Supplementary Information for

Dual-Responsive Drug Release from Oligonucleotide-Capped Mesoporous Silica Nanoparticles

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Experimental details

Materials

Absolute ethanol (EtOH, >99.9%), 2-aminoethythiol hydrochloride, cetyltrimethylammonium bromide (CTAB, 90%), 4',6-diamidino-2-phenylindole (DAPI), diethyl ether, dimethylformamide (DMF, 99%), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dithiothreitol (DTT), doxorubicin (Dox), dulbecco's modified eagle's medium (DMEM), 5' end amine modified 15-mer ssDNA, 33-mer ssDNA，5' end/cyanine (Cy5) modified 33-mer complementary ssDNA (cDNA/Cy5), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), ethylenediaminetetraacetic acid (EDTA), fetal bovine serum (FBS), fluoresceinisothiocyanate (FITC), formaldehyde, hydrochloric acid (HCl, assay 37%), *N*-hydroxysuccinimide (NHS), tetraethylorthosilicate (TEOS, 99%), 3-mercaptopyltrimethoxysilane (MPTMS), methanol (MeOH, 99.5%), 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, phosphate buffered saline (PBS) buffer, sodium hydroxide (NaOH), succinic anhydride, thiopyridyl disulfide, triethylamine (TEA), tris-ammonium acetate-EDTA buffer (TAE), tris-EDTA (TE) buffer (pH=8.0), and trypsin.

Oligonucleotide sequences are indicated as: 15-mer ssDNA: 5'-TTA TCG CTG ATT CAA-3'; 33-mer complementary ssDNA: 5'-TTG AAT CAG CGA TAA TCG AAT AGC CAC TAA GTT-3'.

All above-mentioned materials were purchased commercially.

Instruments

Transmission electron microscopy (TEM) images were taken by JEOL 2010 TEM at 200kV. Specific surface area and pore size distribution of MSNP-SH were measured by ASAP-2020 Mircomeritics. X-Ray diffraction (XRD) pattern was collected by X'Pert powder XRD. Zeta potential value was measured by Mavern Nanosizer. FT-IR spectra were recorded by Fourier transformed infrared spectrometer. The BET isotherms were measured on an autosorp-IQ instrument from Quantachrome Instruments Corporation. Nanodrop 1.0 was used to determine the ssDNA concentration. UV-vis spectra were recorded by UV-vis 2501 Spectrometer. Agarose gel electrophoresis was performed by ENDRO GEL XL E0160 electrophoresis system. A microplate reader (infinite 200 PRO, Tecan) was employed for the MTT assay. Confocal microscopy images were taken by a confocal microscope (Leica TCS SP5, 40× oil objective). Flow cytometry data were collected by BD FACSCalibur Flow Cytometer.

Synthesis of MSNPs

In a typical synthesis, CTAB (500 mg) was dissolved in de-ionized (DI) water (240 mL) containing NaOH (2.00 M, 1.75 mL). The mixture was heated to 80°C under vigorous stirring, and then TEOS (2.5 mL) was added slowly to the solution. The mixture solution was kept stirring at 80°C for 2 h. The synthesized MSNPs were collected by centrifugation at 8000 rpm for 10 min and washed with methanol and DI water. Then as-synthesized MSNP product was dried in vacuum at 50°C. For the FITC-labelled MNSPs, FITC (1.3 mg) was dissolved in absolute ethanol (1.5 mL) containing APTES (3μL) and the mixture solution was mildly stirred for 2 h in the dark before adding with TEOS (2.5mL).

Synthesis of MSNP-SH

The as-synthesized MSNPs (500mg) were suspended in methanol (50 mL) containing MPTMS (0.5 mL). The mixture was refluxed at 80°C under nitrogen atmosphere for 24 h. The thiol (-SH) group grafted MSNPs were collected by centrifugation at 8000 rpm for 10 min and washed with methanol and DI water. Then, the collected nanoparticles were suspended in methanol (100 mL) containing condensed HCl (37%, 6 mL). After the mixture was refluxed at 80°C under nitrogen atmosphere for 24 h, the surfactant removed MSNP-SH was collected by centrifugation at 8000 rpm for 10 min and washed with methanol and DI water.

Synthesis of *S*-(2-aminoethylthio)-2-thiopyridine hydrochloride

Thiopyridyl disulfide (4.41 g) was first dissolved in a mixture solution of methanol (20 mL) and acetic acid (0.8 mL). 2-Aminoethylthiolhydrochloride (1.14 g) in methanol (10 mL) was added dropwise into the above solution within 30 min. The reaction mixture was stirred for another 48 h and then evaporated under high vacuum to yellow oil. The product was washed with diethyl ester (50 mL) and subsequently dissolved in methanol (10 mL). The product was precipitated by the addition of diethyl ether (200 mL), chilled at -20°C for 24 h and collected by vacuum filtration. The product was dissolved again in methanol (10 mL) with the repetition of the previous steps to obtain the product.

Synthesis of MSNP-SS-NH_2

MSNP-SH (200 mg) was first suspended in methanol (30 mL) and *S*-(2-aminoethylthio)-2-thiopyridine hydrochloride (200 mg) was added into the solution. The mixture was stirred at room temperature for 24 h. MSNP-SS-NH_2 was collected by centrifugation at 8000 rpm for 10 min and washed thoroughly with methanol and DI water. The product was dried in vacuum at 50°C for 24 h.

Synthesis of MSNP-SS-COOH

MSNP-SS-NH_2 (100 mg) was suspended in DMF solution (15mL) containing succinic anhydride (2.00 g) and TEA (2.5mL). The solution was kept at room temperature with

continuous stirring for 24 h. The product MSNP-SS-COOH was collected by centrifugation at 8000 rpm for 10 min and washed thoroughly with methanol and DI water.

Synthesis of MSNP-SS-ssDNA

MSNP-SS-COOH (1 mg) was first activated using EDC (1.00 mL, 2 mg mL^{-1}) and NHS (1.00 mL, 2 mg mL^{-1}) in MES buffer (pH 6.0) at room temperature with continuous stirring for 15 min. Then, NH_2-modified 15-mer ssDNA (100 μg) was added at room temperature with mild stirring for 18 h. MSNP-SS-ssDNA was collected by centrifugation at 13000 rpm for 5 min and subsequently washed with PBS buffer (pH 7.4).

Drug loading and complementary ssDNA capping

MSNP-SS-ssDNA was first suspended in a saturated FITC aqueous solution (TE buffer, pH=8.0) at room temperature under continuous stirring for 24 h. Then, complementary 33-mer complementary ssDNA (10μg) was added. The mixture was continuously stirred at room temperature for 1 h to allow the hybridization take place. FITC-loaded MSNP-SS-ssDNA with 33-mer ssDNA capping, noted as FITC-loaded MSNP-SS-dsDNA, was collected by centrifugation and washed with TE buffer. For Dox loading, Dox aqueous solution (1mg mL^{-1}) was used.

Agarose gel electrophoresis

Agarose gel was prepared using agarose gel powder (1.5 wt%) in TAE buffer. Free ssDNA was added in the first well as control. Three different samples of MSNP-SS-dsDNA (20 μL, 1mg mL^{-1}) were added in the following wells: the second well containing no DTT, the third well containing 20 mM of DTT, and the third sample with 10 mM DTT. Appropriate amount of DI water was added to each well, filling up to a fixed volume (25.0 μL) to ensure equal concentration of DNA. All samples were incubated at room temperature for 3 h before the gel electrophoresis was carried out at 100V in TAE buffer for 15 min.

ssDNA hybridization and dehybridization test

MSNP-SS-ssDNA was first suspended in TE buffer and subsequently complementary Cy5

modified 33-mer complementary ssDNA (10 μg) was added. The mixture was mildly stirred at room temperature for 1 h to allow the hybridization take place. Formed MSNP-SS-dsDNA/Cy5 was collected by centrifugation at 13000 rpm for 5 min and subsequently washed with TE buffer. Fluorescence measurements of the MSNP-SS-dsDNA/Cy5 solution before and after heating at 62°C for 15 min were conducted. Excitation wavelength at 620 nm was used for Cy5 detection, and the emission wavelength of 661 nm was recorded.

Controlled drug release test

FITC or Dox-loaded MSNP-SS-dsDNA (0.5mg) was suspended in PBS buffer (1mL) with or without the addition of DTT. The sample was centrifuged at 13000 rpm for 3 min at each interval. The fluorescence intensity of released FITC (or Dox) in the supernatant at each time interval was recorded. The FITC (or Dox) release profile by thermal stimuli was obtained by conducting fluorescence measurements on the supernatant at certain time intervals with temperature maintained at 45°C. Excitation wavelength at 488 nm was used for the FITC detection, and the emission intensity at 518 nm was recorded. For Dox release, the fluorescence was excited at 488nm, and 580nm of emission was recorded.

Confocal laser scanning microscopy (CLSM) images

Intracellular uptake of MSNP-SS-dsDNA and Dox release was investigated with HeLa cells *in vitro*. HeLa cells were seeded in 6-well plate at a density of 2×10^5 cells/well and grown on a cover slide in the DMEM medium for 24h. Then, the cells were exposed to Dox-loaded MSNP(FITC)-SS-dsDNA (20μg mL^{-1}) for 24h. The medium was then removed and cells were washed three times with PBS. The cells were fixed with 4.0% formaldehyde and then stained with DAPI (30 nM). The cells were further washed with PBS for three times before observation with confocal microscopy. The fluorescence of DAPI was excited by 405nm laser. The fluorescence of FITC and Dox were both excited by 488nm laser. The fluorescence of DAPI, FITC, and Dox was collected through 480±10nm, 520±10nm, and 580±10nm channels, respectively.

MTT cytotoxicity assay

HeLa cells were seeded into 96-well plate at a density of 1×10^4 cells/well in DMEM medium. After 24h incubation, the medium was replaced with new medium that contains MSNP-SS-dsDNA or Dox-loaded MSNP-SS-dsDNA at various concentrations. After required incubation time and treatment, the MTT assay was performed. For the MTT assay, the old medium was removed and new medium (100 μL) containing MTT (0.5mg mL^{-1}) was added. After 4h incubation, the medium was replaced with DMSO (100μL). The plate was gently shaked for 15min before measuring the absorbance at 565 nm using a microplate reader (infinite 200 PRO, Tecan). The cell viability related to the control wells that only contain cell culture medium was calculated by $[A]_{test}/[A]_{control}$, where $[A]_{test}$ and $[A]_{control}$ are the average absorption intensities of the test and control samples (n=8), respectively.

Cellular uptake analysis by flow cytometry

HeLa cells were seeded in 6-well plate at a density of 2×10^5 cells/well and grown for 24h. Then, the cells were treated with MSNP(FITC)-SS-dsDNA (20μg mL^{-1}) for 24h. The cells were then collected with trypsin and washed with PBS. HeLa cells without any treatment were used as control. Flow cytometry analysis was performed on both cells by monitoring the fluorescence intensity of FITC.

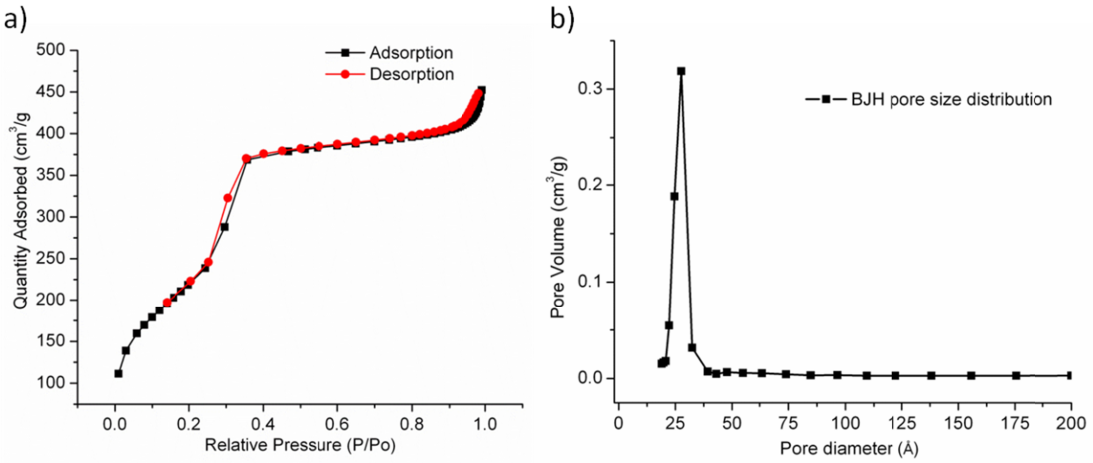

Figure S1. (a) BET isotherm and (b) BJH pore size distribution of MSNP-SH.

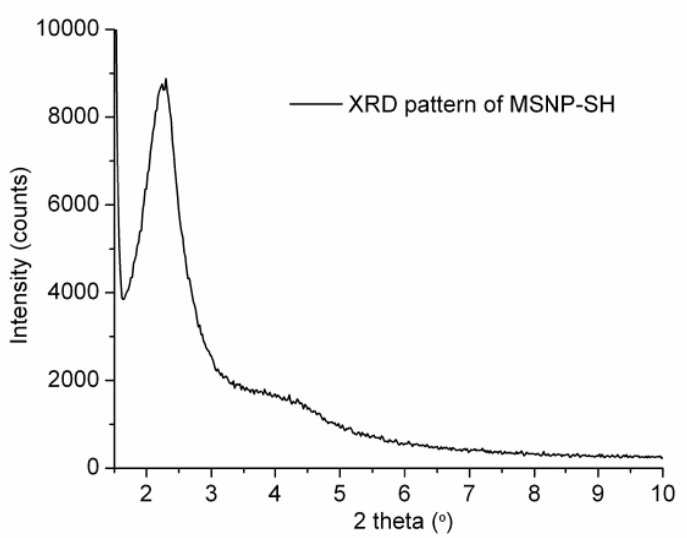

Figure S2. Powder X-ray diffraction pattern of MSNP-SH.

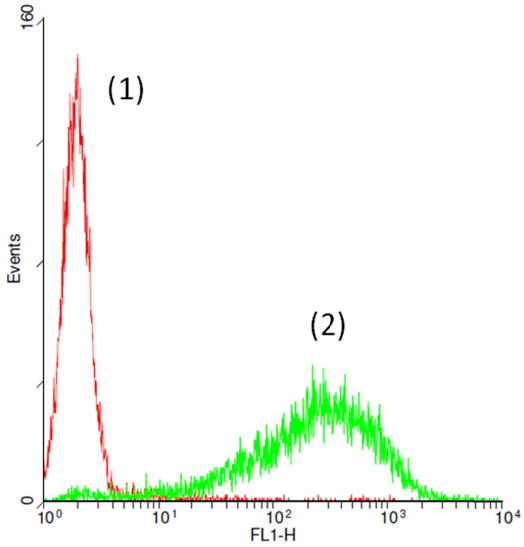

Figure S3. Flow cytometry analysis of MSNP-SS-dsDNA uptake by HeLa cells. (1) Control, (2) HeLa cells after treatment by MSNP(FITC)-SS-dsDNA (20 μg mL^{-1}) for 24h.

Table S1. BET surface area, pore size, and pore volume of MSNP-SH.

BET surface area	808.8 ± 4.8 m^2/g
Pore size	2.8nm
Pore volume	0.78 cm^3/g

Table S2. Zeta potential measurements of MSNP-SH, MSNP-SS-NH_2, and MSNP-SS-COOH.

Sample	Zeta-Potential (mV)
MSNP-SH	-24.8
MSNP-SS-NH_2	+44.2
MSNP-SS-COOH	-45.9