Hollow Silica Nanospheres Coated by Insoluble Calcium Salts for pH-Responsive Sustained Release of Anticancer Drugs

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Experimental

Preparation of monodisperse polystyrene nanospheres (PSNSs)

PSNSs were prepared by emulsion polymerization. For the typical synthesis of PSNSs, sodium dodecyl sulfonate (SDS, 0.05 g), styrene (10 mL), and DD water (28 mL) were added to a three-necked round bottom flask equipped with a condenser and a high purity nitrogen bubbling pipette for deaerating. After the reaction mixture was deaerated at 25℃ for 60 min, potassium persulfate (0.016 g) was added into the mixed solution. Subsequently, the reaction was performed at 80℃ for 8 h under moderate stirring. Finally, the PSNSs emulsion was obtained by centrifugation and washed several times with absolute ethanol.

Synthesis of SiO_2-NH_2 hollow nanospheres

For the synthesis of SiO_2-NH_2 hollow nanospheres, PSNSs emulsion (3.0 g), ammonia solution (0.4 g) and absolute ethanol (15 mL) were mixed completely. Then, tetraethylorthosilicate (TEOS) solution in ethanol was added under moderate stirring.

Subsequently, ethanol dispersion of (3-Aminopropyl) triethoxysilane (APTES) was added dropwise and reacted at room temperature for 12 h under moderate stirring. The precipitates were centrifuged, washed several times, dried at 60℃ for 10 h, and denoted as PS/SiO_2-NH_2. PS/SiO_2-NH_2 were incubated with toluene and ultrasonicated, followed by wash with ethanol for several times to remove the PS template. The obtained products were denoted as SiO_2-NH_2.

Synthesis of SiO_2-COOH hollow nanospheres

SiO_2-NH_2 hollow nanospheres were dispersed in chloroform. Acetic anhydride were added and stirred at room temperature for 24 h. The hollow spheres were centrifuged, washed with ethanol for several times, and dried under vacuum for 10 h at 40℃.

Synthesis of SiO_2-$CaCO_3$ and SiO_2-HAP hollow spheres

SiO_2-COOH hollow nanospheres (0.1 g) were dispersed into DD water and the pH was adjusted to 10.0. Then, aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ was added under stirring and the stirring was continued for another 6 h for the complete binding of Ca^{2+} to SiO_2-COOH. Subsequently, SiO_2-COOH/Ca^{2+} was washed by DD water for several times to remove the weakly absorbed Ca^{2+}. After that, the spheres were redispersed into DD water. For the preparation of SiO_2-$CaCO_3$, the aqueous solution of Na_2CO_3 was added dropwise to the SiO_2-COOH/Ca^{2+} dispersion under moderate stirring. Then the reaction mixture was incubated for 24 h at room temperature. The product was collected by centrifugation, washed, and dried under vacuum at 40℃. The preparation of SiO_2-HAP was performed under the identical conditions to the preparation of SiO_2-$CaCO_3$ except for the replacement of Na_2CO_3 by $(NH_4)_2HPO_4$ with the 5:3 molar ratio between Ca and P.

Characterization

The size and morphology of the samples were characterized by field emission-scanning electron microscopy (FE-SEM, JSM-6390LV, JEOL). High resolution-transmission electron microscopy (HR-TEM) investigations were conducted on a JEOL JEM-2100 transmission electron microscope with the acceleration voltage of 200 kV. The crystal phases of the samples were determined by X-ray diffraction (XRD) using a D8ADVANCE

X-ray diffractometer (Bruker axs Com.) with graphite monochromatized Cu Kα radiation (λ = 0.15406 nm) in the 2θ range of 20-70°. The size distribution analysis of the samples was performed using the log normal function from 100 nanospheres in an arbitrarily chosen area. UV-vis absorption spectra was recorded using a UV-vis spectrophotometer (TU-1900).

Drug loading

In this study, using DOX as drug model, the potential applications of SiO_2-$CaCO_3$ and SiO_2-HAP as DDS were evaluated. SiO_2-$CaCO_3$ or SiO_2-HAP (6.0 mg) were dispersed into DOX aqueous solution (6 mL, 0.1 mg/mL) and shaken in an orbital shaker for 24 h at 30℃ to load the DOX. Then the dispersions were centrifuged, rinsed with DD water for several times until the supernatant changed to colorless. All the supernatants were collected together. The obtained precipitates were dried and denoted as SiO_2-$CaCO_3$/DOX and SiO_2-HAP/DOX. For the determination of the loading efficiency, the amounts of the free DOX in the collected supernatants were quantified by UV-Vis absorbance at 500 nm according to the standard curve. The incorporation efficiency can be expressed as entrapment (%) represented by Equation 1, respectively. The reported data are the mean values of triplicate determinations.

$$Entrapment\ (\%\ w/w) = \frac{mass\ of\ DOX\ in\ SiO_2\text{-}CaCO_3/DOX\ or\ SiO_2\text{-}HAP/DOX}{mass\ of\ DOX\ used\ in\ formulation} \times 100 \quad (1)$$

Evaluation of the *in vitro* drug release performance

To evaluate the *in vitro* drug release performance of the DOX from SiO_2-$CaCO_3$/DOX and SiO_2-HAP/DOX, SiO_2-$CaCO_3$/DOX or SiO_2-HAP/DOX (6.0 mg) were dispersed into PBS buffer solutions (6 mL, pH = 4.0, 5.0, 6.0, 7.4) and shaken at 30℃. At different predetermined intervals, the suspension was centrifuged and the supernatant (3 mL) was taken out and replaced by fresh PBS solution (3 mL) to continue the drug release evaluation. The DOX concentration in the supernatant was determined by the absorbance at 500 nm. The drug release efficiency was calculated by Equation 2. The data were reported as mean ± standard deviation (SD) based on the measurements of the triplicate samples.

$$DOX\ release\ (\%\ w/w) = \frac{mass\ of\ DOX\ in\ supernatant \times 100}{mass\ of\ DOX\ used\ in\ formulation} \quad (2)$$

Cell culture

In this study, Hep G2 human hepatocellular carcinoma cells and V79-4 Chinese hamster lung cells were used as model cancer cell and normal cell, respectively. The cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS, Penicillin (100 units/mL), Streptomycin (100 μg/mL), amphotericin B (0.25 μg/mL) and sodium bicarbonate (3.7 mg/mL) in a humidified incubator at fully humidified atmosphere at 37°C , 5% CO_2 and 95% room air.

Cytotoxic effects evaluation

Culture medium (100 μL) containing Hep G2 or V79-4 cells with initial density of 2.5 × 10^4 cells/mL were seeded separately in the wells of sterile 96-well flat bottom culture microplates and incubated for 24 h. Subsequently, 100 μL of pure DOX, SiO_2-$CaCO_3$/DOX and SiO_2-HAP/DOX with the DOX concentrations of 0.0488, 0.0977, 0.1953, 0.3906, 0.7813 and 1.5625 μg/mL in culture medium were added into the wells and incubated for 72 h. The treatment of cells with culture medium rather than samples was prepared as the control. The cytotoxic effects of the pure DOX, SiO_2-$CaCO_3$/DOX and SiO_2-HAP/DOX on Hep G2 and V79-4 cells were determined by MTT colorimetric assay. Briefly, after 72 h treatment, freshly prepared MTT (20 μL, 5 mg/mL in PBS) was added to each well and incubated at 37°C under 5% CO_2 for 4 h. Then, the supernatant was discarded and DMSO (150 μL) were added to each well to dissolve the dark blue crystals completely. The absorbance of the solution in each well at the wavelength 570 nm was determined by a microplate reader. The cytotoxic effects of the pure DOX, SiO_2-$CaCO_3$/DOX and SiO_2-HAP/DOX were calculated by Equation 3. The data were reported as mean ± standard deviation (SD) based on the measurements of the triplicate samples.

$$Percentage\ of\ inhibition\ (\%) = \left(1 - \frac{O.D_{570nm}\ of\ treatment\ group}{O.D_{570nm}\ of\ control\ group}\right) \times 100 \quad (3)$$

Results and discussion

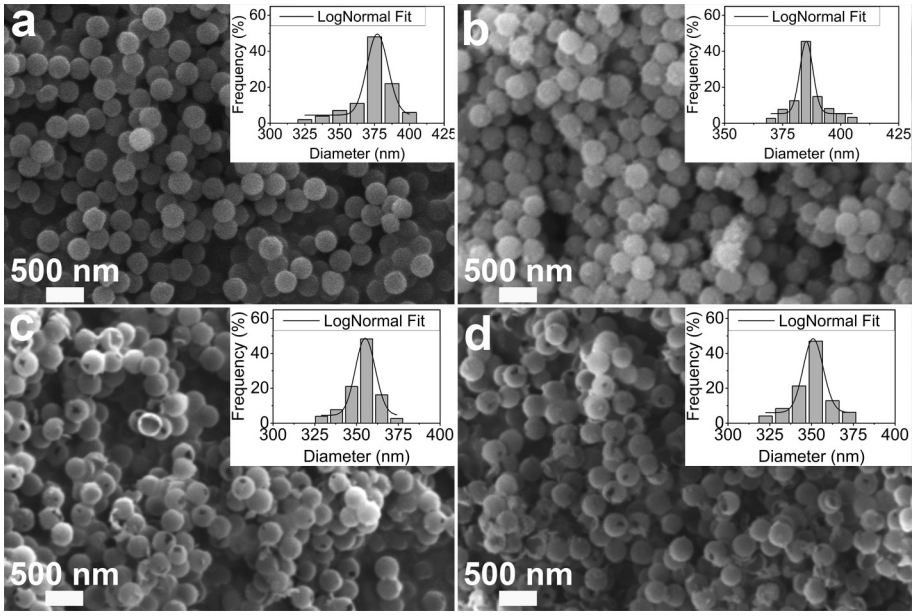

Figure S1. FE-SEM images of (a) PSNSs, (b) PS/SiO_2-NH_2, (c) SiO_2-NH_2, and (d) SiO_2-COOH. Inset: size distribution analysis results.

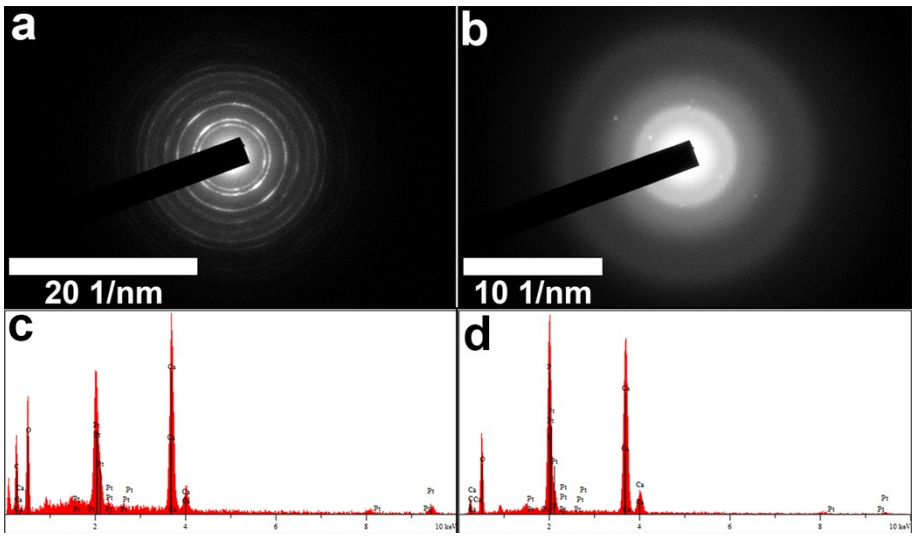

Figure S2. SAED patterns of NaOH treated (a) SiO_2-$CaCO_3$ and (b) SiO_2-HAP. EDX spectra of NaOH treated (c) SiO_2-$CaCO_3$ and (d) SiO_2-HAP.

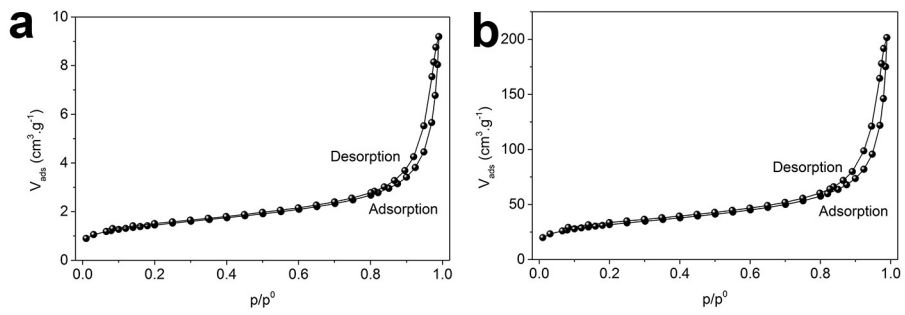

Figure S3. Nitrogen adsorption/desorption isotherms of (a) SiO_2-$CaCO_3$ and (b) SiO_2-HAP hollow spheres.

Through multipoint BET analysis, the specific surface area of the SiO_2-$CaCO_3$ and SiO_2-HAP were determined as 109.18 and 107.21 m^2/g, respectively (Figure S3). The hollow structure of the SiO_2-$CaCO_3$ and SiO_2-HAP might increase the specific surface areas of the samples, higher than that of the solid counterpart. The increase of the specific surface areas might contribute to the effective loading of the anticancer drug.

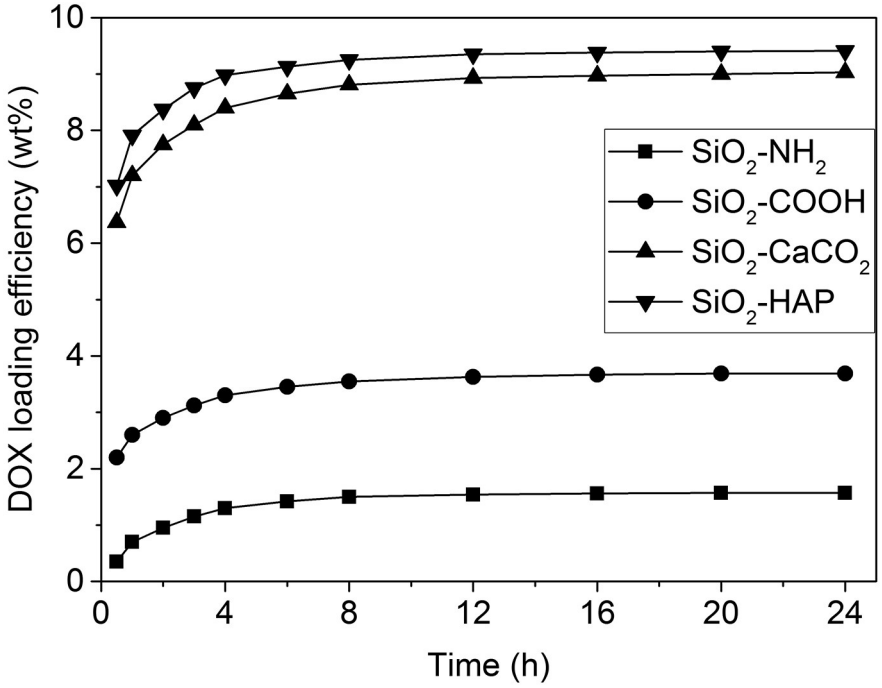

Figure S4. Loading kinetics of DOX into SiO_2-NH_2, SiO_2-COOH, SiO_2-$CaCO_3$, and SiO_2-HAP (6 mg sample added into 6 mL DOX solution with a concentration of 0.1 mg/mL).

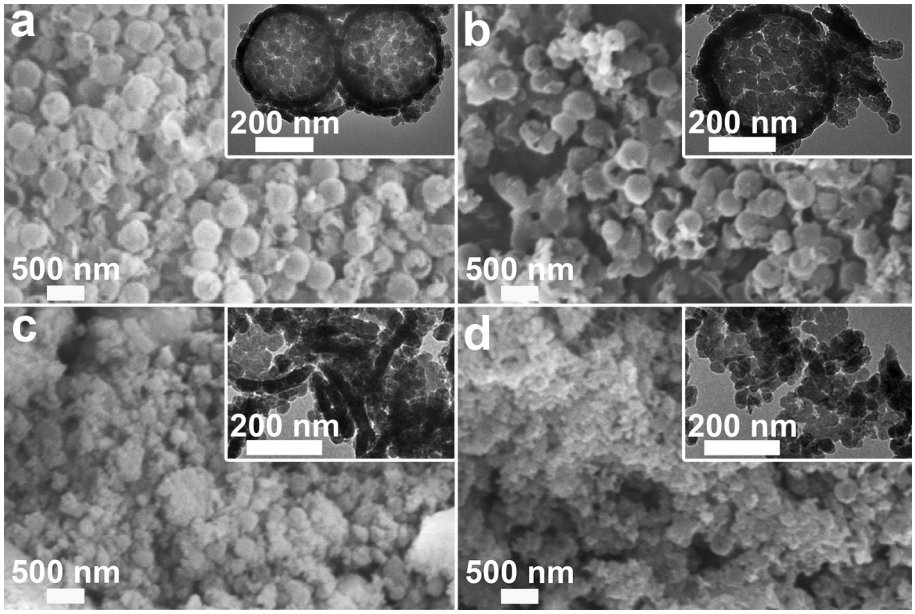

Figure S5. FE-SEM images of SiO_2-$CaCO_3$ incubated in release buffers with pH (a) 7.4, (b) 6.0, (c) 5.0, and (d) 4.0 for 3 days, respectively. Inset: HR-TEM images.

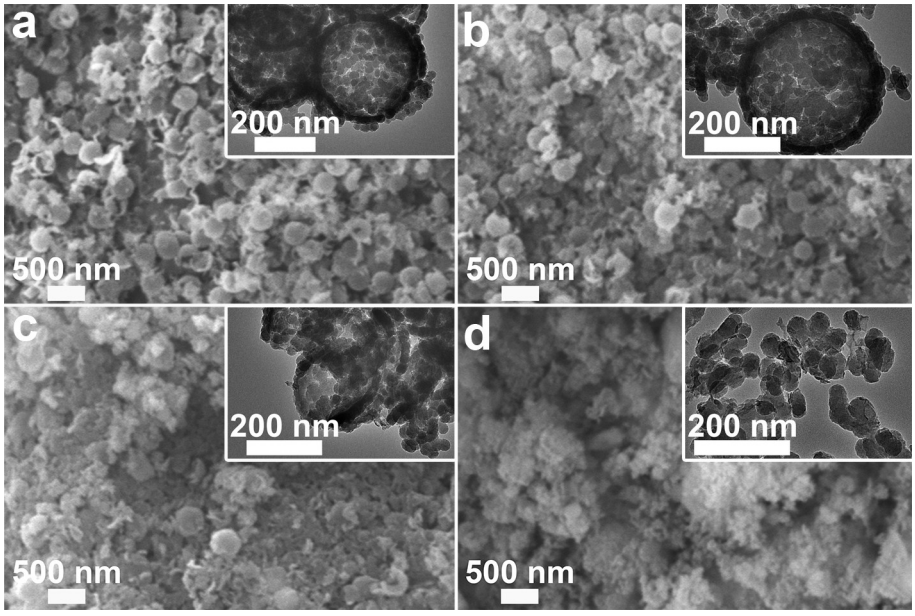

Figure S6. FE-SEM images of SiO_2-HAP incubated in release buffers with pH (a) 7.4, (b) 6.0, (c) 5.0, and (d) 4.0 for 3 days, respectively. Inset: HR-TEM images.

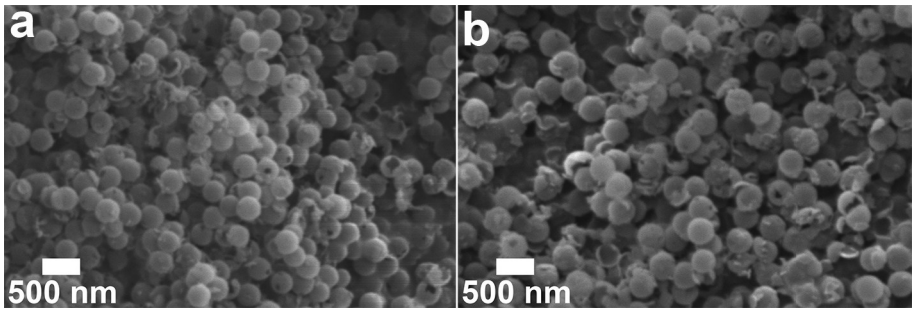

Figure S7. FE-SEM images of (a) SiO_2-$CaCO_3$ and (b) SiO_2-HAP incubated in DMEM medium for 5 d.

The stability of SiO_2-$CaCO_3$ and SiO_2-HAP in cell culture medium were evaluated by FE-SEM observation after incubated in DMEM medium for 5d. From the results shown in Figure S6, after incubated in DMEM medium, SiO_2-$CaCO_3$ and SiO_2-HAP are still well-dispersed spheres, indicating their good stability in culture medium.