Supporting Information

Polymer/silica hybrid hollow nanospheres with pH-sensitive drug release in physiological and intracellular environments

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Experimental Section

Chitosan (CS, Nantong Shuanglin Biological Product Inc.) was refined by dissolving it in dilute acetic acid solution, filtered, precipitated with aqueous NaOH, and finally dried in vacuum at room temperature. The weight average molecular weight of CS was 200 kDa and the degree of deacetylation was about 90%. Potassium persulfate ($K_2S_2O_8$) was recrystallized from distilled water. Acrylic acid (AA, Shanghai Guanghua Chemical Company) was distilled with reduced pressure under nitrogen atmosphere. Tetraethoxyorthosilicate (TEOS) was supplied by Shanghai Chemical Reagent Factory. Doxorubicin hydrochloride was obtained as a gift from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China) in powder. All the other reagents were of analytical grade and used without further purification.

Preparation of CS-PAA nanoparticles was conducted according to our previous work.[1] 0.25 g of purified CS was dissolved in an aqueous acrylic acid solution (25 mL; 0.12 g acrylic acid). Polymerization was then initiated by 0.05 g $K_2S_2O_8$ at 80 ^oC. When the system appeared opalescent, the reaction was continued for another 2 hrs at room temperature. In succession, 0.1 mL glutaraldehyde (GA) was added to the mixture to crosslink CS selectively. The obtained suspension was filtrated and diluted by 2 M ammonia to pH = 10.0. 100 μL of TEOS was mixed with 1 mL ethanol. Then, 1 mL suspension was dropped into TEOS solution, stirred for 24 hrs, and after that, it was kept in oven at 50 ^oC for another 24 hrs. The product was obtained by centrifugation with 5,000 rpm for 10 min, and washed with distilled water for three times. Then these samples were lyophilized to get dry powder.

15 mg of CS-Silica Nps was re-dispersed in 3 mL water, and 1.5 mg doxorubicin was added into it. The mixture was incubated for 48 hrs to fabricate doxorubicin-loaded CS-Silica nanospheres.

2 mL of doxorubicin-loaded CS-Silila nanospheres mentioned above was centrifuged at 10 000 rpm for 20 min and the deposit was washed with distilled water for three times. Then the deposit was re-dispersed in 1 mL phosphate buffer solution (PBS) with different pH values, placed into a dialysis membrane bag (molecular weight cutoff 12000 kDa), and immersed into 5 mL PBS at 37 ^{o}C. At periodic interval, the release media was withdrawn and another 5 mL fresh PBS solution was added. The amount of doxorubicin was determined by measuring the fluorescence at 590 nm (Shimadzu, RF-5301PC, Japan) and using a calibration curve.

LoVo cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT), streptomycin at 100 μg/mL, penicillin at 100 U/mL, and 4 mM L-glutamine at 37 □ in a humidified 5% CO_2-containing atmosphere.

Cytotoxicities of empty CS-Silica Nps, DOX loaded CS-Silica Nps and free DOX were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at pH 7.4, 37 ^{o}C. Cells were seeded at 8×10^{4} cells/mL in 96-well plate. Medium was changed every other day until 80% confluence was reached. Then, medium was changed with 100 μL medium containing empty CS-Silica Nps, DOX loaded CS-Silica Nps and free DOX with different concentrations. One row of the 96-well plates was used as control. After 4 hrs incubation, the medium in each well was removed and the wells were washed three times using PBS. Ten microliters of MTT assay and 90 μL of fresh medium were then added to the wells. After incubation for around 4 hrs, solution was removed, leaving the precipitate. One hundred microliters of inopropanol or DMSO was then added to the wells before the plate was observed using microplate reader (GENios).

For the fluorescence experiment, Free DOX or DOX loaded CS-Silica nanoparticles were incubated with LoVo cells for 4 hrs. Then these cells were washed with cold (4 ^{o}C) and warm (37

^{o}C) PBS three times respectively. After that, these cells were observed with Nikon fluorescent microscopic system C1 under the green channel.

The microstructures of the CS-Silica Nps were observed by Transmission electron microscopy (JEOL TEM-100). FT-IR spectra were measured on a Bruke IFS 66V vacuum-type spectrometer. Nitrogen adsorption measurements were performed at 77 K on the cleaned powder after degassing at 140 ^{o}C under vacuum for 3 hrs using an accelerated surface area and a porosimetry analyzer (Micromeritics, Shimadzu). We used Brunauer-Emmett-Teller (BET) calculations for the surface area and Barret-Joyner-Halenda (BJH) calculations for the pore-size distribution of dry-form powders from the desorption branch of the isotherm.

1 Y. Hu, X. Q. Jiang, Y. Ding, Q. Chen and C. Z. Yang, *Adv. Mater.*, 2004, **16**, 933.

FT-IR measurement

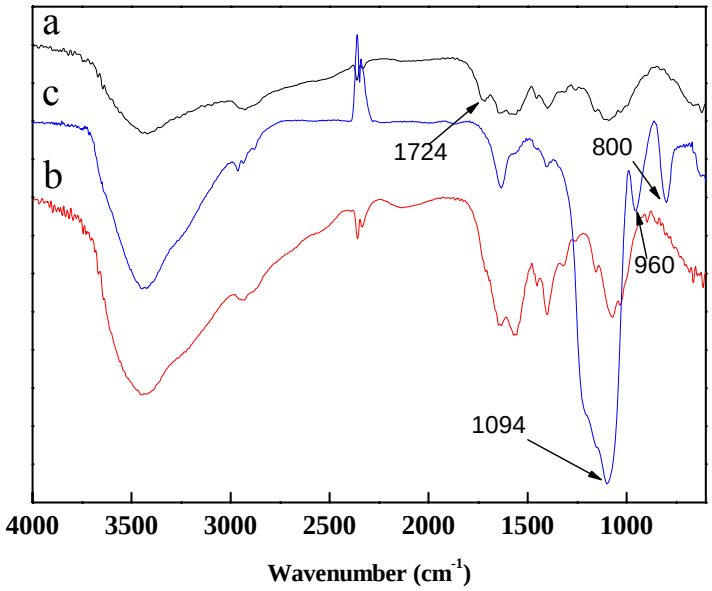

Figure S1: the FT-IR spectra of CS-PAA nanoparticles (a), CS-PAA nanoparticles treated by ammonia (b) and CS-Silica Nps (c).

Figure S1(a) represents the typical FT-IR spectrum of CS-PAA nanospheres. The carboxyl group peaks are clearly observed at 1724 cm^{-1} and 1100 cm^{-1}. After treated with ammonia, the intensity of carboxyl group peaks become very weak and almost disappear (Figure S1 (b)). Figure

S1(c) shows the FT-IR spectrum of CS-Silica Nps. The peaks in 1094 cm^{-1} and 800 cm^{-1} are attributed to ν_{as}(Si-O-Si) and ν_s(Si-O-Si) respectively, and the peak at 960 cm^{-1} is due to ν(Si-OH/Si-O^-). Additionally, carboxyl group peak existed in CS-PAA nanoparticles (1724 cm^{-1}) (Figure S1(a)) was not observed in the CS-Silica Nps, which indicated that the PAA molecules were released out from the CS-Silica Nps during the preparation procedure.

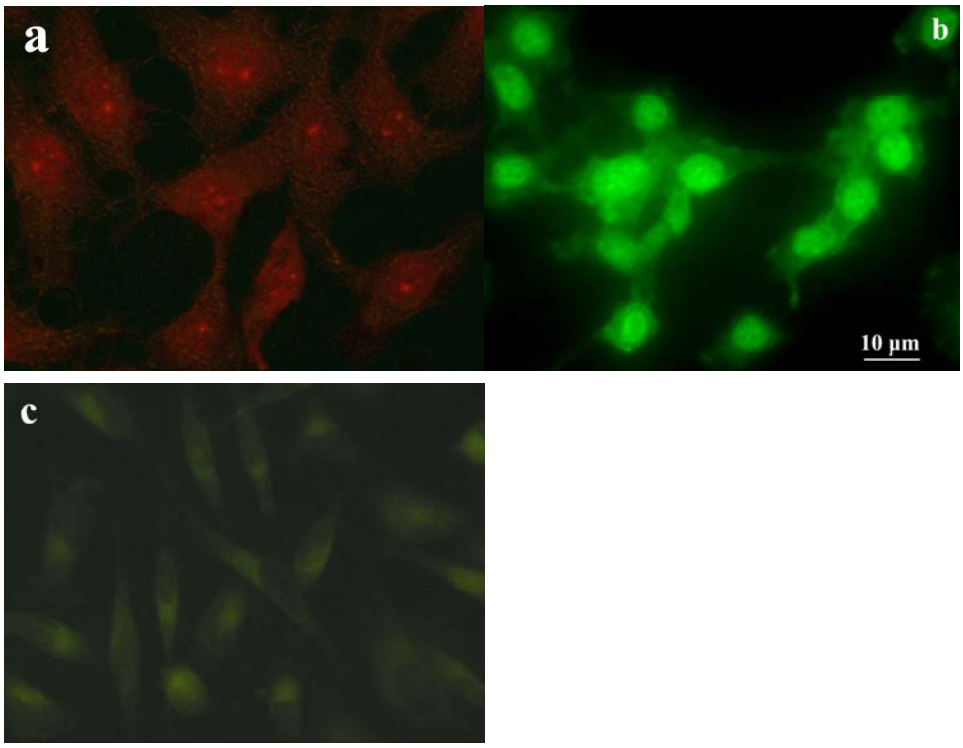

Figure S2 (a) fluorescence image of DOX loaded CS-Silica nanospheres incubated with LoVo cells for 4 hrs observed in red channel; (b) fluorescence image of DOX loaded CS-Silica nanospheres incubated with LoVo cells for 4 hrs observed in green channel; (c) fluorescence image of free DOX incubated with LoVo cells for 4 hrs observed in green channel.

Most of the red spots, which are attributed to DOX loaded CS-Silica nanospheres, are inside the centre of the cells, maybe the nucleus (Figure S1(a)). Figure S2 (c) shows the fluorescence image of free DOX incubated with LoVo cells for 4 hrs. As it can be seen that, even some of the free DOX is inside the cells, autofluorescence of cells is very weak, compared to the DOX loaded CS-Silica nanospheres (figure S2(b)) when they are co-incubated with LoVo cells for the same time.