Electronic Supplementary Information

Carborane Based Mesoporous Nanoparticles as Potential Agent for BNCT

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Experimental

Chemicals

Cetyltrimethylammonium chloride (CTAC) (25 wt % in H_2O), triethanolamine (TEA), Tetraethyl orthosilicate (TEOS), cyclohexane and doxorubicin (DOX) hydrochloride were purchased from Sigma-Aldrich Corp. Ortho-carborane was purchased from Shanghai UCHEM Inc. Anhydrous sodium sulfate and n-Butyllithium (n-BuLi) were obtained from Macklin and J&K, respectively. Polyethyleneimine (PEI, M_w = 10000), N-hydroxysuccinimide (NHS), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and (3-Bromopropyl) trimethoxysilane (Br-PTMS) were purchased from Aladdin Chemistry Co., Ltd. The cRGD (cricoids Arg-Gly-Asp-Phe-Lys) peptide was obtained from Gier Biochemistry Co., Ltd. (Shanghai, China). All the above chemicals were used as received without further purification. Solvents for Schlenk reaction such as diethyl ether were purified, dried and distilled by standard procedures. Deionized water was used for all experiments.

Synthesis of DMSNs

The dendritic mesoporous silica nanoparticles were synthesized as the procedure reported previously with some changes. Briefly, 20 ml (25 wt %) CTAC solution and 326 mg triethanolamine were added to 50 ml deionized water and stirred gently at 60 °C for 1 h in round bottom flask, then 6 ml of TEOS and 20 ml of cyclohexane was carefully added into the above mixture drop by drop and kept at 60 °C with magnetic stirring for 12 h. The products were collected by centrifuging and washed by water and ethanol for several times, and then were dispersed in 50 ml of acidic ethanol and refluxed for 18 h to remove CTAC templates. The final products were washed thrice with ethanol and dried in vacuum at 45 °C for 12 h.

Synthesis of CB-PTMS

In an Schlenk flask under nitrogen, ortho-carborane (300 mg, 2.08 mmol) was dissolved in 10 ml diethyl ether. The solution was cooled at 0 °C and n-BuLi (1.6 ml, 2.50 mmol) was added dropwise. The mixture was stirred for 2 h at room temperature, cooled again at 0 °C, and Br-PTMS (370 µl, 1.98 mmol) was added. The mixture was stirred for 24 h at room temperature. Then it was quenched with 5 ml deionized water, transferred to a separatory funnel and extracted with Et_2O (3 x 10 ml). The organic layer was dried with anhydrous sodium sulfate and concentrated under vacuum to obtain CB-PTMS as colorless oil (531.6 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (s, 1H), 3.55 (s, 9H), 2.25–2.21 (t, J = 8.3 Hz, 2H), 1.62–1.54 (m, 2H), 0.61-0.57 (t, J = 8.1 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 75.2 (cage *C*), 61.2 (cage *C*), 50.5, 40.9, 22.9, 10.2 ppm; ^{11}B NMR δ -1.9, -2.7, -8.9, -9.6, -12.6 (6B); IR (KBr): ν 3063, 2941, 2843, 2593, 1458, 1195, 912, 722, 456 cm^{-1}; HRMS m/z Found: 299.3026, calculated: 299.3023 for $C_8H_{27}B_{10}SiO_3^+$ [M+H]$^+$.

Synthesis of CB@DMSNs

A suspension of DMSNs (60 mg, 1.00 mmol) in toluene (10 ml) was sonicated at room temperature for 15 min. Then, CB-PTMS (62 mg, 0.20 mmol) was added into the mixture. After stirring at 100 °C for 12 h, the products were collected by centrifuging and washed with ethanol for several times. The final silica nanospheres which were partially modified with carborane could be obtained by drying in vacuum at 45 °C for 6 h.

Doxorubicin (DOX) loading

Doxorubicin hydrochloride (20 mg) was dissolved in deionized water (10 ml). CB@DMSNs (20 mg) was added to the solution and the suspension was stirred at room temperature for 48 h. The DOX molecules could be adsorbed in the mesoporous channels. The as-prepared DOX-loaded dendritic mesoporous silica nanospheres modified with carborane were collected by centrifugation. The amount of the adsorbed guests was determined from the difference between the initial amount of DOX and the amount after adsorption of the DOX molecules by

measuring the UV absorbance from the supernatant liquid at 480 nm quantified from a standard curve.

Synthesis of DOX/CB@DMSNs(R)

30 mg PEI was dissolved into 5 ml PBS buffer solution, and then EDC and NHS solution were added with stirring for 2 h. 5 ml cRGD peptide solution (1 mg ml^{-1}) was then added into the mixed solution and stirred for 24 h. Afterwards, 60 mg DOX/CB@DMSNs was dispersed uniformly in the above mixture and agitated for another 24 h at room temperature. Finally, the products were collected by centrifugation and washed with deionized water several times and dried under vacuum.

Material characterization

The morphology of the material was visualized by the scanning electron microscope (SEM, Quanta FEG 250) operating at a voltage of 15 kV. The samples were coated with Au before SEM measurements. Transmission electron microscopy (TEM) images were obtained by using a Talos L120C transmission electron microscope (TALOS, Czech Republic) at an acceleration voltage of 120 kV. Powder X-ray diffraction (PXRD) patterns were recorded on a Smartlab (3KW) X-ray diffractometer using Cu Kα radiation, and operated at 40 kV and 30 mA. The size distribution and Zeta potential of the nanoparticles was measured on a Zetasizer Nano-ZS90 instrument (Malvern). Fourier transform infrared (FTIR) spectra were acquired on a Thermo Fisher Scientific Nicolet iS10 Spectrometer using spectroscopic grade KBr. Solid state ^{29}Si and ^{11}B magic angle spinning (MAS) NMR spectra were recorded on an Agilent 600M spectrometer with 4 mm MAS probes at a spinning rate of 8 kHz. UV-vis spectra were measured using a Nanodrop One spectrophotometer (Thermo Fisher Scientific). Nitrogen sorption isotherms were measured at 77 K using a Quantachrome Autosorb-iQ3 instrument. Confocal microscopic images were obtained using a Leica TCS SP8 apparatus with a 400× oil objective. An inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x) was used to analyze boron concentrations in the nanoparticles and the mice organs.

ICP-MS for DOX/CB@DMSNs(R)

DOX/CB@DMSNs(R) were mixed with 1 ml HF-HNO_3 (1:1, vol/vol) solution and then digested for 24 h at room temperature. Afterwards, a certain amount of deionized water was added to the eppendorf tube. The boron content was detected using ICP-MS.

Cell lines and culture

Pancreatic cancer cell lines including PANC-1 (human) and Panc02 (mouse) were obtained from the Institute of Biochemistry and Cell Biology (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with penicillin (100 units ml^{-1}), 10% heat-inactivated fetal bovine serum and streptomycin (50 units ml^{-1}) at 37 °C in a humidified incubator with 5% CO_2 atmosphere.

CCK-8 assay

Cell viability was determined by Cell Counting Kit-8 (CCK-8) assay. In a typical experiment, PANC-1 cells (2 x 10^4 cells ml^{-1}) were seeded in 96-well tissue culture plates and allowed to attach overnight. Different concentrations of CB@DMSNs and DOX/CB@DMSNs(R) were added to each well and allowed to incubate for additional 12 h, respectively. The cells were washed with PBS three times, and then, 100 μl DMEM and 10 μl CCK-8 solution were added to each well. After incubation for another 2 h at 37 °C, the optical density (OD) was measured at the wavelength of 450 nm using a V Max kinetic microplate reader. The viability of cell growth was calculated by the following formula: Viability (%) = (mean of OD value of treatment group/mean OD value of control) × 100.

Intracellular uptake

PANC-1 cells (2 x 10^4 cells ml^{-1}) were seeded onto sterile glass coverslips in 24-well plates. After 12 h of incubation, the cells were attached to the surface of the plate. Then 200 μg ml^{-1} of DOX/CB@DMSNs(R) were added to each well and incubated for 12 h at 37 °C. The culture medium was removed, and the cells were then washed with PBS. The images were

obtained by using a confocal laser scanning microscopy (CLSM) after cell nucleus were stained with DAPI, which was excited at 360 nm with the emission at 460 nm. The red fluorescence at 590 nm of products obtained above was observed by using a 480-nm laser.

In vitro drug release

Briefly, 10 mg DOX/CB@DMSNs(R) was dispersed in 10 ml PBS (pH 7.4) or PANC-1 cell lysate with constant shaking at 37 ˚C in a glass vial. At specific time after incubation, a certain volume of supernatant was taken out from the vial and the same volume of fresh PBS or cell lysate were replaced. PANC-1 cell lysate was prepared by incubating 6×10^6 cells in 10 ml RIPA lysis buffer obtained from Thermo Fisher Scientific. All samples were determined by Nanodrop One spectrophotometer at the absorbance of 480 nm.

Animal and orthotopic mouse model of pancreatic cancer

Female C57BL/6 mice (four to six weeks old) were used to build orthotopic mouse model of pancreatic cancer. Mice were anesthetized with 2.5% isoflurane. The general area of the spleen (left upper quadrant of the abdomen) was located. Surgical scissors was used to make an incision of approximately 1.0 cm to create a pocket. The caudal end of the spleen was gently grabbed and pulled out of the body. The pancreas would be attached to the spleen. Panc02 cells (1×10^6 cells per mouse) were injected into the tail of pancreas, leaving the needle inside for 10 sec. The experiments were started at day 14 after the tumor models were established. All mice were bred and housed in a specific pathogen-free facility of Peking University Shenzhen Graduate School and all experiments were carried out according to protocols of the Care and Use of Laboratory Animals published by the China National Institute of Health.

***In vivo* uptake experiments**

The tumor-bearing mice were intravenously injected with DOX/CB@DMSNs(R). The mice were sacrificed and the major organs (heart, liver, spleen, kidney and tumor) were collected after different uptake times (12, 16, 24, 30 h). The frozen organs were sectioned into 4 μm

thickness slice and stained with DAPI. Afterwards, the tissue sections were examined for DOX channel under confocal microscopy.

***In vivo* biodistribution of DOX/CB@DMSNs(R) by ICP-MS**

DOX/CB@DMSNs(R) were administered to each mouse by intravenous injection at a dose of 100 mg kg^{-1}. The mice organs, such as, blood, heart, liver, spleen, kidney, tumor were collected at different time points (12, 16, 24, 30 h) after injection. HF-HNO_3 (1:60, vol/vol) solution was added to each organ of mice and digested using a microwave digestion apparatus. The element ^{10}B was detected using ICP-MS with multi-element solution (GSB 04-1767-2004, containing B) as standard with respect to ^{10}B concentration. Each experimental condition was done in triplicate.

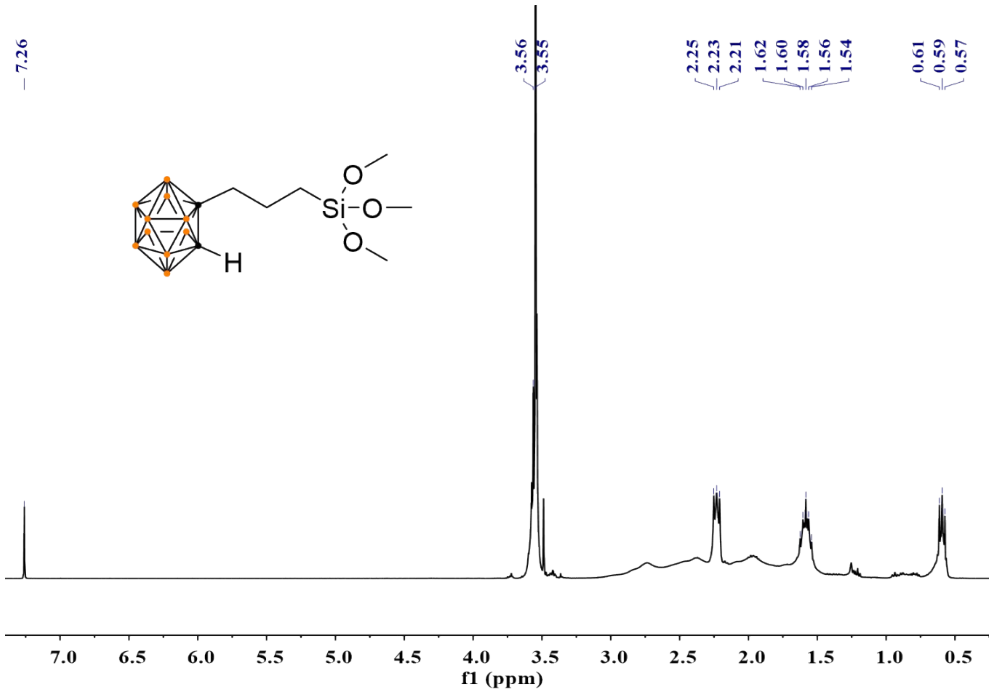

Fig. S1 ^{1}H NMR spectrum of CB-PTMS.

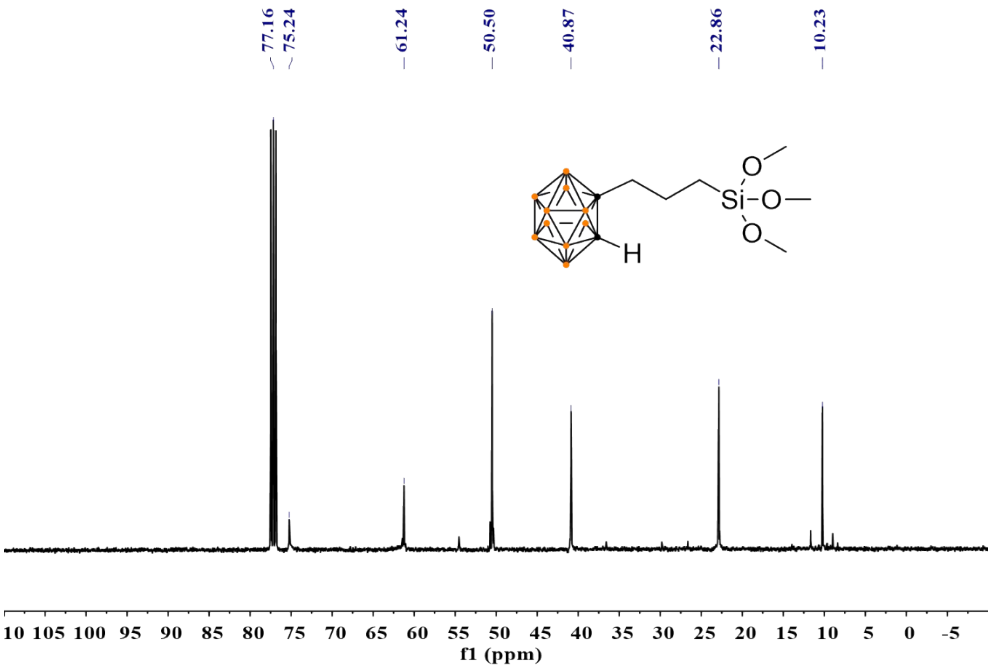

Fig. S2 ^{13}C NMR spectrum of CB-PTMS.

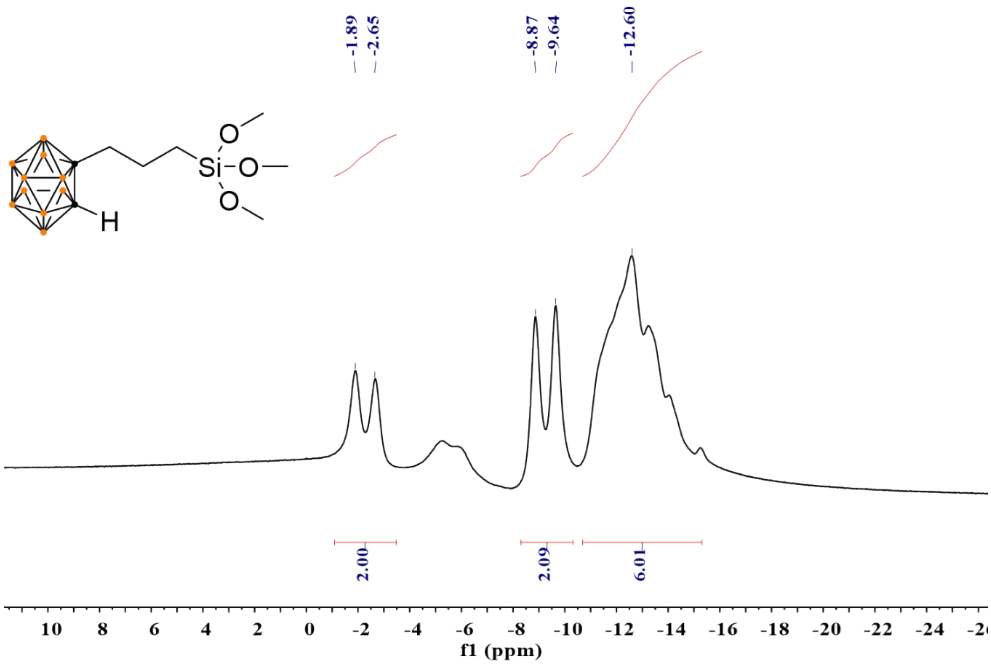

Fig. S3 ^{11}B NMR spectrum of CB-PTMS.

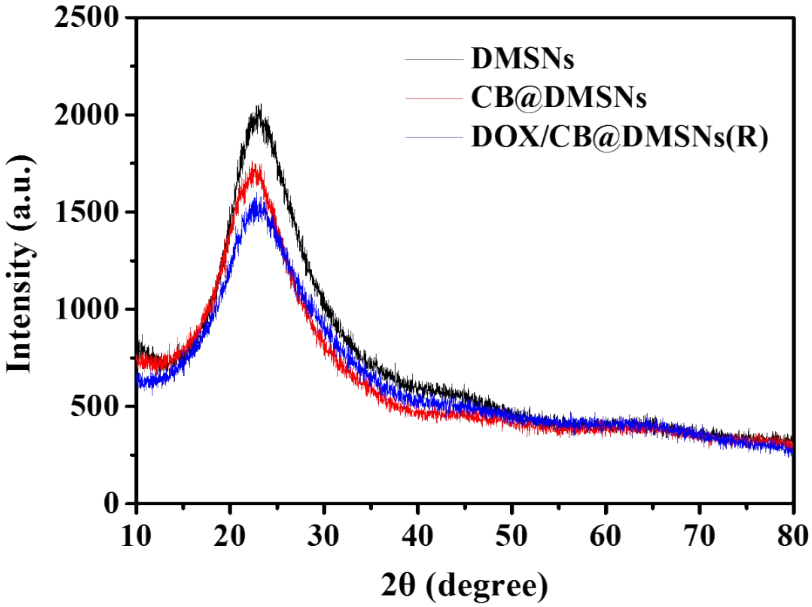

Fig. S4 Powder X-ray diffraction patterns of DMSNs, CB@DMSNs and DOX/CB@DMSNs(R), respectively.

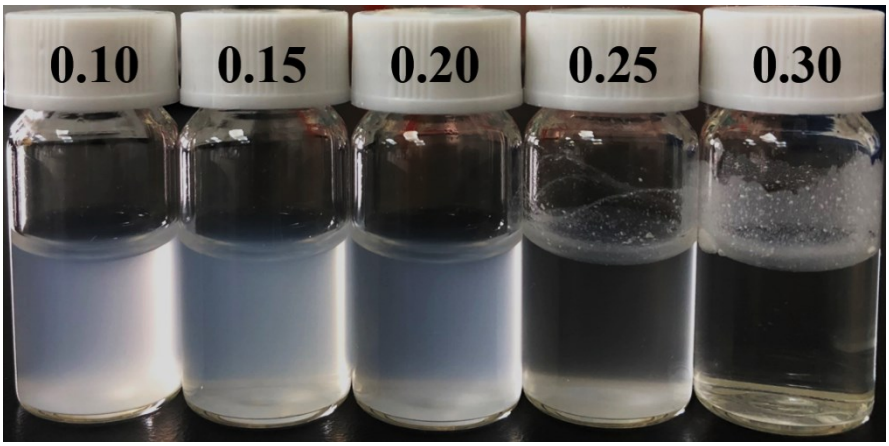

Fig. S5 Dispersion behaviors of CB@DMSNs obtained in different molar ratios of CB-PTMS to DMSNs in aqueous phase.

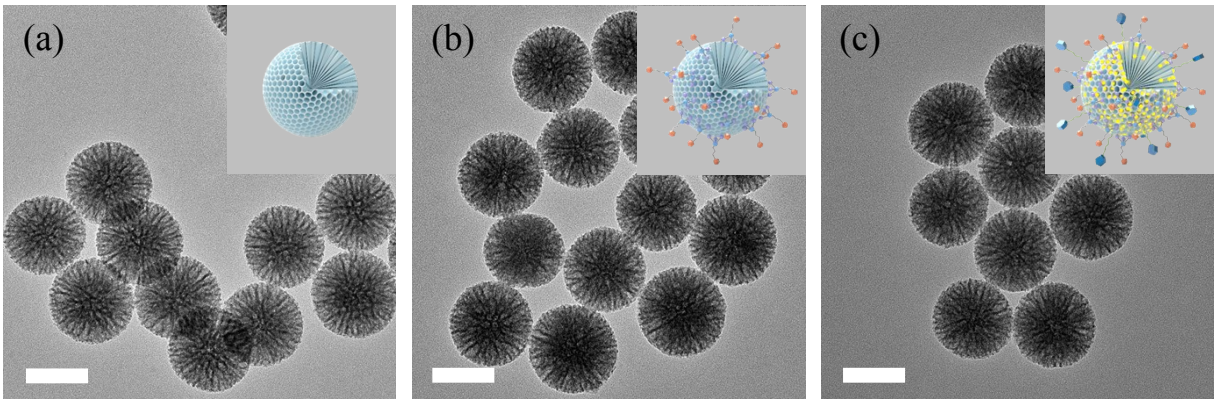

Fig. S6 TEM images of DMSNs after CTAC extraction (a), CB@DMSNs (b,) and DOX/CB@DMSNs(R) (c), respectively. Insets are relevant schematic models. The bars represent 100 nm.

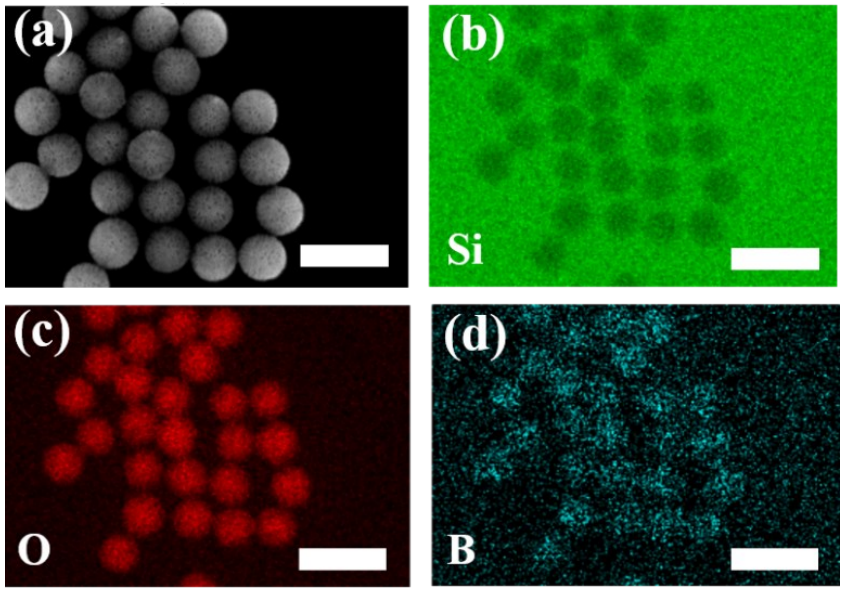

Fig. S7 FE-SEM images of DOX/CB@DMSNs (a). EDS mapping of DOX/CB@DMSNs (b, c, d). The scale bar in each image is 250 nm.

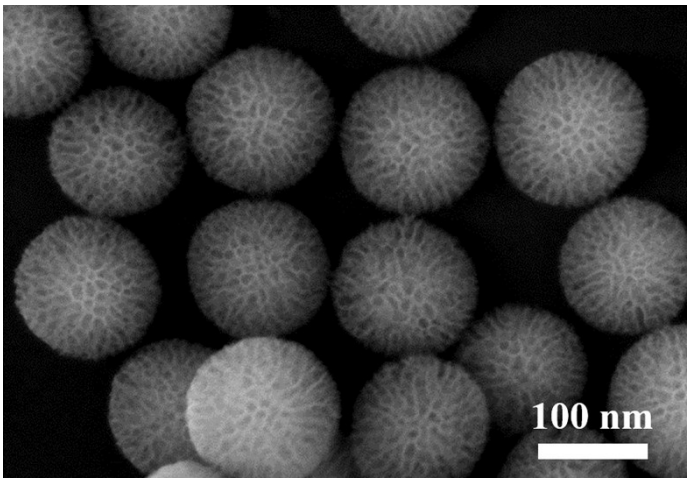

Fig. S8 FE-SEM image of DOX/CB@DMSNs after immersion in simulated body fluid for one week.

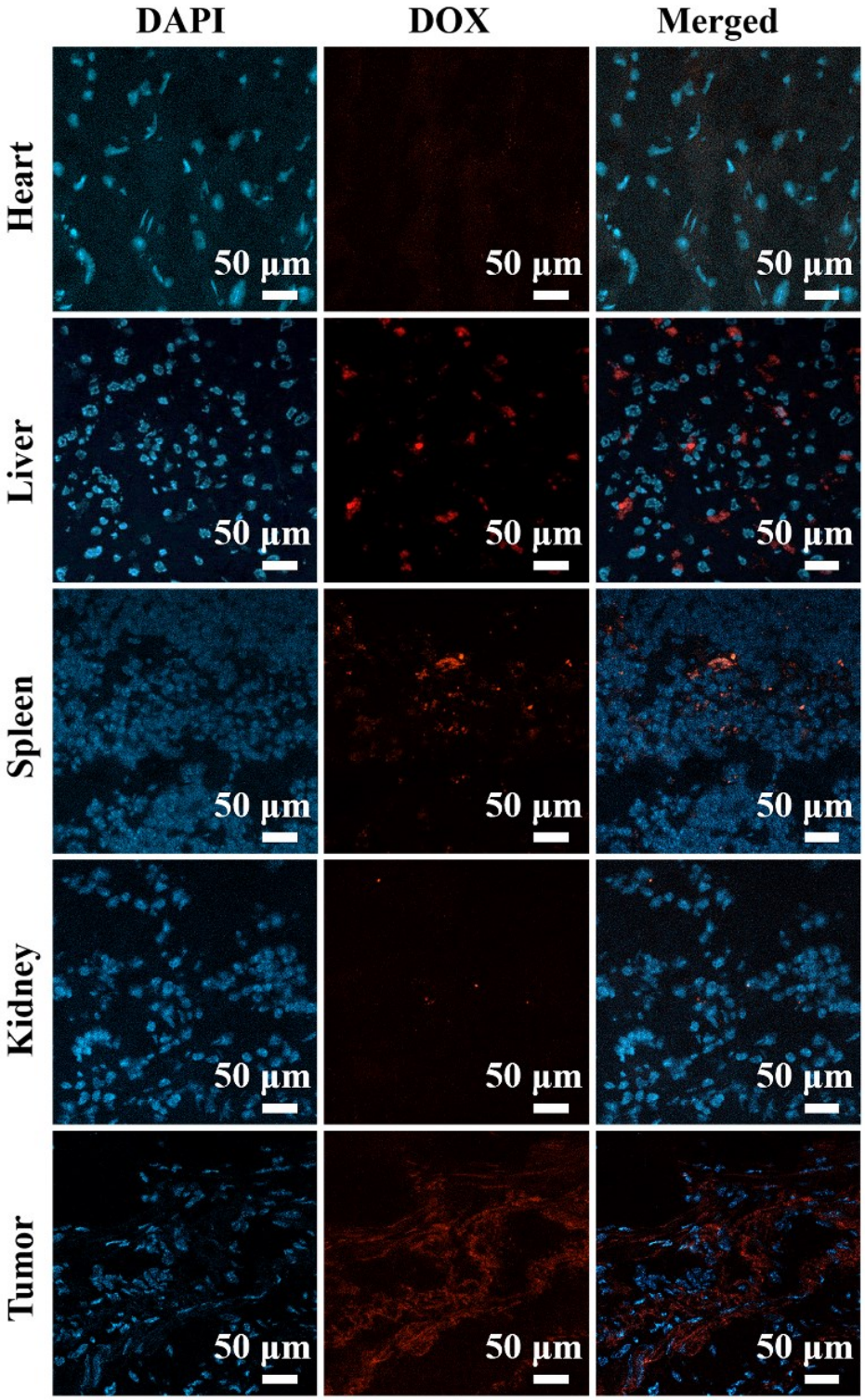

Fig. S9 Confocal laser scanning microscopy (CLSM) images of heart, liver, spleen, kidney and tumor sections dissected from the mice intravenously injected with DOX/CB@DMSNs(R) after 16 h.

Table S1. Composition of simulated body fluid

Reagent	**Amount in grams**
NaCl	7.90 g
$NaHCO_3$	0.35 g
KCl	0.22 g
$K_2HPO_4 \cdot 3H_2O$	0.22 g
$MgCl_2 \cdot 6H_2O$	0.30 g
$CaCl_2$	0.27 g
Na_2SO_4	0.07 g
1 M HCL	40 ml
Tris buffer	6 g
water	945g