ethanol several times.

Drug loading and Encapsulation Efficiency

DOX loading content (LC) and encapsulation efficiency (EE) in the Fe_3O_4@YPO_4:Re hybrid spheres were determined by the centrifugation method. First, Fe_3O_4@YPO_4:Re hybrid spheres were loaded with DOX in PBS (pH=7.4) for 24 h and then separated from the aqueous suspension medium by centrifugation at 40 000 r/min for 40 min at 4 °C. The obtained DOX-loaded Fe_3O_4@YPO_4:Re (DOX-Fe_3O_4@YPO_4:Re) pellets were incubated at 60 °C in vacuum overnight and were weighted. DOX concentration in supernatant was analyzed by the ultraviolet absorption (UV) at the wavelength of 480 nm, a strong absorption band of DOX, with reference to a calibration curve on a UV-Vis-NIR spectrophotometer. The measurements were performed in triplicate. The amount of the drug in the DOX-Fe_3O_4@YPO_4:Re spheres could be calculated by addition amount of DOX

subtracting DOX one in supernatant. Drug-loading content and encapsulation efficiency were obtained by eqs 1 and 2, respectively.

$$\text{drug-loading content\%} = \frac{\text{weight of the drug in hybrid spheres}}{\text{weight of the hybrid spheres}} \times 100\% \quad (1)$$

$$\text{encapsulation efficiency\%} = \frac{\text{weight of the drug in hybrid spheres}}{\text{weight of the feeding drug}} \times 100\% \quad (2)$$

Drug Release from the DOX-Fe_3O_4@YPO_4:Re spheres in Vitro

Two hundred milligrams of DOX-Fe_3O_4@YPO_4:Re spheres were resuspended in 10 mL PBS (pH=7.4) and was placed in a dialysis membrane bag (8~14 kDa cut off), and then tied, and sank into PBS (300 mL). The entire system was kept at 37 °C with continuously magnetic stirring. After a predetermined period, the PBS medium (3 mL) was drawn out from release system for analysis, and fresh medium (3 mL) was added into the release system. Release kinetics of DOX from DOX-Fe_3O_4@YPO_4:Re in PBS at 37 °C was measured by filtering out DOX-Fe_3O_4@YPO_4:Re spheres from the PBS medium at each time point using the centrifugal filter and measuring UV absorbance of free DOX in the supernatant at 480 nm. The released DOX amount was determined by UV analysis with a calibration curve as described above.

In Vitro Cellular Uptake of Fe_3O_4@YPO_4:Re spheres

Hela cells were used in this study. The RPMI Medium containing 10% fetal bovine serum (FBS) was utilized as cell culture medium. Cells were precultivated in 6 well plates (Costar) with 5% CO_2 in the medium at 37 °C until confluence has

reached. Fe_3O_4@YPO_4:Re spheres diluted in the medium were added into the chamber. The cells were then incubated at 37 °C for 96 h to allow the cells to internalize the spheres. Then the cells were rinsed three times with PBS in order to remove the spheres remained in the chambers. Further, 80% acetone was used to fix the cells before fluorescent imaging.

Characterization

XRD studies were conducted on a Rigaku D/max-2500 X-ray powder diffractometer using Cu Kα radiation (λ=1.5406 Å). The morphological investigations were carried out with field-emission scanning electron microscopy (FESEM, JEOL JSM-6700F), and transmission electron microscopy (TEM, JEOL JEM-2100F). The magnetic properties were measured on a SQUID magnetometer (Quantum Design, MPMS XL-5) at 300 K. Fluorescent properties were performed at room temperature by an F-4600 fluorescence spectrophotometer. UV analysis was characterized on a Varian Cary500 UV-Vis-NIR spectrophotometer (Varian, USA). An inverted microscope (Eclipse TE2000S, Nikon) was used to image the cellular uptake of the Fe_3O_4@YPO_4:Re spheres by Hela cells.

2. Figure S1-S3

Figure S1. XRD pattern of Fe_3O_4@YPO_4:Re (Re=Tb, Eu) magnetic-fluorescent hybrid spheres.

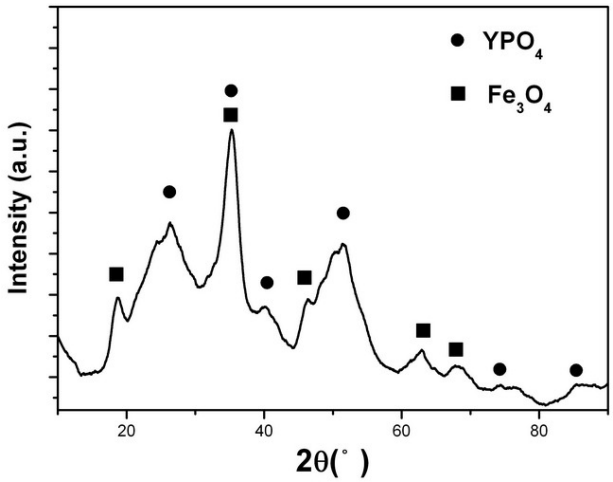

Figure S2. EDS spectra of Fe_3O_4@YPO_4:Re hybrid spheres: (A) Re=Tb, (B) Re=Eu.

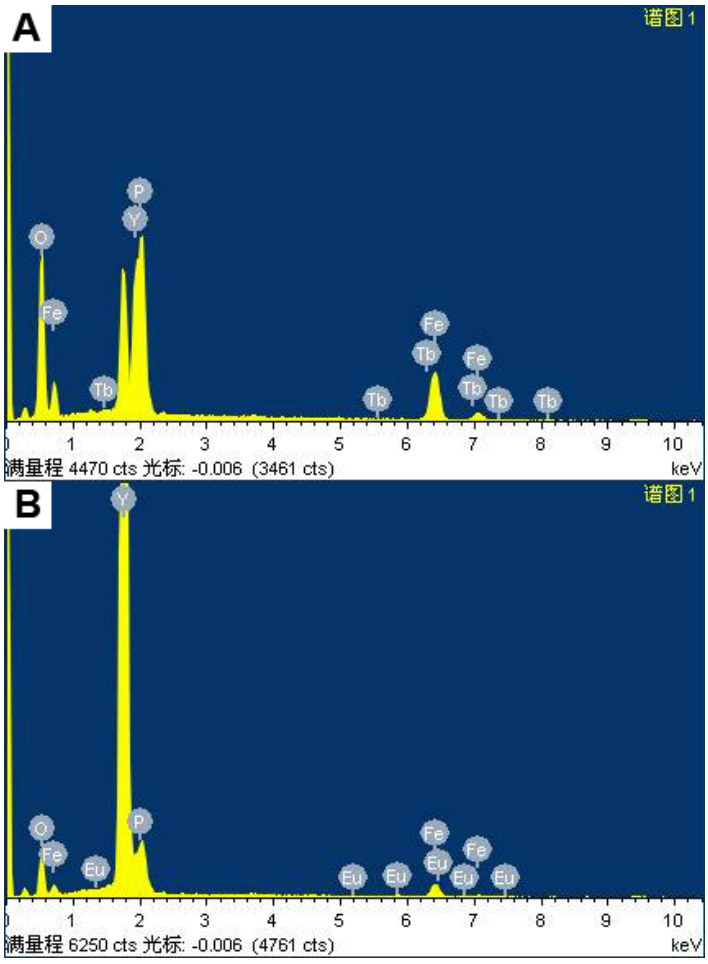

Figure S3. In vitro release behavior of DOX from Fe_3O_4@YPO_4:Tb hybrid spheres in PBS solution at 37 °C.