Supporting Information

A H^+-Triggered Bubble-Generating Nanosystem for Killing Cancer Cells

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† *Electronic Supplementary Information (ESI) available: Detailed experimental procedures and materials; Fig. S1 – S3*

Experimental Section

Reagents and materials: The chemical reagents used in this study were of analytical grade and used without further purification. Doxorubicin hydrochloride was purchased from Melonepharma (Dalian, China). Trimethoxyoctadecylsilane (C_{18}TMS） were acquired from Aladdin (Shanghai, China), tetraethylorthosilicate (TEOS) was purchased from Sigma (St. Louis, MO, USA). Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Laboratories (Kumamoto, Japan). Alexa Fluor 488-dextran (10,000 MW) was purchased from Thermo Fisher Scientific Inc. (MA， USA). Caspase-3 Activity Assay Kit was obtained from Beyotime Institute of Biotechnology (Haimen, China)

Preparation of HMSNs: HMSNs were prepared according to a previous report (Ref. S1). Briefly, ethanol (71.4 mL), H_2O (10 mL), and ammonium solution (3.14 mL) were mixed and stirred at 30°C. And then, TEOS (6 mL) was added into the mixture, and the reaction kept for another 1 h. TEOS (5 mL) and C_{18}TMS (3 mL) were premixed and added into the reaction medium rapidly afterward, and the reaction kept for another 1 h. Finally, the obtained nanoparticles were dispersed into Na_2CO_3 aqueous solution (0.2 M, 100 mL) for 2 h at 80 °C. The product was collected by centrifugation at 8,000 rpm for 10 min and washed with water three times thoroughly. The C_{18}TMS was removed by calcination at 550°C for 6 h. The pore size distribution and surface areas were calculated by the typical N_2 absorption/desorption measurements (Autosorb-1, Quantachrome, USA).

Preparation of DMSNs and BGNS: For the preparation of BGNS, 100 mg HMSNs was dispersed into DOX aqueous solution (1 mg/mL, 10 mL). After stirring in the dark for 12 h, DMSNs were harvested by centrifugation at 8,000 rpm for 10 min and washed with ultrapure water for 3 times. Then, DMSNs were dispersed into $NaHCO_3$ aqueous solution (0.2 M, 20 mL) and incubated for 12 h. The precipitate was collected by centrifugation at 8,000 rpm for 10 min, and then treated with DOX solution, $NaHCO_3$ solution successively. To obtain BGNS, this process was repeated 4 times. At last, BGNS was collected by centrifugation at 8,000 rpm and washed three times with water. After vortex mixing, the obtained BGNS was stored at 4°C in dark. BGNS kept its cytotoxicity against cancer cells after two-month storage. To evaluate the DOX loading efficiency, the residual DOX content in the supernatant was determined using the calibration curve of DOX standard solutions by the absorbance measurement at 490 nm. The loading efficiency of DOX in DMSNs or BGNS was calculated as follows:

$$\frac{initial\ mount\ of\ DOX - residual\ DOX}{initial\ mount\ of\ DOX} \times 100\%$$

In vitro drug release study: DMSN, BGNS solution (450 μg/mL, 100 μL) was added into 48-well plates that contained 900 μL of PBS with different pH values (pH 7.4, 5.0). The 48-well plates were gently shaken in a thermostatic rotary shaker at 250 rpm at 37 °C. Samples were then taken out at pre-determined time points, and the concentration of DOX released from DMSNs, BGNS was quantified using a UV-2450

spectrophotometer (Shimadzu, Japan). Finally, after incubated at different pH condition for 24 h, DMSNs and BGNS were imaged by TEM (JEM-1200EX, JEOL Technics, Tokyo, Japan), scanning electron microscopy (SEM, S-4800, Hitachi, Japan). The generation of CO_2 bubbles from BGNS at acidic conditions was visualized by an ultrasound imaging system with a 7 MHZ transducer (HD11XE, Philips, Holland).

Cell culture: MCF-7 and MCF-7/ADR cells were purchased from ATCC, and were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 100 units ml^{-1} of penicillin and streptomycin. The medium and supplements were purchased from Gibco. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Inhibitory effects of cancer cell proliferation: MCF-7 and MCF-7/ADR cells were seeded at a density of 8,000 cells/well in 96-well plates and were incubated in 5% CO_2 at 37 °C for 24 h. The growth media were replaced by fresh complete medium containing free DOX, DMSNs, or BGNS at a series of concentration and incubated for 24 or 48 h. Cell viability was evaluated by the CCK-8 assay. Each data point was represented as the mean ± standard deviation (SD) of three independent experiments. In each experiment, all drug concentrations were tested in six replicates.

Cellular colocalization imaging: For fluorescence colocalization imaging, MCF-7 cells were seeded in 35-mm glass-bottom culture dishes (NEST Science Co, Ltd, China) at a concentration of 1×10^5 cells/dish, and then treated with free DOX, DMSNs, or BGNS at a DOX concentration of 2 μg/mL for 4 or 24 h. After incubation

for a specified time, the cells were washed several times with pre-chilled PBS (pH = 7.4) to remove residual drug or nanoparticles. Then, cells were stained with Hoechst 33342 (cellular nucleus dye), lyso-tracker green (lysosome dye). Finally, the culture dishes were visualized under a CLSM (Leica TCS SP5, Germany). The fluorescence images were taken under 60× oil-immersion objective (NA = 1.42). Blue, green, and red luminescent emissions from Hoechst 33342, lyso-tracker green and DOX were excited at the wavelength of UV, 488 and 503 nm, respectively. The emission wavelengths were ranged from 450 to 500 nm for Hoechst 33342, 500 to 520 nm for lyso-tracker green, and 580–610 nm for DOX. There is no interference between these three channels.

Visualization of LMP: In order to measure lysosomal membrane permeabilization, MCF-7 cells were seeded in 35-mm glass-bottom culture dishes (NEST Science Co, Ltd, China) at a concentration of 8,000 cells/dish, then, cells were incubated with 100 μg/mL Alexa Fluor 488-dextran for 1 h, the growth media were replaced by fresh complete medium containing free DOX, DMSNs or BGNS at DOX concentration of 1 μg/mL and incubated for another 4 h. Fluorescence images were obtained by a fluorescence microscope (IX70, Olympus) equipped with a 100× oil immersion objective (NA = 1.30) and a blue-illumination filter set (450-480/500/525/39 nm). The percentage of cells with LMP was obtained by counting randomly chosen areas, with a minimum of 100 cells for each condition, and three independent triplicate experiments were carried out.

Caspase-3 activity: A commercial caspase-3 Activity Assay Kit was used to

determine caspase-3 activity. First, 1×10^5 MCF-7 cells were seeded in a 3.5 cm Petri dish. After a 48-h incubation with free DOX, DMSNs, or BGNS at a DOX concentration of 1 μg/mL, cells were centrifuged, washed, and lysed with cell lysis solution. After incubating with reaction buffer and caspase-3 substrate at 37 °C for 4 h, caspase-3 activity was determined at 405 nm by a microplate reader (Multiskan Mk3, Thermo Fisher Scientific Instrument Co., Ltd., Shanghai, China). The detail analysis procedure was described in the manufacturer's protocol.

Statistical Analysis: Data are described as the mean ± standard deviation, and statistical analysis was performed using One-Way ANOVA. The differences were considered significant for p values < 0.05, and $p < 0.01$ was indicative of a very significant difference.

Reference

S1. Y. Gao, Y. Chen, X. Ji, X. He, Q. Yin, Z. Zhang, J. Shi, Y. Li, *ACS nano*, 2011, **5**, 9788.

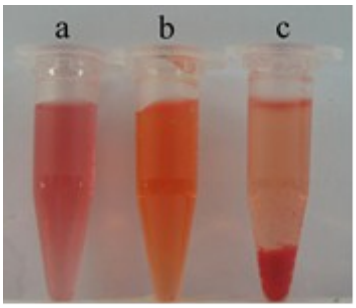

Fig. S1 Typical photographs of DOX dispersed in different solution. (a) Sodium hydroxide solution (pH 8.5), (b) Ultrapure water, (c) 0.2 M $NaHCO_3$.

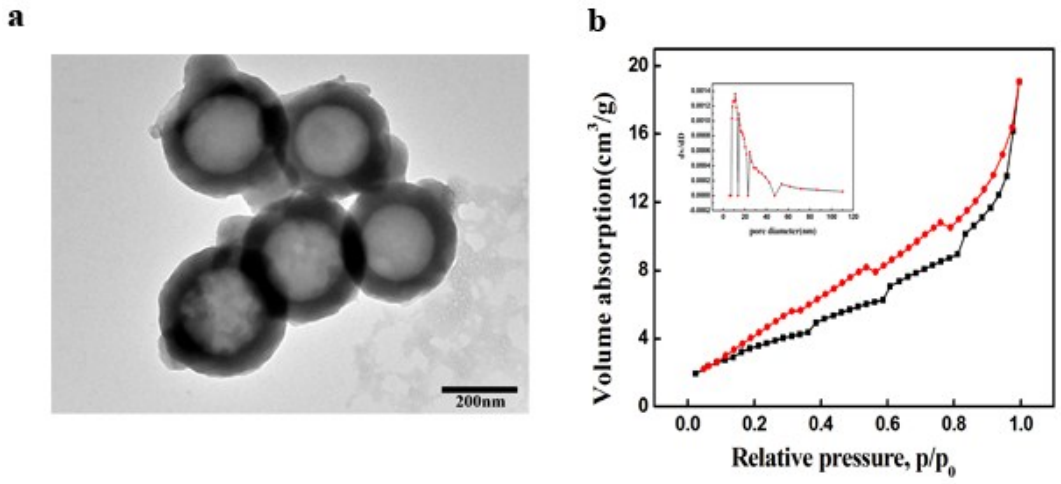

Fig. S2 Characterization of HMSNs. (a) TEM image of HMSN, (b) N_2 adsorption–desorption isotherms and pore size distribution of HMSNs (inset).

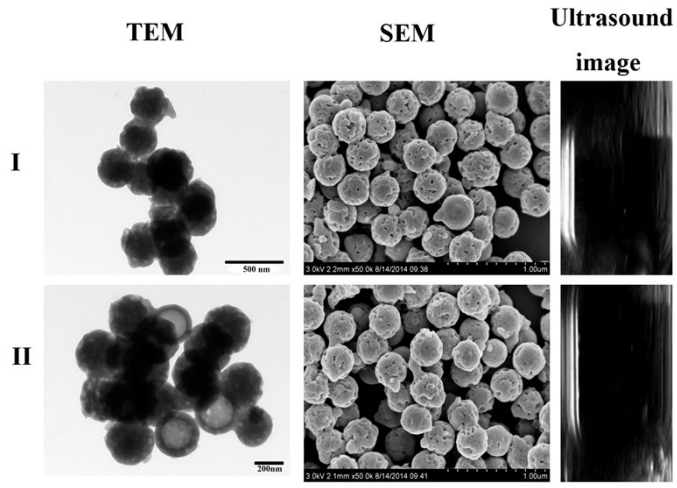

Fig. S3a

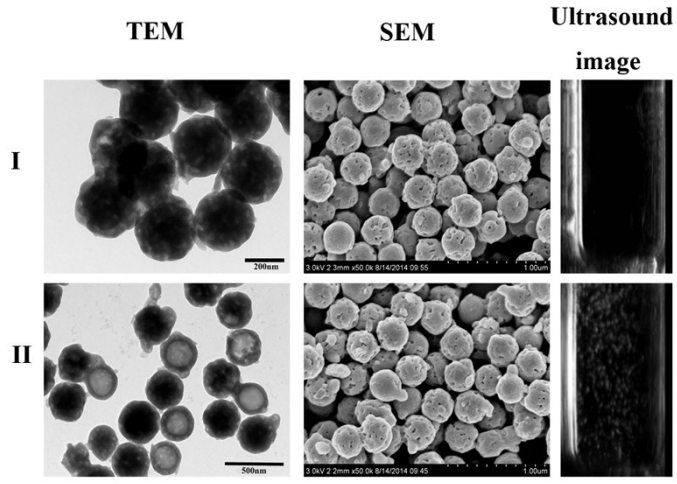

Fig. S3b

Fig. S3 TEM, SEM and ultrasound images of DMSNs and BGNS. (a) TEM, SEM micrographs and ultrasound images of DMSNs after incubation in PBS 7.4 (I) or PBS 5.0 (II). (b) TEM, SEM micrographs and ultrasound images of BGNS after incubation in PBS 7.4 (I) or PBS 5.0 (II).