An organosilane route to mesoporous silica nanoparticles with tunable particle and pore sizes and their anticancer drug delivery behavior

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Electronic Supplementary Information

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Experimental section

Preparation of the size/pore-controllable mesoporous silica spheres

The typical preparation of MSNs is as follows. The silica precursors, C_{18}TMS and TEOS, were firstly mingled at the molar ratio of 1:4.7 and dropped slowly into the starting reaction solution containing H_2O, ethanol, and ammonia under vigorous stirring, followed by reaction for 4 hours at ambient temperature (25 ^oC). The resultant C_{18}TMS-incorporated MSNs were centrifuged, washed by ethanol, dried, and calcined to remove organic groups at 550 ^oC for 6 hours.

Tab.S1. Reaction molar ratios, the mesoporous structural parameters and particle sizes of MSNs.

Sample	Reaction molar ratios H_2O: EtOH: NH_3 :Si[a]	S_{BET} [m^2/g]	V_{BJH} [cm^3/g]	D_{BJH} [nm]	Particle size (TEM) [nm]
A1	1: 3.86: 0.34: 0.0041	593	0.63	2.3	90 ± 10
A2	1: 2.29: 0.34: 0.0041	570	0.59	3.2	120 ± 20
A3	1: 1.78: 0.34: 0.0041	505	0.49	4.0	300 ± 20
A4[b]	1: 1.24: 0.34: 0.0041	532	0.52	4.6	230 ± 20
A5	1: 0.77: 0.34: 0.0041	322	0.39	4.0	150 ± 20
B1	1: 1.24: 0.34: 0.00125	581	0.66	4.3	80 ± 10
B2	1: 1.24: 0.34: 0.0025	552	0.54	4.4	150 ± 15
B3[b]	1: 1.24: 0.34: 0.0041	532	0.52	4.6	230 ± 20
B4	1: 1.24: 0.34: 0.0056	551	0.55	4.5	310 ± 30

[a] Molar quantity of TEOS and C_{18}TMS mixture and the TEOS/C_{18}TMS kept at 4.7.

[b] A4 and B3 are the same sample.

Characterization

TEM (Field Emission Transmission Electron Microscopy) analysis was conducted on JEM 2100F electron microscope operated at 200 KV. SEM (Scanning

Electron Microscopy) analysis was conducted on JEOL JSM6700F electron microscope. The confocal images were obtained on Olympus FV1000-IX81 confocal microscopy. The UV-vis absorbance spectra were obtained on Shimadzu UV-2550. Nitrogen adsorption-desorption and pore size distributions were obtained at 77 K on Micromeritics Tristar 3000 analyzer after the samples being pre-treated at 120 ^{o}C for dehydration. The surface areas and pore volumes were calculated by the Brunauer-Emmett-Teller (BET).

1. Anticancer drug of DOX loading and release experiment

The resultant MSNs (50 mg) were suspended and stirred in DOX aqueous solution (1mg/ml) for 24 h at room temperature in dark place. The resulting suspension was centrifuged at 10000 rpm for 20 min and dried at 218 K under vacuum over night. The effective DOX storage capacities of the obtained sample were determined by measuring the difference in absorption band of DOX (490 nm) in the supernatant before and after the loading by UV-vis absorbance spectrometry. DOX-loaded MSNs (10 mg) were suspended in 4 mL of Phosphate Buffered Saline (PBS). The suspension was placed into dialysis bags with a molecular weight cut-off of 3500 Da which were pretreated to remove heavy metal and sulfur contaminants, and subsequently placed in a beaker containing 35 mL PBS. The dissolution media was constantly stirred (~100 rpm) at 37 ^{o}C and the release process was determined by taking an aliquot of the dissolution at different times. The UV-Vis absorbance of the DOX in the dissolution was measured to give information on the quantity of released DOX.

2. TEM observations of MCF-7 cells incubated with MSNs

The MCF-7 cells were incubated with 200 μg/mL of MSNs for 24 h. Then cells were immersed with media and incubated in 5% CO_2 at 37 ^{o}C for 5 min. Then the cells were scraped off and centrifuged for 10 min at 2500 r/min to remove incubation medium. After that, the MFC-7 cells were fixed by glutaraldehyde at 0 ^{o}C for 2 h. The

sample was rinsed with PBS and dehydrated through a graded ethanol series, then cleared with propylene oxide. Finally, the cell sample was embedded in EPOM812 and polymerized in the oven at 37 ^{o}C for 12 h, 45 ^{o}C for 12 h and 60 ^{o}C for 48 h. Ultrathin sections of approximately 50 nm thick were cut with a diamond knife on a Leica UC6 ultramicrotome and transferred to the copper grid. The images were viewed on JEM-1400 electron microscopy.

3. In vitro biocompatibility of MSNs with L929 (mouse connective tissue fibroblast cells)

For the biocompatibility studies of parent MSNs with L929 cells, cells were seeded in a 96-well plate at a density of 10^4 cells per well and cultured in 5% CO_2 at 37 ^{o}C for 24 h. Then, parent MSNs were added to the media, and the cells were incubated in 5% CO_2 at 37 ^{o}C for 24h and 72h. The concentrations of parent MSNs were 3, 6, 15, 60 and 120 μg/mL, respectively. Cellviability was determined by the standard 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

4. In vitro cytotoxicity against MCF-7 cells

For the cytotoxicity analysis of free DOX, parent MSNs and DOX-loaded MSNs against MCF-7 cells, cells were seeded in a 96-well plate at a density of 10^4 cells per well and cultured in 5% CO_2 at 37 ^{o}C for 24 h. Then, free DOX dispersed in DMSO, parent MSNs and DOX-loaded MSNs were added to the media, and the cells were incubated in 5% CO_2 at 37 ^{o}C for 24 h and 72h. The concentrations of DOX were 0.5, 1.0, 2.5, 10 and 20 μg/mL, respectively. The concentrations of parent MSNs were 5, 10, 20, 40, 80 and 160μg/mL, respectively. Cell viability was determined by MTT assay. The statistical analysis of experimental data utilized the Student's t-test. A p-value of less than 0.05 was considered statistically significant. Each data point is represented as mean ± standard deviation (SD) of eight independent experiments (n = 8, n indicates the number of wells in a plate for each experimental condition).

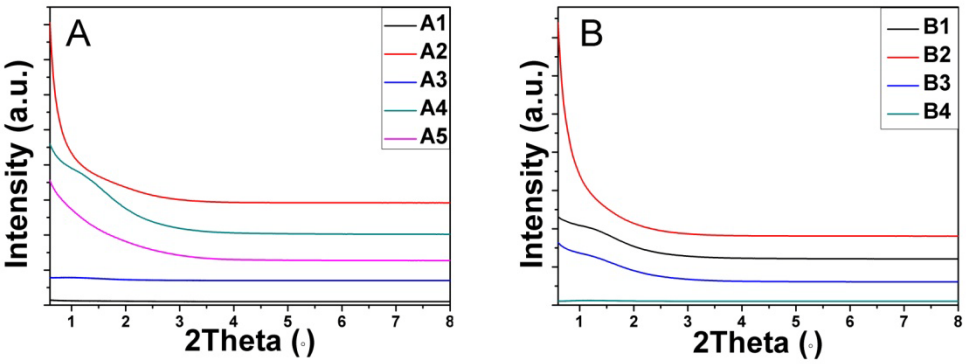

Fig. S1. Low-angle XRD patterns of all the MSNs samples.

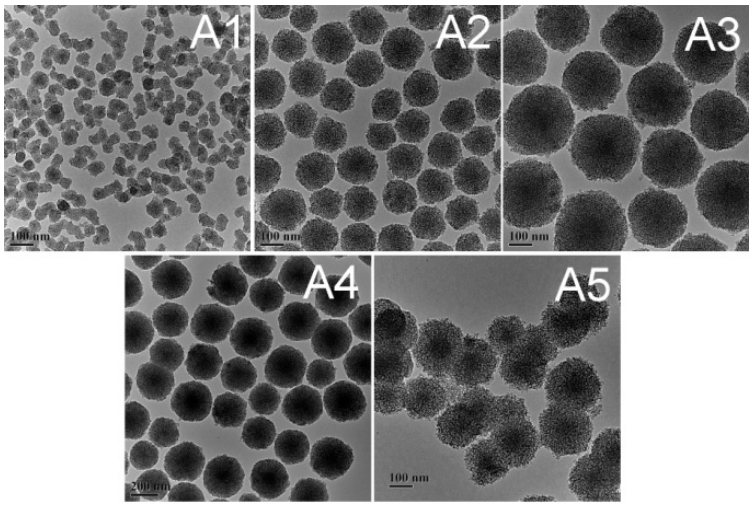

Fig. S2. TEM images of MSNs synthesized atdifferent H_2O/EtOH molar ratios.

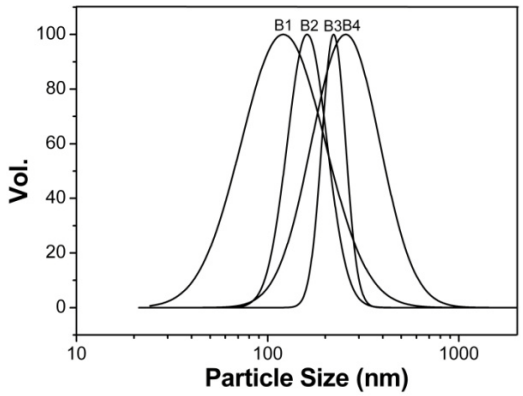

Fig. S3 The dynamic light scattering pattern of MSNs fabricated by adding 200 (B1), 400 (B2), 650 (B3), 900 (B4) μl of TEOS/C18TMS mixture to 48.2 ml of starting solution, respectively, while other reaction conditions were kept constant.

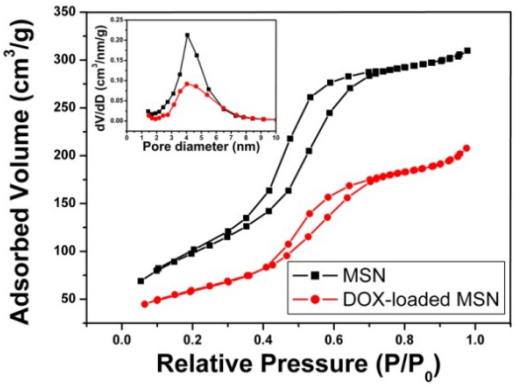

Fig. S4. Nitrogen adsorption-desorption isotherm and pore distributions of MSN and DOX-loaded MSN

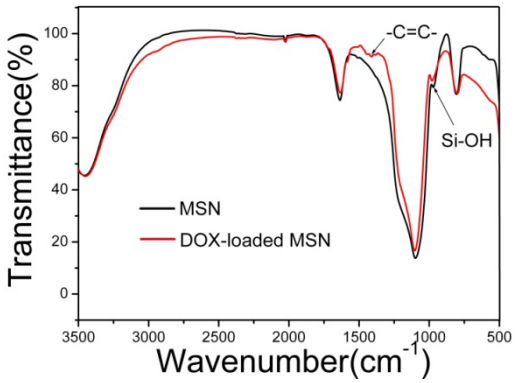

Fig. S5. The FTIR spectra of MSN before and after the DOX loading.

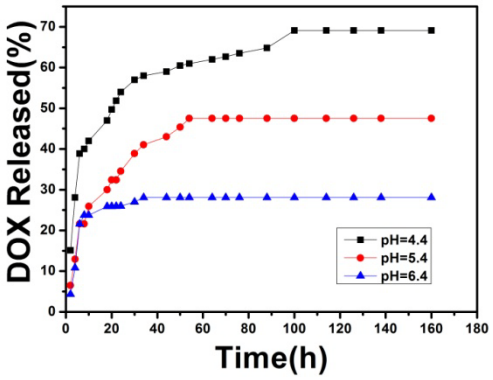

Fig. S6 The DOX release behaviors in vitro from the DOX-loaded MSNs system over a 160 hour period in PBS solutions with pH 4.4, 5.4, and 6.4.

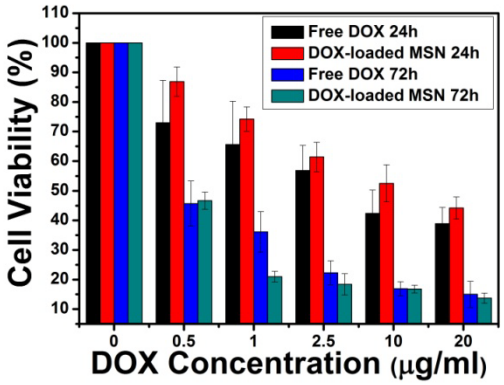

Fig. S7. MCF-7 cell viability incubated with free DOX and DOX-loaded MSNs at different concentrations for 24 hours and 72 hours, respectively.

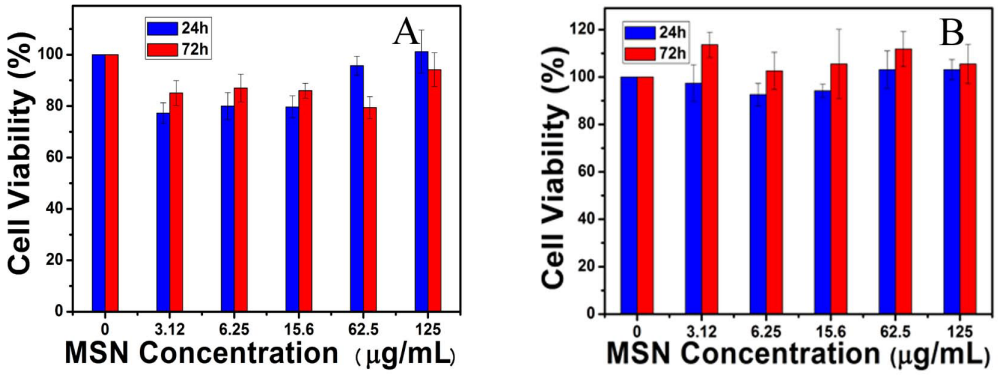

Fig. S8. MCF-7 cell (A) and L929 cell (B) viability incubated with MSNs for 24 h and 72 h, respectively.