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Electronic Supplementary Information (ESI)

A facile microemulsion template route for producing hollow silica nanospheres as imaging agents and drug nanocarriers

Nirun Jatupaiboon[ab]‖, Yanfang Wang[ab]‖, Hao Wu[ab], Xiaojie Song[ab], Yizhe Song[ab], Jianbin Zhang[ab], Xiaojun Ma[a]* and Mingqian Tan[a]*

[a] *Division of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China. Fax & Tel: +86-411-84379139; E-mail:* maxj@dicp.ac.cn, mqtan@dicp.ac.cn

[b] *University of the Chinese Academy of Sciences, Beijing 100049, China*

‖Nirun Jatupaiboon and Yanfang Wang contributed equally to this work.

Section 1. Experimental

1.1 Materials and Instrumentals

The ligand of europium complex, 4,4'-Bis (1",1",1",2",2",3",3",-heptafluoro-4",6"-hexanedion-6"-yl) chlorosulfo-*o*-terphenyl (BHHCT), was synthesized by using a previous method.[1] (3-aminopropyl) triethoxysilane (APS), tetraethylorthosilicate (TEOS), and Triton X-100 were purchased from Acros Organics. Fluorescein isothiocyanate (FITC) and cetyltrimethylammonium bromide (CTAB) were purchased from Aladdin Reagent Com. China. All other reagents were used as received. Doxorubicin (Dox) was purchased from Meilun Biotech. Co. (Dalian, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). De-ionized water was prepared with a Milli-Q-Plus system (18.2MΩ). Transmission electron microscopy (TEM) was performed using a JEOL JEM-2000EX transmission electron microscope with a beam voltage of 120 kV from Dalian Medical University, China. Fluorescence spectra were measured on a Perkin-Elmer LS 55B spectrofluorometer.

1.2 Synthesis of hollow silica nanospheres (HSNs)

The water phase was prepared by mixing appropriate amount of APS (1, 2 and 3 μL) into 1.1 mL of water. To 14.5 g of cyclohexane were added 4.47 g of Triton X-100 and 3.64 g of n-octanol to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 200 μL of TEOS and 200 μL of NH_4OH to trigger the

polymerization reaction. After reaction at room temperature for 24 h, the raw HSNs were isolated from the microemulsion by adding acetone. The final product of HSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.

APS
Water
Triton X-100
n-octanol
cyclohexane
W/O microemulsion
TEOS, NH_4OH
$H_2N(H_2C)_3$ Si O Si $(CH_2)_3NH_2$
O O
$H_2N(H_2C)_3$ Si O Si $(CH_2)_3NH_2$ n
APS: $(EtO)_3Si(CH_2)_3NH_2$ TEOS: $Si(OEt)_4$

Scheme S1. Schematic illustration of the synthesis of HSNs.

1.3 Synthesis of solid silica nanospheres (SSNs)

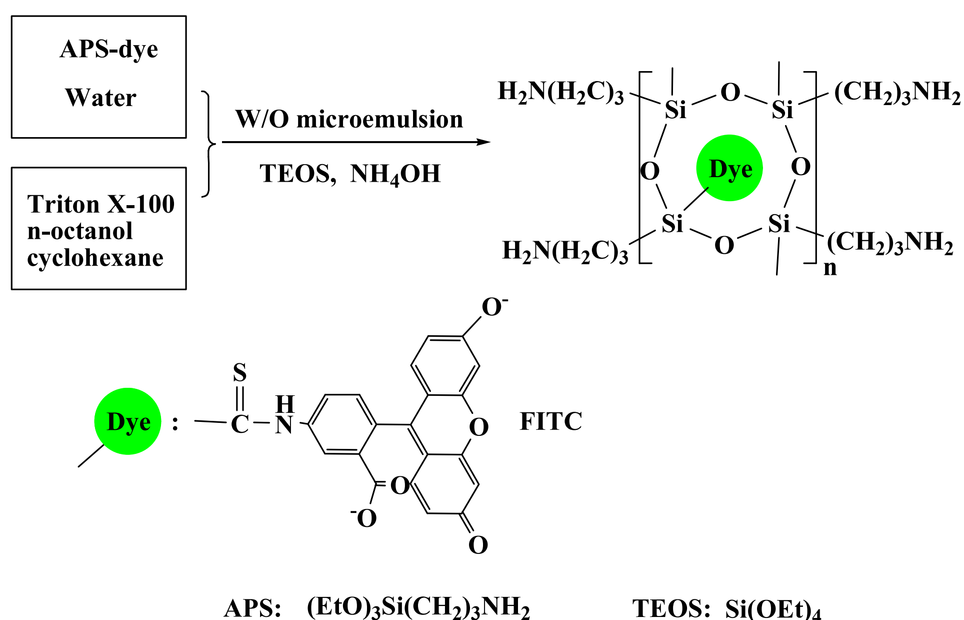
The water-in-oil microemulsion was prepared by adding1.1 mL of water into the organic phase containing 14.5 g of cyclohexane, 4.47 g of Triton X-100 and 3.64 g of n-octanol. After adding 3 μL of APS and 200 μL of TEOS, the copolymerization reaction was trigged by adding 200 μL of NH_4OH. The mixture was stirred at room temperature for 24 h and the reaction was stopped by adding 15 mL of acetone. The product of SSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.

Water
Triton X-100
n-octanol
cyclohexane
APS
W/O microemulsion
TEOS, NH_4OH
$H_2N(H_2C)_3$ Si O Si $(CH_2)_3NH_2$
O O
$H_2N(H_2C)_3$ Si O Si $(CH_2)_3NH_2$ n
APS: $(EtO)_3Si(CH_2)_3NH_2$ TEOS: $Si(OEt)_4$

Scheme S2. Schematic illustration of the synthesis of solid silica nanospheres.

1.4 Synthesis of FITC-doped HSNs (FITC-HSNs)

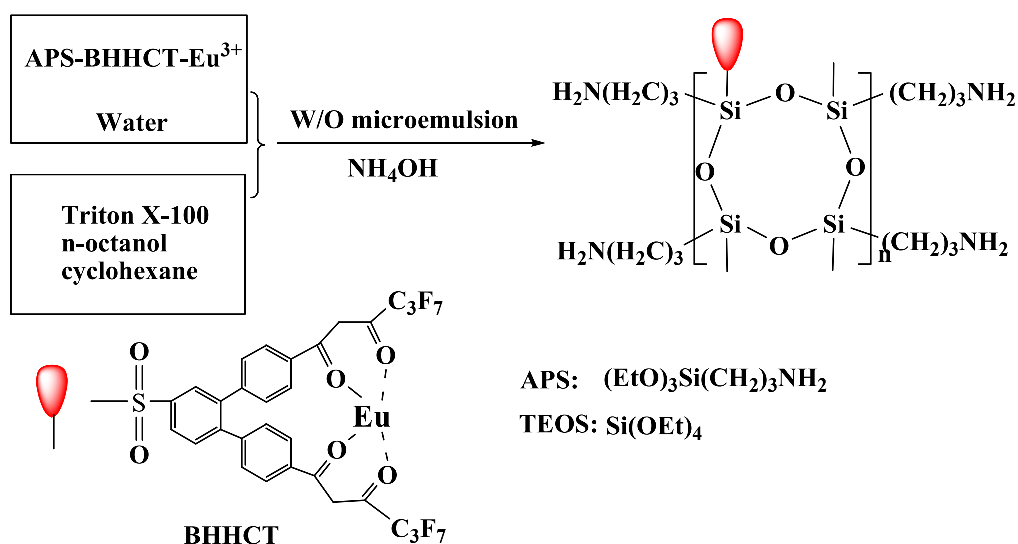
To 15 μL of APS were added 3 mg of FITC and 15 μL of triethylamine. The mixture was ultrasonicated at room temperature for 15 min under dark conditions to avoid photobleaching. The water phase was prepared by mixing the above APS-FITC conjugate solution into 1.1 mL of water. To 14.5 g of cyclohexane were added 4.47 g of Triton X-100 and 3.64 g of n-octanol to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 200 μL of TEOS and 200 μL of NH_4OH to trigger the polymerization reaction. After reaction at room temperature for 24 h, the FITC-doped HSNs were isolated from the microemulsion by adding 15 mL of acetone. The final product of FITC-HSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.



Scheme S3. Schematic illustration of the synthesis of FITC-HSNs.

1.5 Synthesis of BHHCT-Eu^{3+}-doped HSNs (Eu-HSNs)

To 2 mL of cyclohexane were added 102 mg of BHHCT, 23 mg of $EuCl_3 \cdot 6H_2O$ and 220 μL of APS. The mixture was ultrasonicated at room temperature for 15 min under dark conditions to avoid photobleaching. The water phase was prepared by mixing 10 μL of APS-BHHCT-Eu^{3+} conjugate solution into 1.1 mL of water. To 14.5 g of cyclohexane were added 4.47 g of Triton X-100 and 3.64 g of n-octanol to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 200 μL of TEOS and 200μL of NH_4OH to trigger the polymerization reaction. After reaction at room temperature for 24 h, the BHHCT-Eu^{3+}-doped HSNs were isolated from the microemulsion by adding 15 mL of acetone. The final product of BHHCT-Eu^{3+}-doped HSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.



Scheme S4. Schematic illustration of the synthesis of BHHCT-Eu^{3+}-doped HSNs.

1.6 Synthesis of Fe_3O_4-doped HSNs (Fe_3O_4-HSNs)

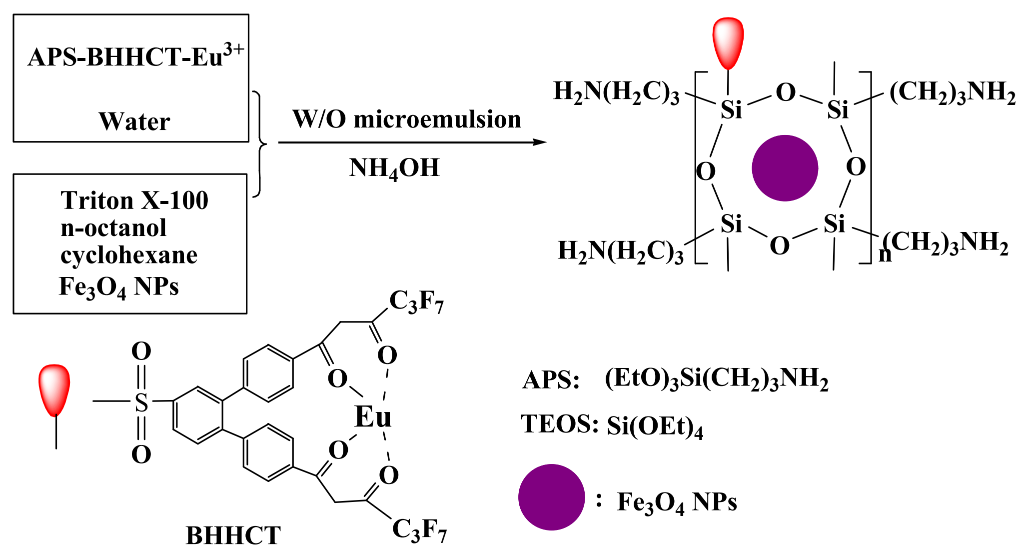
The water phase was prepared by mixing 5 μL APS into 1.1 mL of water. To 18.2 mL of cyclohexane were added 4.47 g of Triton X-100 and 4.41 mL of n-octanol to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 13 mg of Fe_3O_4 nanoparticles, 200 μL of TEOS and 200 μL of NH_4OH to trigger the polymerization reaction. After reaction at room temperature for 24 h, the raw Fe_3O_4-doped HNSs were isolated from the microemulsion by adding 10 mL of acetone. The final product of Fe_3O_4-HSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.

Scheme S5. Schematic illustration of the synthesis of Fe_3O_4-HSNs

1.7 Synthesis of BHHCT-Eu^{3+}@Fe_3O_4-doped HSNs (Eu@Fe_3O_4-HSNs)

The water phase was prepared by mixing 10 μL of APS-BHHCT-Eu^{3+} conjugate (prepared in section 1.5) into 1.1 mL of water. To 18.2 mL of cyclohexane were added 4.47 g of Triton X-100 and 4.41 mL of n-octanol to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 13 mg of Fe_3O_4 nanoparticles, 200 μL of

TEOS and 200 μL of NH_4OH to trigger the polymerization reaction. After reaction at room temperature for 24 h, the raw Eu@Fe_3O_4-HSNs were isolated from the microemulsion by adding 10 mL of acetone. The final product of Eu@Fe_3O_4-HSNs was obtained by centrifuging, washing with ethanol and water under ultrasonication for three times to remove impurities.



Scheme S6. Schematic illustration of the synthesis of BHHCT-Eu^{3+}@Fe_3O_4-doped HSNs

1.8 Synthesis of hollow mesoporous silica nanospheres (HMSNs)

The water phase was prepared by mixing 80 μL of APS into 17.6 mL of water. To 290 mL of cyclohexane were added 71.52g of Triton X-100, 72 mL of n-octanol and 60 mg CTAB to prepare the oil phase. The water-in-oil microemulsion was prepared by adding the water phase into oil phase under vigorous stirring, followed by adding 3.2 mL of TEOS and 3.2 mL of NH_4OH to trigger the polymerization reaction. After reaction at room temperature for 24 h, the raw HMSNs were isolated from the microemulsion by adding 100 mL of acetone. The white precipitate was then

thoroughly washed with ethanol and water under ultrasonication for three times to remove impurities. Finally, the HMSNs were obtained after extraction with an acetic acid/ethanol mixture at room temperature for 4 h to remove the pore-generating template.

1.9 Magnetic resonance imaging measurements.

Magnetic resonance imaging measurements of the Eu@Fe_3O_4-HSNs were performed in a 0.5 T MiniMR-60 system at 32 ^oC (Suzhou Niumag Electronic Technology Corporation). The T_2-weighted images were acquired using spin echo acquisition with the following parameters: point resolution = 156 × 156 m^2, section thickness = 1 mm, TE =60 ms, TR = 4,000 ms, number of acquisitions = 2.

1.10 Cell fluorescence imaging

Human SH-SY5Y neuroblastoma cells cultured in DMEM medium were incubated with 0.4 mg/mL of FITC-HSNs after treating with 0.22 μm of filter. The cells were submitted for fluorescence imaging with confocal laser scanning microscope after incubation for 1, 2, 4, 6 days, respectively. Confocal laser scanning microscope (Leica, TCS-SP2, Germany) was equipped with Ar (488 nm/5 mW) and He-Ne (543 nm/1.2 mW) laser sources and an inverted microscope (Leica, DMIRE2, Germany). All confocal fluorescence pictures were acquired with a 20 × objective (HC PLAN APO 20 x / 0.40 PH1 0.17/A 2.2).

1.11 Loading Dox into HMSNs and in vitro drug release

The Dox-loaded HMSNs (Dox-HMSNs) were prepared by mixing 1 mL of Dox aqueous solution (5 $mg \cdot mL^{-1}$) with 1 mL of HMSNs (5 $mg \cdot mL^{-1}$) for 72 h and then centrifuged at 10000 rpm for 10 min to remove the free Dox. The Dox loading content and entrapment efficiency was calculated using the following equations: 1) Dox loading content (%) = $Wt_{Dox\ in\ MHSNs} / Wt_{MHSNs} \times 100\%$; 2) Dox entrapment efficiency (%) = $Wt_{Dox\ in\ MHSNs} / Wt_{Dox}$ (Wt = weight). The content of residual Dox in supernatant was determined by UV-Vis measurements at 480 nm with the comparison to calibration curve created by a serial of Dox standard solutions of different concentration.

In vitro release of Dox from Dox-HMSNs nanocarriers was evaluated using a dialysis bag (8-14 kDa molecular weight cutoff) after loading 2 mL of Dox-loaded HMSNs solution. The dialysis bag was placed into 10 mL of PBS (pH 7.4) at 37 ^{o}C with gentle shaking. The released Dox content in PBS at 0.25, 0.5, 1, 2, 3, 6, 12, 24, 48, 240, 480 and 720 h was measured by the UV-Vis measurements as mentioned above. SSNs and HSNs were used as control in this experiment. The release of Dox from Dox-SSNs and Dox-HSNs were also evaluated and used as control in this experiment.

1.12 Cell uptake of Dox- HMSNs

Hela (human cervical cancer) cells were cultured and maintained in 1640 medium containing 10 % FBS, antibiotic-antimycotic solution L-glutamine (2 mM),

and non-essential amino acids (1%) in 5 % CO_2 at 37 °C. After the cells were trypsinized and seeded in tissue culture plates at an initial cell density of 1 × 10^5 cells/well (1mL of the medium), they were treated with the Dox-HMSNs and free Dox, respectively, at a Dox concentration of 10.0 μg /mL. After incubation at 37 °C for 1 or 4 h, the Hela cells were washed three times with fresh medium and imaged with an Olympus FV1000 laser confocal fluorescent microscope equipped with a 488 nm laser and its emission was collected at 540–600 nm.

1.13 Cytotoxicity study of HMSNs

Hela cells were seeded in 24-well plates at a density of 5×10^3 cells/well and cultured for 24 h in 10% serum containing 1640 medium supplemented with penicillin (100 units/mL) and streptomycin (100 μg/mL) in a humidified atmosphere with 5% CO_2 at 37°C. After incubation with different concentrations (0, 3.13, 6.25, 12.5, 25, 50, 100 and 200 μg/mL, n=3) of HMSNs, Dox-doped mesoporous HSNs and free Dox, 20 μL of MTT (5 mg/mL) reagent was added to each cell well. After further incubation for 24, 48 and 72 h, respectively, the cells were washed with PBS three times and 100μL of dimethylsulfoxide (DMSO) was added. The optical density (OD) of the mixture was measured at 570 nm using a Microplate Reader (Wellscan MK3, Labsystems). The cell viability was estimated according to the following equation: Cell Viability [%] = ($OD_{treated}$ /$OD_{control}$) × 100% ($OD_{control}$ was measured in the absence of agent, and $OD_{treated}$ donates the intensity of HMSNs, Dox-doped HMSNs and free Dox, respetively. The data was expressed as the percentages of viable cells compared to the survival of a control group (untreated cells as controls of 100%

viability).

Section 2. Table and Figures

Table S1. Physicochemical properties of various nanospheres.

Nanospheres	APS (μL)	Size (nm)	Core size (nm)	Shell thickness (nm)	Size distribution (nm)
HSNs-0 (Solid)	0	35.0	N/A	N/A	28.0 ~ 44.0
HSNs-1	1	34.0	7.0	13.5	32.0 ~ 38.0
HSNs-2	2	32.0	10.0	11.0	25.0 ~ 35.0
HSNs-3	3	26.0	16.0	5.0	21.0 ~ 38.0
Eu-HSNs	10	32.0	16.0	8.0	26.0 ~ 36.0
FITC-HSNs	15	23.0	10.0	6.5	17.0 ~ 27.0
Fe_3O_4-HSNs	5	40.0	18.0	11.0	33.0 ~ 47.0
Eu@Fe_3O_4-HSNs	10	38.0	17.0	10.5	30.0 ~ 49.0
HMSNs	5	36.0	12.0	12.0	31.0 ~ 46.0

N/A: Not applicable. SHNs: silica hollow nanoparticles. HSNs-0, HSNs-1, HSNs-2 and HSNs-3: HSNs prepared with 0, 1, 2, and 3 μL of APS, respectively.

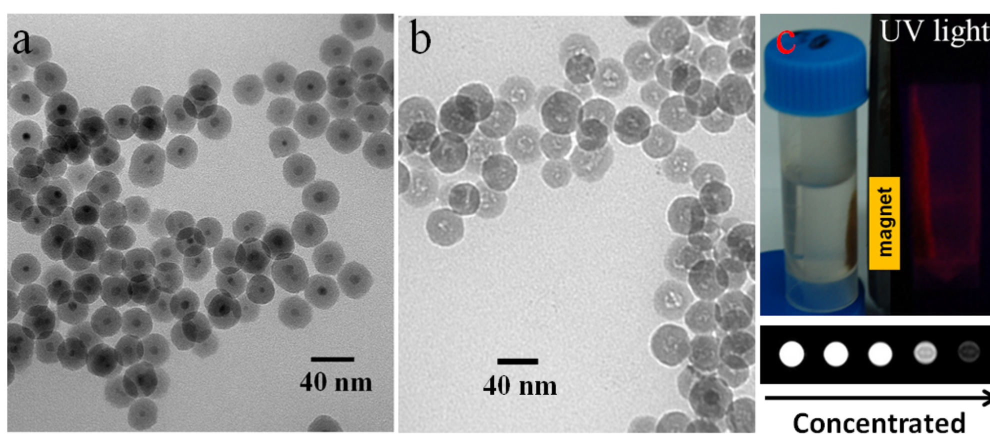


Fig. S1 TEM images of Eu@Fe_3O_4-SSNs (a) and Eu@Fe_3O_4-HSNs (b). (c)Visual photographs of Eu@Fe_3O_4-HSNs dispersion in water in response to a NdFeB magnet under ambient light and red emission under the 365 nm UV light excitation, as well as T_2 weighted magnetic resonance imaging along with the increased concentration Eu@Fe_3O_4-HSNs.

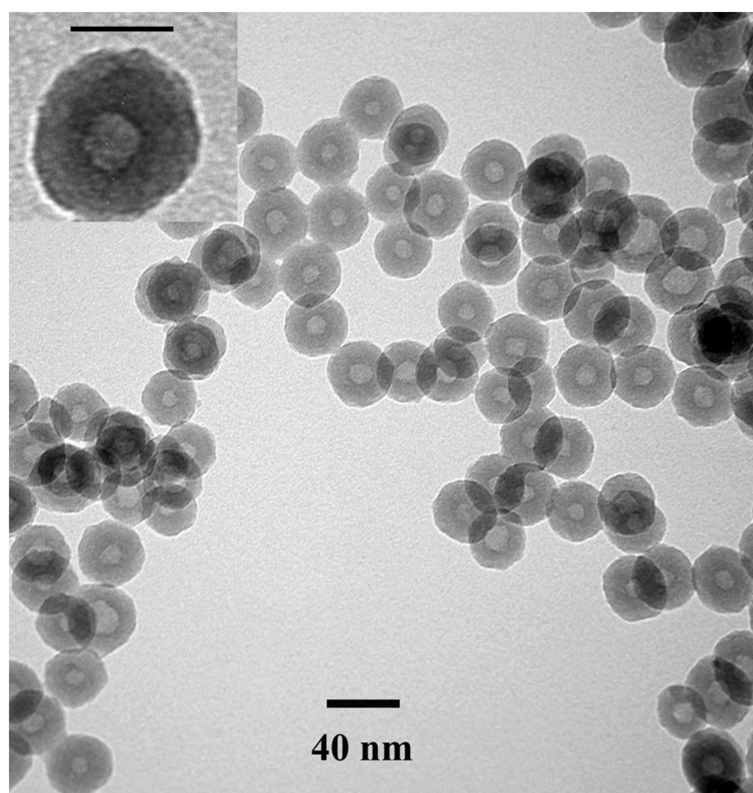


Fig. S2 TEM image of HMSNs. Inset shows the enlarged TEM image of the HMSNs. Scale bar=20 nm.

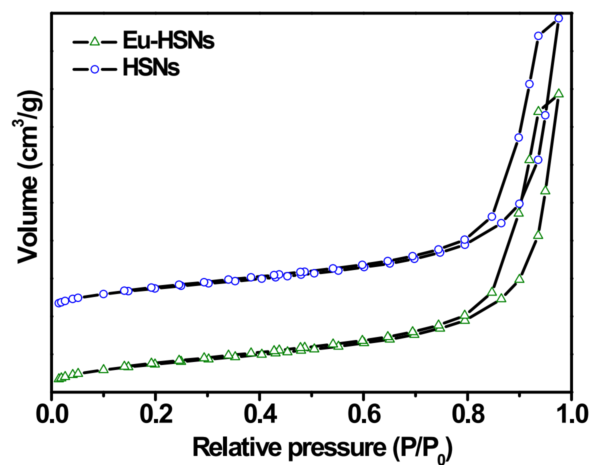


Fig. S3 Nitrogen absorption-desorption isotherms for HSNs and Eu-HSNs.

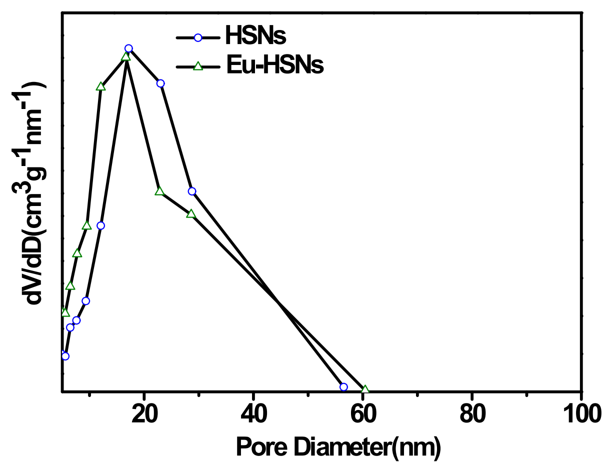


Fig. S4 The pore size distribution curves of HSNs and Eu-HSNs measured from BJH model applied to the absorption branch of HSNs.

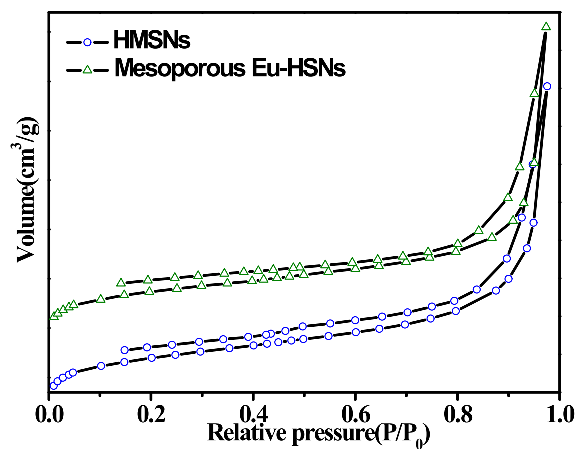


Fig. S5 Nitrogen absorption-desorption isotherms for HMSNs and mesoporous Eu-HSNs.

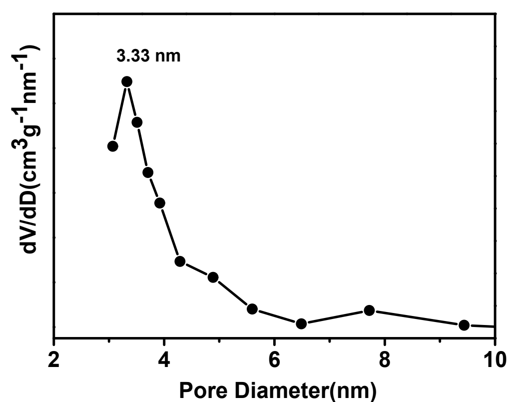


Fig. S6 Pore size distribution curve of HMSNs measured from BJH model.

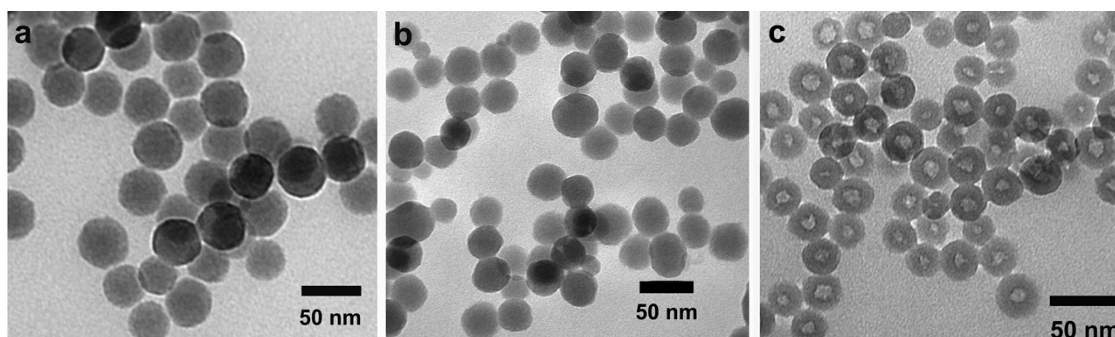


Fig. S7 TEM images of BHHCT-Eu^{3+}-doped silica nanospheres synthesized by method 2 (a) and by method 1 before washing (b) and after washing (c).

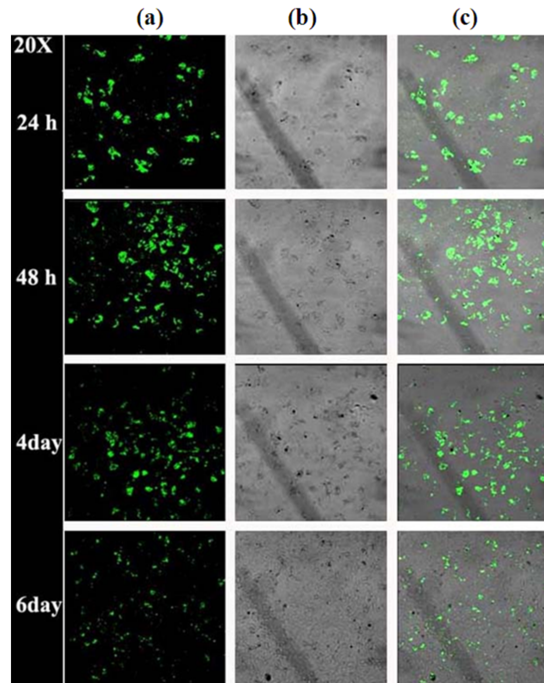


Fig. S 8. Confocal laser scanning microscopy images of cell line SHSY5Y incubated with FITC-HSNs for 6 days in (a) fluorescence (b) bright-field (c) overlay.

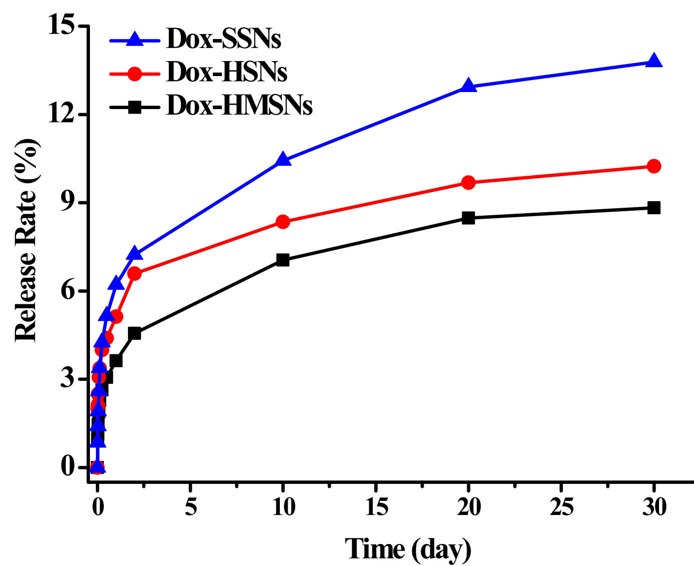


Fig. S 9. Dox-release profiles for Dox-HMSNs, Dox-HSNs and Dox-SSNs measured at pH 7.4 in PBS buffer at 37 ^{o}C.

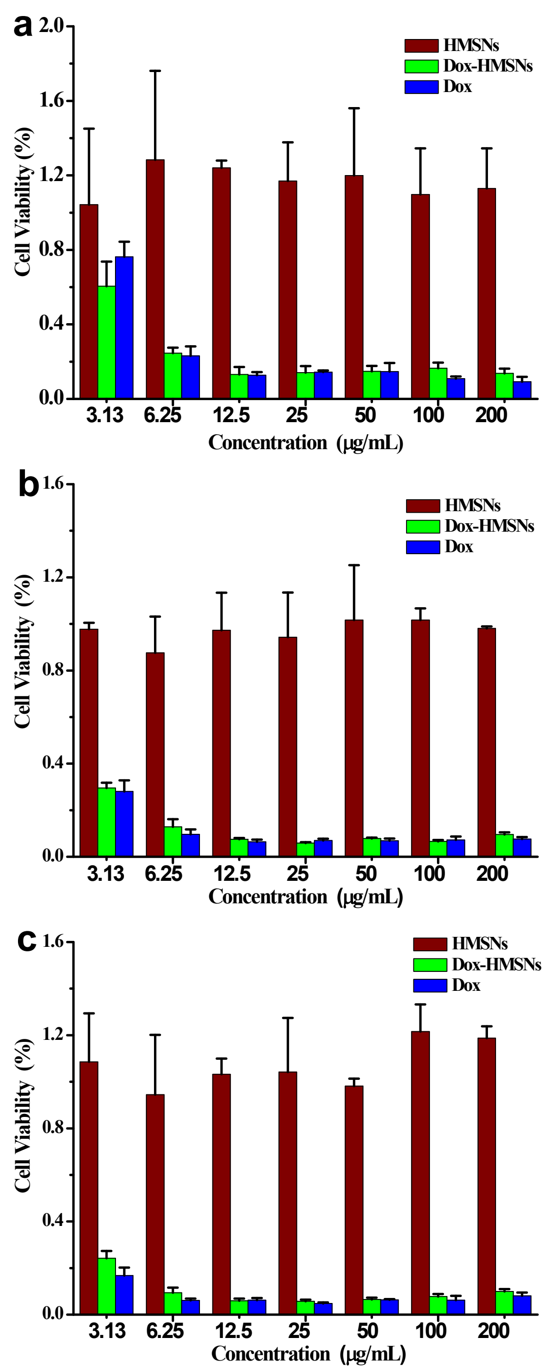


Fig. S10. *In vitro* cytotoxicity of empty HMSNs, Dox-HMSNs and free Dox against Hela cells at different concentrations after (a) 24 h, (b) 48 h and (c) 72 h incubation.

Reference

[1] M.Q. Tan, G.L. Wang, X.D. Hai, Z.Q. Ye, J.L. Yuan, *J Mater Chem*, 14 (2004) 2896-2901.