Supporting information

Preparation of fluorescent mesoporous hollow silica-fullerene nanoparticle via selective etching for combined chemotherapy and photodynamic therapy

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Experimental Section

Chemicals

C60 fullerene (99%), tetraethyl orthosilicate (TEOS, 98%), (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide), 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), cyclohexane, 1-hexanol, andTriton X-100were purchased from Sigma-Aldrich. All chemicals were used as received without purification.

Synthesis of SSF

SSF was synthesized according to a literature method with slight modification.[1] In a typical synthesis, C60 fullerene (16 mg in 8 mLtoluene) were added to a microemulsion solutions composed ofcyclohexane (20 mL), hexanol (8 mL), distilled water (2 mL), andTriton X-100 (6.8 mL). After stirring for 30 min,tetraethyl orthosilicate (TEOS, 400 μL) was added and stirred for another 30 min before addition of ammonium hydroxide(28 wt%, 240 mL).The mixture was stirred at room temperature for 24 h. At the end of reaction, ethanol (40 mL) was added to break microemulsion and the particle were collected using centrifugation at 20000 rpm and washed with ethanol twice.

Synthesis of MHSF

~ 25 mg of SSF was mixed with 50 ml ethanol and sonicated for 30 min. Then, the suspension of SSF in ethanol was stirred at 45 °C for 24 h. After treatment, SSF was collected using centrifugation and dispersed in 0.1 M ammonium hydroxide for selective etching at 30 °C for 3h. After centrifugation and washing with water and ethanol each for three times. The MHSF particles wereobtained after drying in vacuum at room temperature overnight.

Characterizations

Transmission electron microscopy (TEM) images were obtained with a JEOL 1010 operated at 100 kV. Dynamic light scattering (DLS) was carried out in D.I. water at 298 K using a Zetasizer Nano-ZS from Malvern Instruments.Nitrogen adsorption–desorption isotherms were measured at 77 K using a Micromeritics ASAP Tristar II 3020 system. The samples were degassed at 393 K overnight on a

vacuum line. The pore size distribution curve was derived from the desorption branch of the isotherms using the Barrett–Joyner–Halanda (BJH) method. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface area. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure (P/P_0) of 0.99.Fourier transform infrared (FTIR) spectra were recorded on ThermoNicolet Nexus 6700 FTIR spectrometer equipped with Diamond ATR (attenuated total reflection) Crystal. For each spectrum, 1024 scans and 4 cm^{-1} resolution was applied over the range of 400–4000 cm^{-1}.Elemental analysis was performedwith an CHNSO elemental analyses (Intertek), andcarbon contents were obtained by averaging the results of triplicatemeasurements.UV-vis transmittance spectra were measured with a Shimadzu UV-2450double beam spectrophotometer.

Detection of Singlet Oxygen

Generation of singlet oxygen can be usually detected by chemical method using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) as a singlet oxygen sensor.We have used deuterium oxide (D_2O) as a solvent because it extends the lifetimeof singlet oxygen compared to water.[2] The amount of singlet oxygen generated by nanoparticles are recorded based on the decrease in absorbance of ABDA at 376 nm via UV-vis spectroscopy. 100 μL of ABDA solution (2 mg/mL) was mixed with SSF(0.5 mg/mL) / MHSF (0.5 mg/ml) and placed in a cuvette, respectively. The ABDA only in D_2O was used as thecontrol. The absorption intensity of ABDA at 376 nm was monitored under different UV light irradiation periods.

DOX loading and release

For the drug loading, 0.5 ml DOX-watersolution(2 mg ml^{-1}) was mixed with 0.5 ml nanoparticle suspension (2 mg ml^{-1}) and stirred at room temperature for 24 h followed by centrifugation. The amount of DOX adsorbed to SSF or MHSF was further quantified by measuring the absorbance of the supernatant at 480 nm. The amount of DOX adsorbed was calculated by the concentration difference between before and after adsorption.

For the drug release test, DOX loaded MOSF were immersed in 1 ml of PBS solution (pH=7.4) or acetate buffer soloution (pH=5.5) and gently shaken at 100 rpm at 37°C. At a predetermined time point, the solution was centrifuged and the supernatant was removed and replaced by the same amount of fresh buffer solution. The release amount of drug was quantified by UV-vis measurement.

Cell culture

Cell culture reagents were purchased from GIBCO Invitrogen Corporation/Life Technologies Life Sciences unless otherwise specified. MCF-7 (ATCC® HTB-22™) cells were maintained as monolayer cultures at 310 K and 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The media were changed every two to three days and the cells were passaged by trypsinization.

Cellular uptake assay

MCF-7 cells were seeded onto 24-mm glass coverslips in 6 well-plates at 1×10^5 cells per well one day before the assay. MHSFwas incubated with cells at a concentration of 160 μg/ml for 6 h in serum-free medium. Afterwards, cells were washed with PBS twice and fixed with 4% paraformaldehyde in PBS for 30 minutes, following two more washes with PBS. The cells were stained by Alexa Fluor® 488 phalloidin (Life technologies, Australia) according to the protocol of manufacturer’s protocol. Then,

the coverslips were washed three times with PBS and mounted onto glass slides by fluoroshield with DAPI (Sigma). The uptake performance of nanoparticles was assessed using a confocal microscope (LSM 710, ZEISS).

Cytotoxicity assay

The cytotoxicity of SSF and MHSF were determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric assay. Cells (8×10^3 per well) were seeded in a flat-bottomed 96-well plate one day before the assay, and exposed to SSF and MHSF with a series of concentrations (20, 40, 80, 160 ug/ml) in serum containing medium. After incubation for 36 hours, 10 μL MTT reagent (5 mg/ml in PBS) was added and incubated at 37 °C for 4 hours. Then, the medium was removed, and dimethyl sulfoxide (DMSO) (50 μl) was added to dissolve the formazan crystal. Cell viability was determined by reading the absorbance at 570nm using a microplate reader.

In vitro Chemotherapy and Photodynamic Therapy Studies

To study the phototoxicity effect, MCF-7 cellswere seeded at a density of 8×10^3 in 96-well cell culture plates and incubated for 24 h. Cells were thentreated with a planned amount of DOX or MHSF-DOX for 6 h in the culture medium, followed by PBS wash, and then treated withUV irradiation (365 nm, 150 $mWcm^{-2}$) for desired period of time. For cell viability test, the irradiated plates were returned to the incubator and cell viability wasmeasured 36 h later with MTT assay.

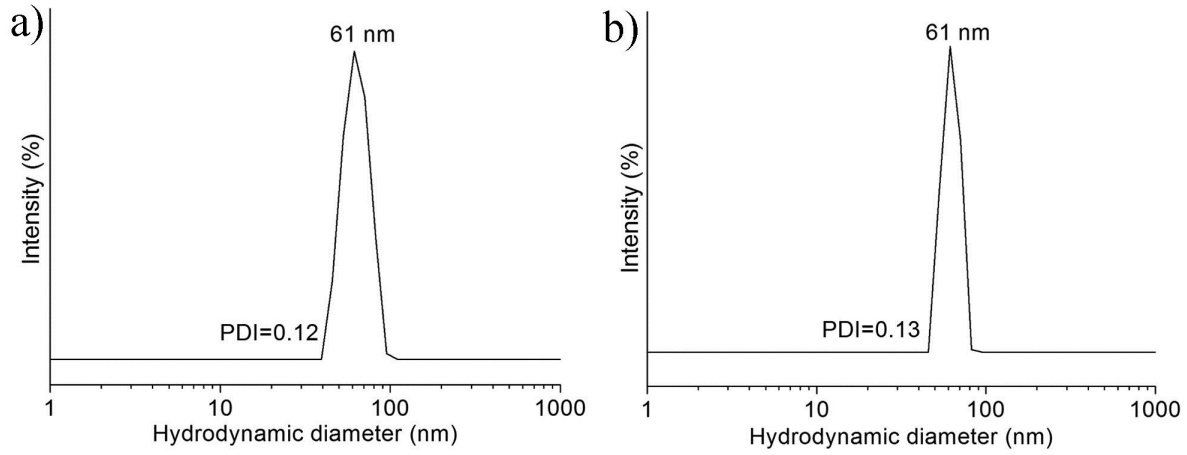

Fig. S1 Particle size distribution curves of SSF (a) and MHSF (b) obtained from DLS.

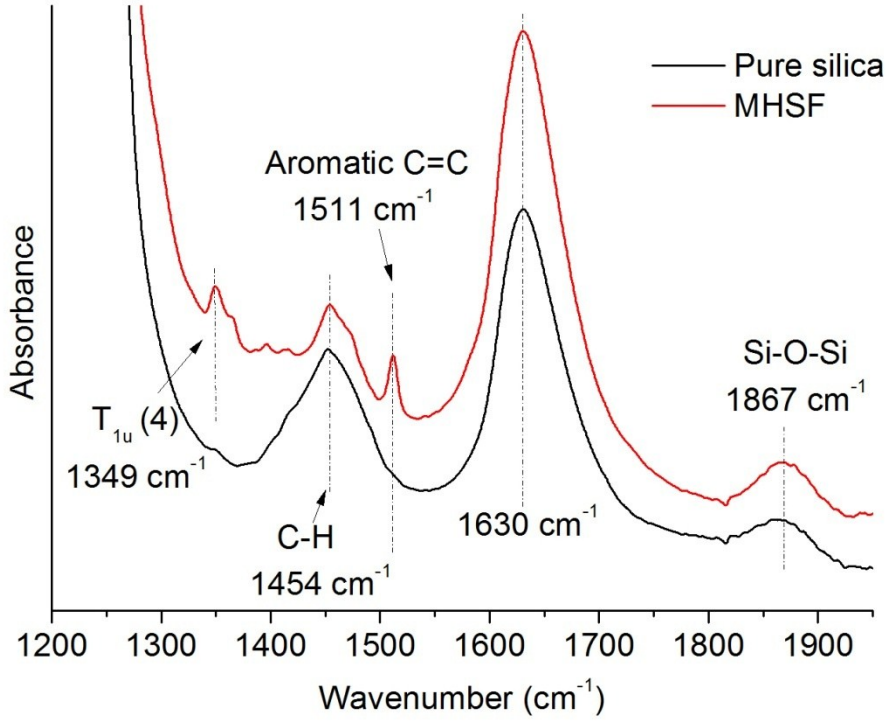

Fig. S2FTIR spectra of MHSF and pure silica.

Figure S2 shows the FTIR spectrum MHSF and pure silica. The peaks exclusively observed for MHSF at 1349 and 1511 cm^{-1} are ascribed to $T_{1u}(4)$ mode vibration[3]and aromatic C=C, indicating the successful incorporation of C_{60}.[1] The peaks at 1454 and 1630 are attributed to δ(C-H),[4] due to the presence of organic residues. A broad peak centred at 1867 cm^{-1} is ascribed to Si-O-Si deformations.[5]

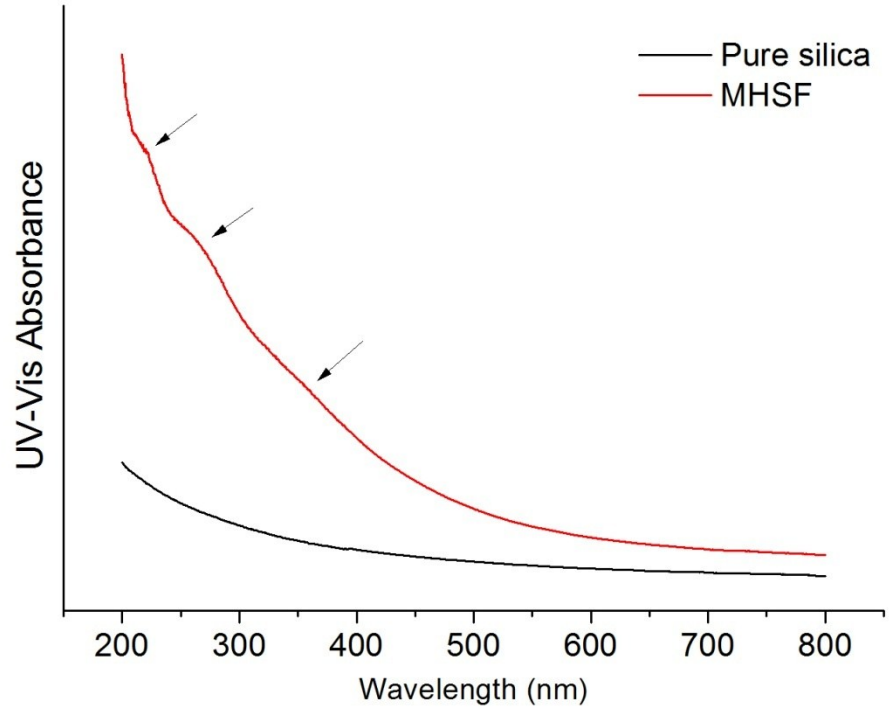

Fig. S3 UV-Vis absorption spectra of the MHSF and pure silica.

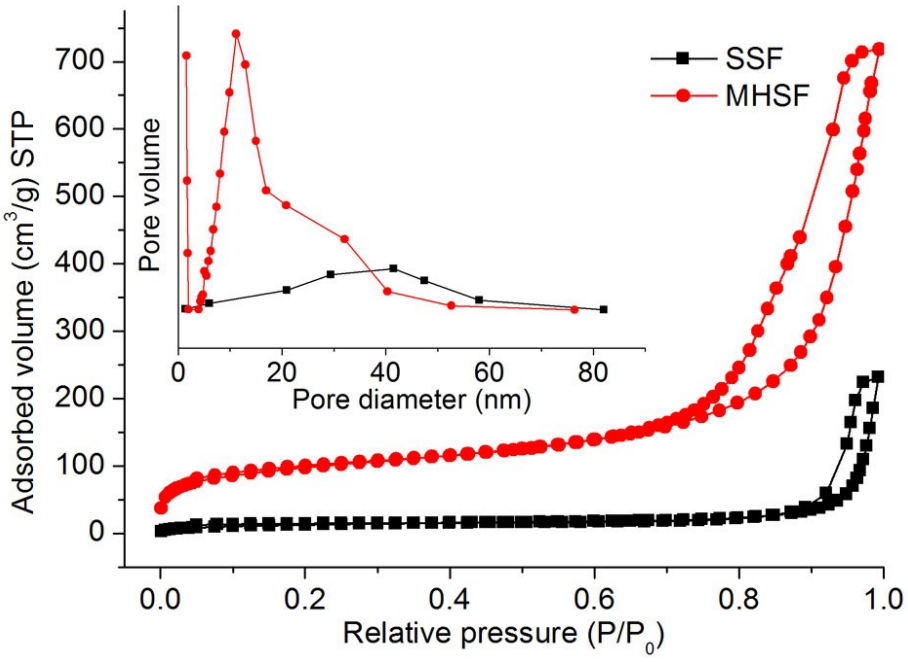

Fig.S4 N_2 adsorption-desorption isotherms of SSF and MHSFand corresponding pore size distribution curves (inset) calculated from the desorption branches using the Barrett–Joyner–Halanda (BJH) method.

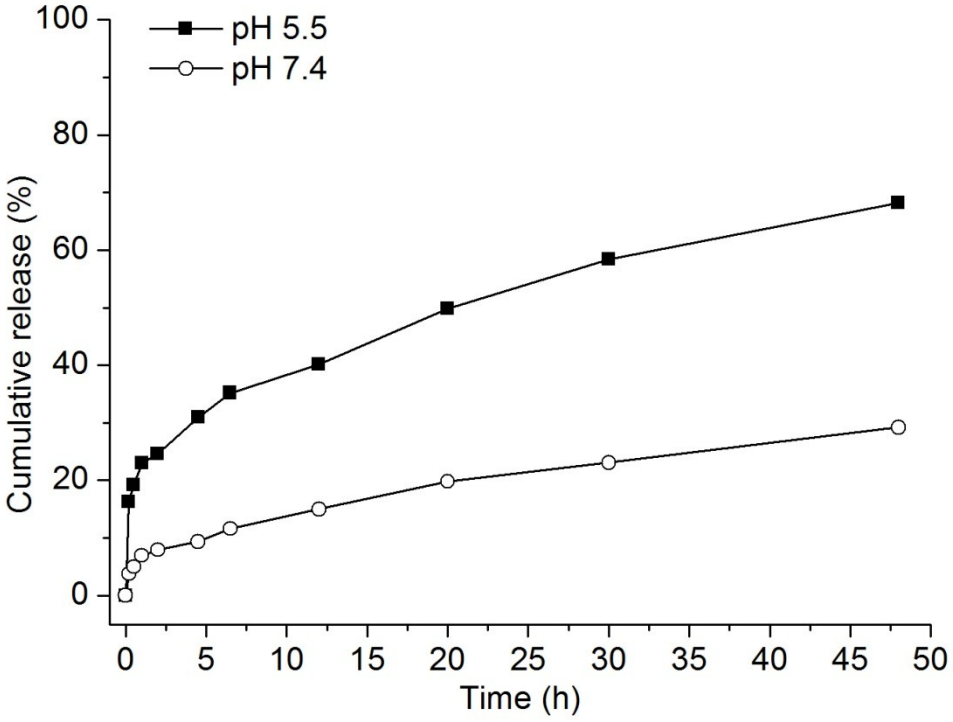

Fig. S5DOX release profile from MHSF in PBS (pH 7.4) and acetate buffer (pH 5.5) as a function of time.

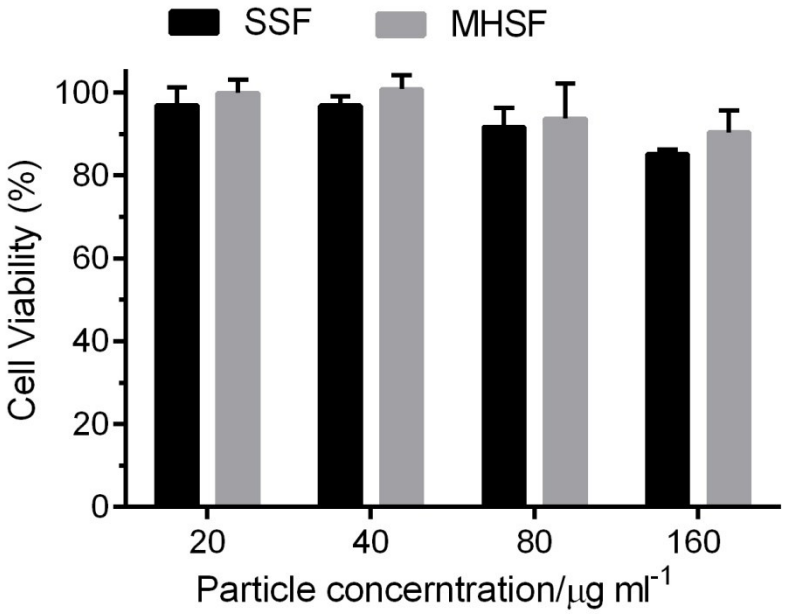

Fig. S6 Cytotoxicity of SSF and MHSF as a function of particle concentration in MCF-7 cells after 36 h.

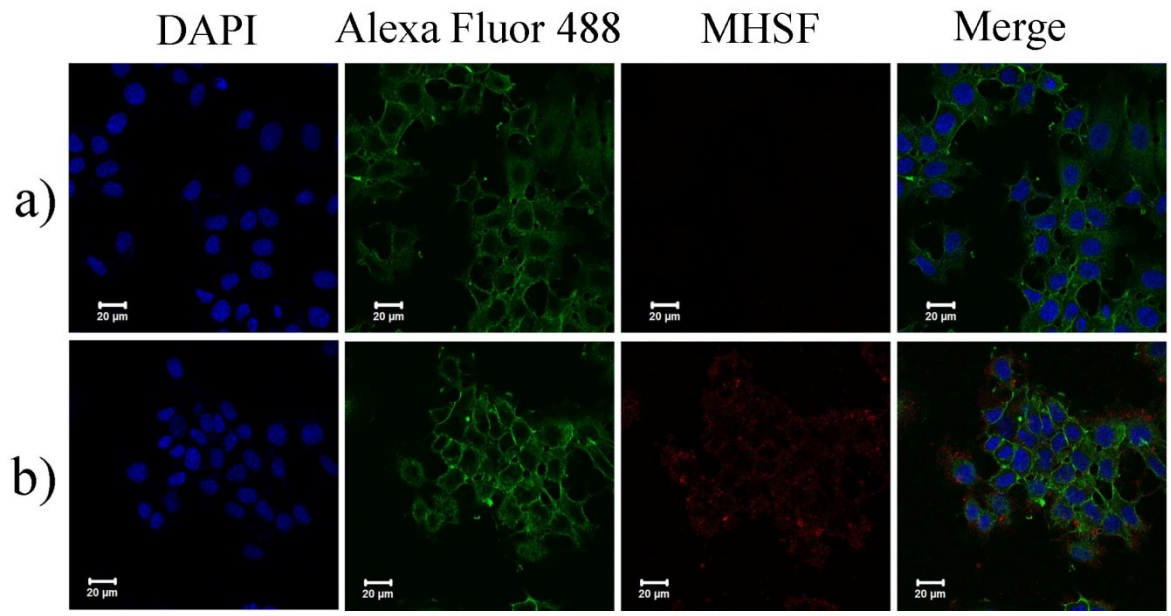

Fig.S7 Confocal microscopy images of MCF-7 cells (a) without adding nanoparticles and (b) with treatment of MHSF (red) with 160 μg/mL for6 h. The cytoskeleton and nuclei in cells were stained by Alexa Fluor® 488 phalloidin(green) and DAPI (blue), respectively.

Table S1 Structural properties of SSF and MHSF.

Sample Name	Entrance size (nm)	V_p ($cm^3\ g^{-1}$)	S_{BET} ($m^2\ g^{-1}$)	*t*-Plot Micropore Area (m^2/g)
SSF	-	0.36	45.6	4.7
MMSF	11.2	1.1	331.9	107.2

Note: V_p: total pore volume; S_{BET}: BET surface area.

References

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