Supporting information

AIEgens-functionalised mesoporous silica nanoparticles as FRET donor for monitoring drug delivery

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Experimental

Materials. Tetraethoxysilane (TEOS, Beijing Beihua Chemical Co., Ltd.), cetyltrimethylammonium bromide (CTAB, China National Pharmaceutical Industry Co.), sodium hydroxide (NaOH, Tianjin Guangfu Fine Chemical Research Institude.), 3-aminopropyltriethoxysilane (APTS, Sigma-Aldrich), doxorubicin hydrochloride (DOX, J&K Scientific Ltd.), dimethyl sulfoxide (DMSO, Tianjin Guangfu Fine Chemical Research Institude.), anhydrous toluene (Beijing Beihua Chemical Co., Ltd). All agents used in cell culture were obtained from Invitrogen, USA. Both mesoporous silica nanoparticles (MSNs) and 1,2-bis[4-(bromomethyl)phenyl]-1,2-diphenylethene (BTPE) were prepared according to the literature.[1,2]

Instruments. Small angle Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku D/MAX 2500/PC X-ray diffractometer with CuKα radiation (λ=0.15405 nm), scanning speed was 1^{o} min^{-1}. Scanning electron microscopy (SEM) images were obtained using a field-emission scanning electron microscope (JSM-6510). Transmission-electron-microscopy (TEM) images were recorded with a Tecnai F20 electron microscope. N_2 adsorption-desorption isotherms were measured on a

Micromeritics ASAP 2420 apparatus at 77 K. The surface areas were estimated according to the Brunauer-Emmett-Teller (BET) method, and the pore size distributions were calculated via the density functional theory (DFT) method. Thermogravimetric analysis (TGA) of NH_2-MSNs was performed on TGA Q500 system in air with a heating rate of 10 K min^{-1}. Infrared (IR) measurements of the samples dispersed in KBr pellets were performed on a Perkin-Elmer spectrum 430 FT-IR spectrometer. CHN elemental analyses were carried out on a vario MICRO elemental analyser. UV-Vis adsorption spectra were obtained on a Shimadzu UV-2550 spectrophotometer for liquid test and UV-4100 spectrophotometer for solid measurement. The photoluminescence excitation and emission spectra were obtained on a Shimadzu RF-5301PC spectrofluorometer. The quenching tests of FMSNs by DOX were measured on FLUOROMAX-4 spectrofluorometer. The surface potentials of the drug carriers in different pH solution were analyzed by Malvern Nano ZS90.

Synthesis of amine group modified mesoporous silica nanoparticle (NH_2-MSNs). 1 g mesoporous silica nanoparticles were loaded into a 50 mL flask, then 20 mL anhydrous toluene and 3 mL APTS were mixed and added into the bottle at room temperature. The slurry was heated at 120 °C with stirring under N_2 atmosphere for 6 h and centrifuged, washed with ethanol, then dried in vacuum freeze dryer for 24 h, marked as NH_2-MSNs.

Synthesis of AIEgens-functionalised mesoporous silica nanoparticles (FMSNs). 250 mg NH_2-MSNs and 3 mg BTPE were added into 20 mL DMSO solution, and the mixture was stirred at 80 °C for 24 h, then the solid was centrifuged, washed with acetone, deionized water, ethanol in turn for several times and dried under vacuum, marked as FMSNs.

Drug loading and in vitro release. 40 mg FMSNs sample was added into 6 mL deionized water solution containing 0.5 mg mL^{-1} DOX, which was stirred in a vial in the dark at room temperature for 24 h. The products were collected by centrifugation and washed with deionized water until the supernatant became colourless. The amount of DOX adsorbed into FMSNs was determined by UV-vis absorbance at 480 nm, marked as DOX@FMSNs. Then 3 mg DOX@FMSNs was immersed into 3 mL

phosphate buffer solution (PBS) at pH=7.4 and pH=5.0, respectively. The drug release medium solution was taken out by centrifugation for UV-vis analysis at 480 nm after given time intervals. The same volume of fresh PBSs was added and ultrasound for a while, then the fluorescence emission of the dispersion was measured immediately to observe the fluorescence resonance energy transfer (FRET) phenomenon.

Cell viability experiment. HeLa cells were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin under a humidified atmosphere containing 5%/95% CO_2/air at 37 °C. The cultured medium is refreshed every 3 days. Cells were ready for treatment when reached 70% confluence in the plates.

The in vitro cytotoxicity of the FMSNs and in vitro cell-growth inhibition efficacy of DOX@FMSNs and free DOX were investigated by 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-pheny-tetrazoliumromide (MTT) assays. HeLa cells were seeded into 96-well plates at 2×10^4 mL^{-1} per well for 24 h. After treated with FMSNs at doses of 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 μg mL^{-1} for 24 h, the total volume of the cell culture fluid is 200 μL, and then the cells were incubated with 0.5 mg mL^{-1} MTT for 4 h at 37 °C in darkness. Purple formazan crystals were solubilized by adding 100 μL of DMSO and the absorbance was measured using a microplate reader at the wavelength of 492 nm. The in vitro cell-growth inhibition experiment of DOX@FMSNs and free DOX were carried out with the same procedure.

Confocal laser scanning microscopy (CLSM). HeLa cells were cultured in DMEM supplemented with 10% FBS at 37 °C and 5% CO_2. To check cellular uptake, HeLa cells (20–50% confluent) seeded on cover slips in a 12-well plate were incubated with FMSNs (25 μg mL^{-1}) in culture medium at 37 °C and 5% CO_2. After 6 h post incubation, the medium was removed and the cells were washed several times with PBS (pH = 7.4) and fixed with 2% formaldehyde in PBS for 10 min at room temperature. The CLSM measurement of DOX@FMSNs is similar with that of FMSNs except that the concentration of DOX@FMSNs is 212.86 μg mL^{-1} and incubation time is 10 min, 1 h, 6 h, respectively.

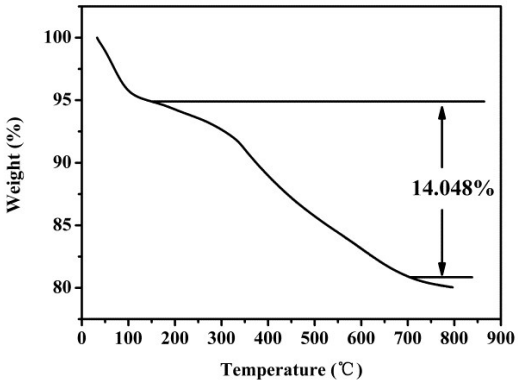

Figure S1. TG profile of NH_2-MSNs.

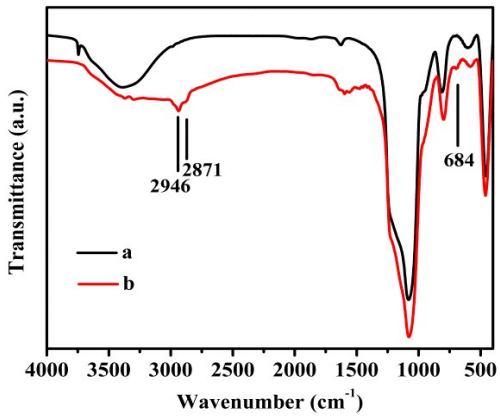

Figure S2. FT-IR spectra of MSNs (a) and NH_2-MSNs (b).

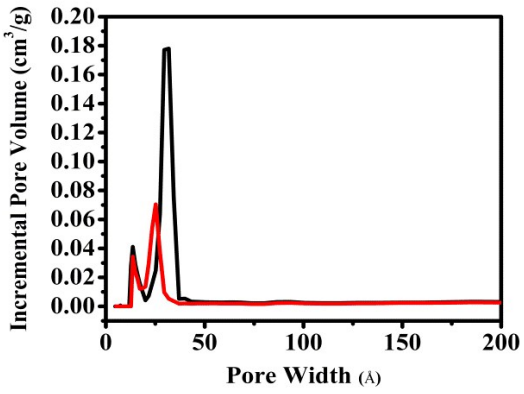

Figure S3. The pore size distribution of MSNs and FMSNs determined on the basis of the density functional theory (DFT).

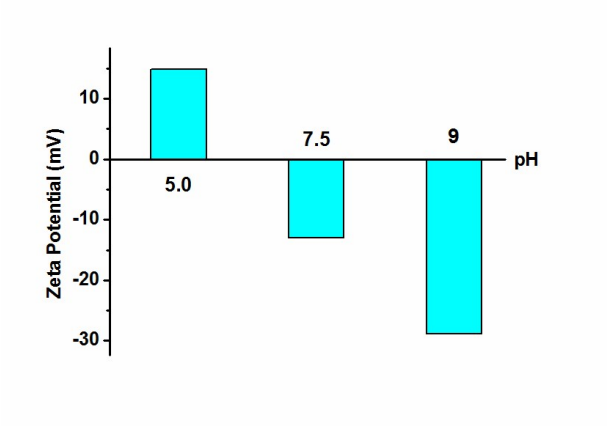

Figure S4. The surface potentials of the FMSNs in different pH solution.

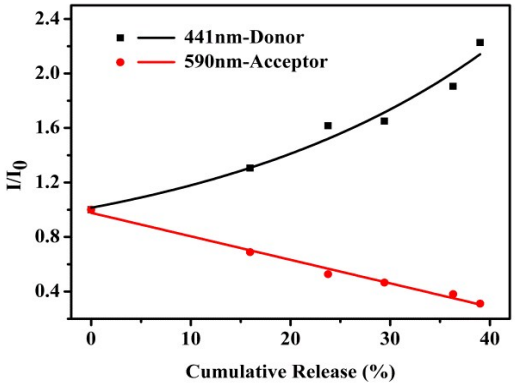

Figure S5. PL intensity at 441 nm and 590 nm as a function of cumulative release amount of DOX at different time intervals in PBS at pH=5.0.

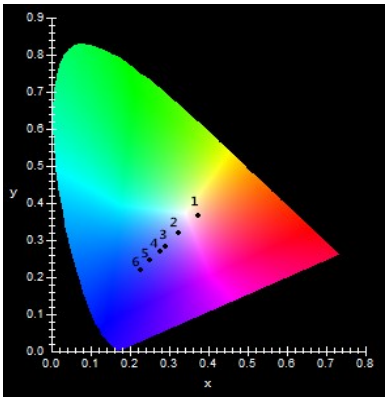

Figure S6. The emission colours of FMSNs loading with different amount of DOX in

the CIE 1931 chromaticity diagram.

Table S1. Textural parameters of MSNs and FMSNs

Sample	S_{BET} (m^2 g^{-1})	D_{DFT} (nm)	V (cm^3 g^{-1})
MSNs	1139	3.08	1.09
FMSNs	837	2.51	0.56

Table S2. Corresponding parameters of the plots in Figure S5

Number	Time (h)	Cumulative Release (%)	PL Intensity at 441nm I_i/I_1	Colour Coordinate (x,y)
1	0	0	1	0.38, 0.37
2	1	15.95	1.31	0.33, 0.32
3	3	23.77	1.62	0.29, 0.28
4	5	29.42	1.65	0.28, 0.27
5	12	36.31	1.91	0.25, 0.25
6	24	39.02	2.23	0.23, 0.22

Reference

[1] X. B. Xu, S. Y. Lü, C. M. Gao, X. G. Wang, X. Bai, N. N. Gao and M. Z. Liu, *Chem. Eng. J.*, 2015, **266**, 171.

[2] A. J. Qin, L. Tang, J. W. Y. Lam, C. K. W. Jim, Y. Yu, H. Zhao, J. Z. Sun and B. Z. Tang, *Adv. Funct. Mater.*, 2009, **19**, 1891.