Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

A Simple Transformation from Silica Core-Shell-Shell to Yolk-Shell Nanostructures: A Useful Plateform for Effective Cell Imaging and Drug Delivery

Guosheng Song, Chun Li, Junqing Hu, Rujia Zou, Kaibing Xu, Linbo Han, Qian Wang, Jianmao Yan, Zhigang Chen, Zongyi Qin, Kangcheng Ruan, Ronggui Hu

Experimental section

Materials: Cetyltrimethylammonium bromide (CTAB), sodium carbonate (Na_2CO_3), dodecanol, ammonia aqueous ($NH_3 \cdot H_2O$, 25-28 wt%), sodium hydroxide (NaOH), and ethanol are analytically pure and were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Fluorescein isothiocyanate (FITC, 90%), 3-aminopropyl triethoxysilane (APS, 98%), and tetraethylorthosilicate (TEOS, GR) were obtained from Aladin. Methoxy poly ethylene glycol succinimidyl carbonate (mPEG-SC, MW = 5000) was purchased from Kaizheng Biotech., Beijing, and doxorubicin hydrochloride (DOX) was got from Huafeng United Technology CO., Ltd, Beijing. All reagents were used as received without further purification.

Synthesis of silica solid nanospheres: Monodisperse SiO_2 nanospheres with a various diameter were prepared by using a modified Stöber method.[1] A various amount of $NH_3 \cdot H_2O$, 5 mL of H_2O, and 50 mL of ethanol were mixed in a 100-mL flask with magnetic stirring, and the resulted solution was heated to 60 °C. Then, a various amount of TEOS was diluted in 10 mL of ethanol and then added dropwise into the above solution at a speed of ~ 0.4 mL min^{-1}. Subsequently, the solution was kept at 60 °C under continuous magnetically stirring for 4 h. After that, the product was collected by centrifugation, washed with ethanol three times, and dried at 60 °C overnight. The volumes used of $NH_3 \cdot H_2O$ and TEOS were 5 mL and 2 mL, 3 mL and 2 mL, and 5 mL and 1 mL, respectively, to prepare silica solid nanospheres with a various diameter of ~ 170 nm, ~ 140 nm, ~ 100 nm, respectively.

Synthesis of silica core-shell-shell nanostructures (CSSNs): The silica CSSNs were prepared by using CTAB and dodcanol as soft template and slowly adding TEOS dropwise under a mild alkali condition. In a typical procedure, 0.15 g of as-prepared silica solid nanospheres was dispersed in 50 mL of H_2O by stirring and ultrasonication. Then, 0.1 g of CTAB, 30 mg (or 37 μL) of dodecanol, 1 mL of NaOH aqueous (30 mg mL^{-1}) were added in the above solution under gentle magnetically stirring, avoiding to produce a large quantity of foams. After the solution was heated and kept at 60 °C for 1 h, 0.75 mL of TEOS was added dropwise at a speed of ~ 25 μL min^{-1} under stirring, followed by 2 h of further stirring at 60

°C. Finally, the resultant silica CSSNs were separated by centrifugation, washed with ethanol three times, dried at 60 °C overnight. The silica CSSNs of ~ 200 nm, ~ 170 nm and ~ 120 nm were obtained using silica solid nanospheres of ~ 170 nm, ~ 140 nm and ~100 nm as a core, respectively. For synthesis of core-shell nanospheres covered with a half of outermost shell, silica solid nanospheres of ~ 170 nm as a core, 0.5 mL of TEOS under a dropping speed of ~ 0.3 mL min^{-1} were used while the other synthetic parameters were kept constants, compared with the synthesis of silica CSSNs.

Synthesis of silica yolk-shell nanostructures (SYSNs): The SYSNs were fabricated by Na_2CO_3 aqueous selective etching more porous interlayer of the CSSNs. Typically, 300 mg of as-prepared CSSNs was dispersed in a solution of 100 mL of H_2O and 200 mL of ethanol by stirring and ultrasonication, then 100 mL of Na_2CO_3 aqueous (0.5 M) were added into the above the solution, followed by stirring at room temperature for 40 min. The final product was centrifuged and washed with H_2O, and then dispersed in 40 mL of ethanol for later use. The SYSNs with a diameter of ~ 200 nm, ~ 170 nm, and ~ 120 nm were obtained by selectively etching silica CSSNs with a diameter of ~ 200 nm, ~ 170 nm and ~ 120 nm, respectively.

Preparation of FITC-labeled SYSNs/PEG: For modification of the SYSNs' surfaces, FITC-APS conjugates were firstly synthesized by referring to the literature.[2] Briefly, 60 μL (250 μmol) of APS and 10 mg (25 μmol) of FITC were mixed in 5 mL of absolute ethanol under a dry nitrogen atmosphere and magnetically stirred overnight. The conjugates were later used as the fluorescent and silane reagent with amine groups. After that, 0.3 mL of FITC-APS conjugates and 0.4 mL of $NH_3 \cdot H_2O$ were added into the as-prepared 40 mL of SYSNs/ethanol solution and the mixture was stirred for 12 h at room temperature. After the reaction, the resultant FITC-labeled SYSNs/NH_2 nanocomposites were purified by three cycles of centrifugation and dispersion in DMSO, and then dispersed into 20 mL of DMSO. Finally, 80 mg of mPEG-SC was dissolved in 10 mL of DMSO and then added to the above 20 mL of DMSO solution of the FITC-labeled SYSNs/NH_2, followed by further stirring for 12 h at room temperature.[3] The resultant FITC-labeled SYSNs/PEG were centrifuged, washed with ethanol several times to remove the remained PEG, and then dried under vacuum at room temperature for over night. All the products were protected from light during the sample preparation and storage to prevent photobleaching.

Loading DOX into FITC-labeled SYSNs/PEG: The obtained 80 mg of the FITC-labeled SYSNs/PEG was dispersed in 10 mL of PBS by stirring and ultrasonication, and then was mixed with 10 mL of DOX /PBS solution (0.5 mg mL^{-1}), followed by 12 h of stirring in the dark under room temperature. The DOX-loaded FITC-labeled SYSNs/PEG were centrifuged, washed with the PBS 3 times and then dried under vacuum at room temperature overnight. To evaluate the DOX loading efficiency, the supernatant was collected, and the residual DOX content was determined by the UV-Vis measurement at 482.4 nm. The loading efficiency of DOX in FITC-labeled SYSNs/PEG was calculated as follows:

$$\frac{\text{initial amount of DOX - residual DOX}}{\text{initial amount of DOX}} \times 100\%$$

***In vitro* release of DOX from DOX-loaded FITC-labeled SYSNs/PEG:** *In vitro* release of DOX-loaded FITC-labeled SYSNs/PEG was evaluated using a semi-permeable dialysis bag diffusion technique. 10 mg of as-prepared DOX-loaded nanocomposites was dispersed in 4 mL of PBS (pH 7.4) and 4 mL of acetate buffer (pH 4.8) by stirring and ultrasonication, respectively. Then, both of the release mediums were placed into pre-treated semi-permeable dialysis bags and immersed into 6 mL of PBS and 6 mL of acetate buffer, respectively, at 37 °C with gentle shaking (120 rpm). At several intervals, 4 mL of release medium out of semi-permesble dialysis bags was taken out to be determined the amount of released DOX by UV-Vis spectroscopy at a wavelength of 482.4 nm and then replaced with an equal volume of the fresh medium after measurement.

Cellular uptake and internalization: The protocol of cellular uptake and internalization were referred to the literature.[3] Breast cancer cells of MDA-MB-231 (kindly provided Shanghai Institute of Biological Sciences, CAS, China) were maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Hyclone), supplemented with 10% FBS (Hyclone) at 37 °C in a humidified atmosphere of 5% CO_2. Cell culture media were supplemented with 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin, and the MDA-MB-231 cells were seeded on glass-bottom dishes (35 mm, Corning Incorporated). A final concentration of 100 μg mL^{-1} FITC-labeled SYSNs/PEG was added to the cells and incubated for 6 h and 12 h, respectively. The cells were washed with PBS four times, stained with 60 nM Lysotracker Red DND-99 (from Beyotime Institute of Biotechnology, Haimen, China) for 1 h, and then washed with PBS four times, fixed with 4% paraformaldehyde, and stained with 10 μg mL^{-1} DAPI (2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride, Sigma), and washed with PBS three times. Micrographs were pre-observed on a Nikon fluorescence microscope (Nikon Eclipse Ti-S, CCD: Ri1), finally taken on a laser scanning confocal microscope (Leica TCS sp5, Germany) for confocal luminescence imaging on a 63 × oil-immersion objective lens.

***In vitro* cytotoxicity of FITC-labeled SYSNs/PEG and DOX-loaded FITC-labeled SYSNs/PEG:** To test the cytotoxicity of the FITC-labeled SYSNs/PEG, breast cancer MDA-MB-231 cells were seeded in a 96-well plate at a density of 2000 cells per well and cultured in 5% CO_2 at 37 °C for 24 h. Then FITC-labeled SYSNs/PEG with different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125 μg mL^{-1}) were added to the medium, and the cells were incubated in 5% CO_2 at 37 °C for 24 h or 48 h, respectively. Cell viabilities were determined using 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide (MTT) reduction assay. For the cytotoxicity of free DOX and DOX-loaded nanocomposites, breast cancer

MDA-MB-231 cells were seeded in a 96-well plate at a density of 5000 cell and cultured in 5% CO_2 at 37 °C for 24 h. Then, free DOX and DOX-loaded nanocomposites with different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125 μg mL^{-1}) were added to the medium, respectively, and then the cells were incubated in 5% CO_2 at 37 °C for 24 h and 48 h, respectively. Finally, the cell viability was assessed using the MTT assay.

Characterization: The Morphologies and structures of the products were characterized by a field-emission scanning electron microscope (S-4800) and a field-emission transmission electron microscope (JEOL JEM-2100F) at an accelerating voltage of 5 kV and 200 kV, respectively. The surface area, pore volume, and pore-size distribution of the products were determined by Brunauer-Emmett-Teller (BET) and Barett-Joyner-Halenda (BJH) methods (Quantachrome, Autsorb-1MP). Powder x-ray diffraction was conducted by a D/max-2550 PCX–ray diffractometer (Rigaku, Japan). Fourier transform infrared (FTIR) spectra were recorded using the KBr pressed pellets on an IRPRESTIGE-21spectrometer (Shimadzu). UV-Vis absorption spectra were measured on UV-Vis 1901 Spectrophotometer (Phoenix). Photoluminescent (PL) spectra were measured on FP-6600 research NIR fluorescence spectrometer (Jasco). X-ray photoelectron spectroscopy (XPS) spectra were conducted on an Axis Ultra DLD X-ray photoelectron spectroscopy (Kratos Co., Britain). The hydrodynamic diameter (DLS) and zeta potential measurements were carried out with the Zetasizer Nano Z (Malvern, Britain).

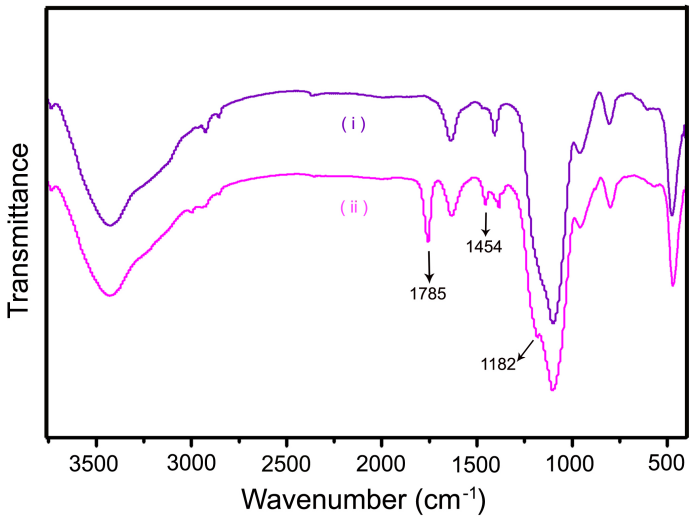

Figure S1 (i) FTIR spectra of the SYSNs and (ii)FITC-labeled SYSNs/PEG.

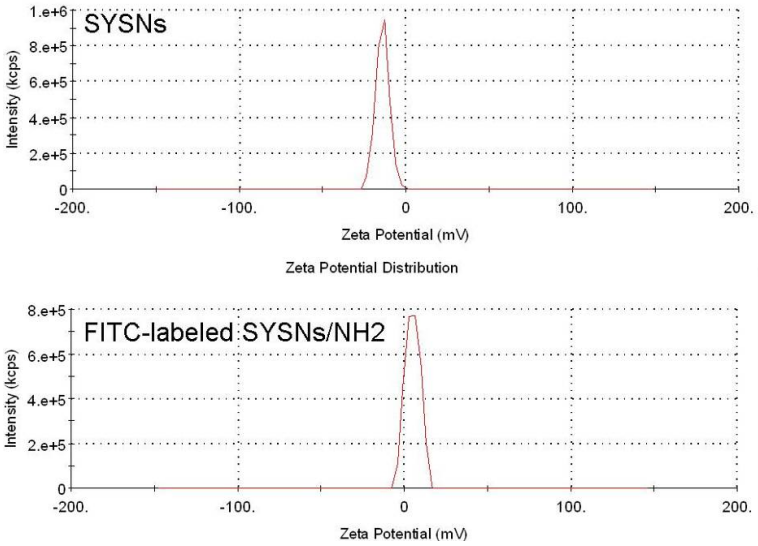

Figure S2. The zeta potential of SYSNs and FITC-labeled SYSNs/NH_2. The zeta potential of SYSNs is -13.7mv and then increased to 5.294mv after being grafting with the APS and APS-FITC (FITC-labeled SYSNs/NH_2).

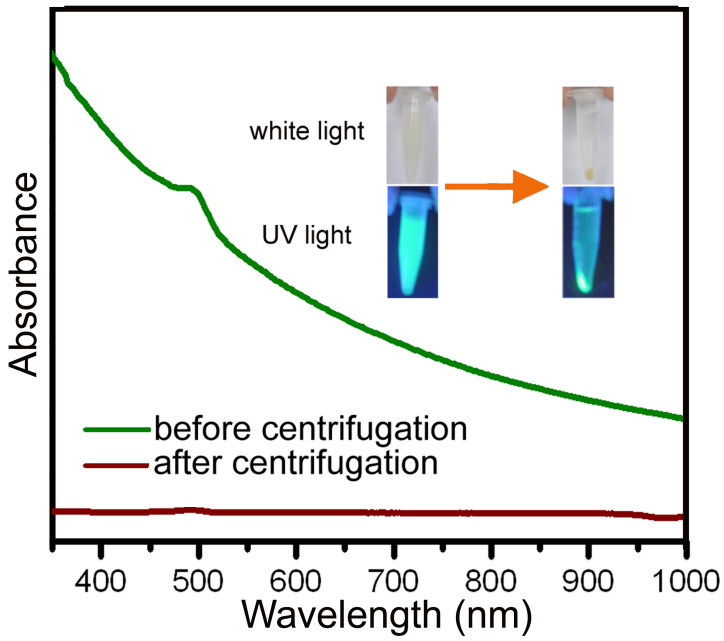

Figure S3. UV-Vis spectra of FITC-labeled SYSNs/NH_2 before and after centrifugation (inset, left: digital photo of FITC-labeled SYSNs/NH_2 dispersed in water; right: the solution after centrifugation under white light and UV light respectively.).

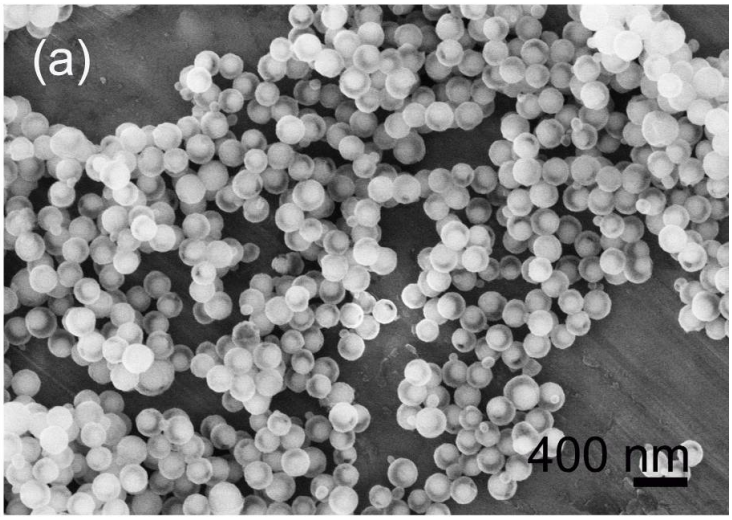

Figure S4. SEM image of as-prepared SYSNs by the Na_2CO_3 solution etching of the silica CSSNs with the diameter of 200 nm.

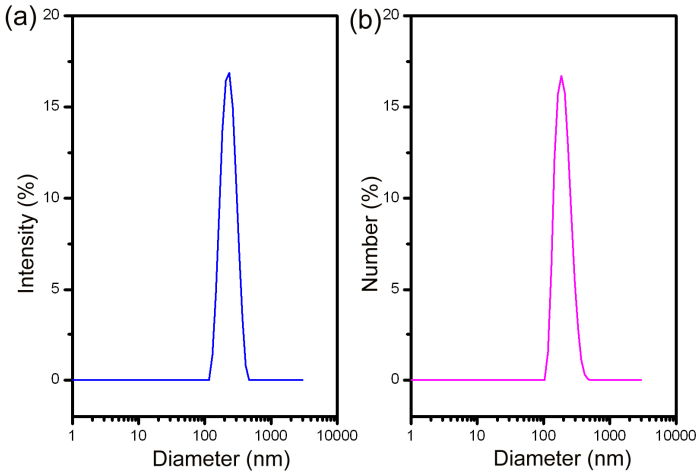

Figure S5. The dynamic light scanning (DLS) diameter of 200 nm SYSNS is determined by intensity (a) and number (b). DLS: 221.1 nm and PDI: 0.051, indicating their uniform size distribution. The dynamic light scanning (DLS) diameter was larger than the TEM and SEM diameters to some varied extents, due to the presence of the hydrated layers around the particle surfaces [4].

Reference:

1 J. Liu, S. Z. Qiao, S. B. Hartono and G. Q.Lu, *Angew. Chem. Int. Ed.*, 2010**, 49**, 4981.

2 J. Y. Lei, L. Z. Wang and J. L. Zhang, *ACS Nano*, 2011**, 5**, 3447.

3 L. L. Li, F. Q.Tang, H. Y. Liu, T. L. Liu, N. J. Hao, D. Chen, X. Teng and J. Q. He, *ACS Nano*, 2010**, 4**, 6874.

4 L. Pan, Q. He, J. Liu, Y. Chen, M. Ma, L. Zhang, J. Shi, *J. Am. Chem. Soc.,* 2012, 134, 5722.