Supporting Information

Multifunctional magnetic silica nanotubes for MR imaging and targeted drug delivery

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Experimental section

Materials

Sodium hyaluronan (MW~100 kD) was purchased from Liuzhou Sheng Qiang Biotechnology Corporation. Hydrazine hydrate (64% hydrazine), polyoxyethylene (20) cetyl ether (Brij 58), and tetraethyl orthosilicate (TEOS) were purchased from Acros. Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) and diethylamine were purchased from Fisher. Iron (III) acetylacetonate ($Fe(acac)_3$), tetraethylene glycol (TEG) and dopamine hydrochloride were purchased from Sigma-Aldrich. Doxorubicin (DOX) was purchased from Melone Pharmaceutical Co., Ltd. Hydrochloric acid (HCl, 37.5%), cyclohexane, and isopropanol were purchased from Sinopharm.

Synthesis of Silica Nanotube (SNT)

SNT was synthesized according to previous report[1] using nickel-hydrazine complex nanorods as templates. Briefly, 8.5 g of Brij 58 in 15 mL of cyclohexane was stirring in a water bath at 50 °C. When the Brij 58 completely dissolved, a certain volume of $NiCl_2$ solution (0.8 M) was added. After stirring for several minutes, 0.45 mL of hydrazine hydrate was added dropwise and aged for 3h. Then, 1 mL of diethylamine and 3mL of TEOS were successively added and the reaction was proceeded for 2 h for the silica coating. The adding of 0.9 and 1.2 mL of $NiCl_2$ solution resulted in the final SNT sizes of 50×38 nm and 75×45 nm, respectively. The product was centrifuged down, washed with isopropanol several times and dried overnight at 70 °C. To obtain SNTs, the dry powder was dissolved in 300 mL of HCl (1M), stirred at room temperature for 3 h, centrifuged and washed with water until a neutral pH.

Synthesis of SNT@SPIONs

About 1/4 of as-synthesized SNTs was dispersed by 1 mL of ethanol and added with 30 mL of TEG and 400 mg of $Fe(acac)_3$ in a two-neck flask. The mixture was kept at 80 °C under vacuum for 15 min to remove the ethanol, and then heated up to 210 °C under argon flow and vigorously stirred for 2 h. The solution was further heated to 290 °C and stirred for 1 h. After cooling down to room temperature, the mixture was diluted with acetone and the product was separated by a magnet and washed with acetone and ethanol for several times.

Modification and Drug Loading of SNT@SPIONs

SNT@SPIONs (20 mg) were dispersed into 10 mL of ethanol containing 5 mg/mL dopamine. The mixture was sonicated for 30 min and subsequently stirred overnight. The SNT@SPIONs were centrifuged, washed with water for several times and then dispersed into 20 mL of DOX aqueous solution (0.8 mg/mL). After stirred at room temperature for 24 h in the dark, the DOX loaded SNT@SPIONs were separated from free DOX by a magnet and washed with water. The supernatant was measured by UV-vis absorption spectrum to determine the drug loading percentage, which was defined as [weight of the loaded drug/(weight of the loaded drug + weight of SNT@SPIONs)]×100%.

Preparation of SNT@SPIONs-DOX-HA

The HA coating on SNT@SPIONs-DOX was performed by drop-wise adding 5 mL of HA aqueous solution (1 mg/mL) into 5 mL of SNT@SPIONs-DOX dispersion (1 mg/mL) under vigorous stirring for 30 min. The obtained SNT@SPIONs-DOX-HA were harvested by a magnet and washed with water.

Characterization

Transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDX) were carried out on FEI-F20 electron microscopy operated at 200 kV. The room temperature magnetic hysteresis loop was determined by a superconducting quantum interference device (SQUID) magnetometer (MPMS-7, Quantum Design) with magnetic field up to 20 kOe. Nitrogen sorption isotherms were measured at 77 K with an Autosorb-IQ surface area and pore size analyzer (Quantachrome Instruments). Dynamic light scattering (DLS) and Zeta-potential were acquired with a Malvern Zetasizer NanoZS Instrument. Fe content was determined by a Perkin-Elmer/OPTIMA 7000DV inductively coupled plasma optical emission spectrometry (ICP-OES). T_2 relaxivity was measured using a 3T clinical MR scanner (Siemens) at room temperature.

Cell Culture

4T1, A549 and Beas-2B cells were regularly cultured in DMEM supplemented with 10% heat-inactivated FBS, and 1% penicillin and streptomycin at 37 °C and 5% CO_2, respectively.

In Vitro Drug Release

The prepared SNT@SPIONs-DOX-HA solution (1 mL) was transferred into a dialysis tube (MWCO 3500K) and immersed in 20 mL PBS (with pH~7.4 and 5.5, respectively) at 37 °C under shaking (200 rpm). At certain time intervals, 1 mL of the external buffer was withdrawn and replenished with an equal volume of fresh PBS. The amount of the drug release was measured by UV-vis absorption spectrum at 488 nm.

In Vitro Cytotoxicity

In vitro cytotoxicity of SNT@SPIONs-HA and SNT@SPIONs-DOX-HA were evaluated by MTT viability assay. Cells with a seeding density of 5×10^3 cell/well were cultured in 96-well plates in 100 μL DMEM medium supplemented with 10% FBS at 37 °C for 24 h. Then the medium was removed and replaced with fresh medium containing SNT@SPIONs-HA, SNT@SPIONs-DOX-HA and free DOX at various DOX concentrations, respectively. After 48 h incubation, 10 μL of MTT solution (5 mg/mL in PBS) was added to each well and the plates were incubated for another 4 h. The medium was replaced and 100 μL of DMSO was added. The optical absorbance was measured using a microplate reader at the wavelength of 490 nm.

Confocal Laser Scanning Microscope Observation

4T1 cells were seeding in 8-well plate in FBS containing DMEM and incubated for 24 h at 37 °C. The medium was then discarded and the cells were incubated with fresh medium containing SNT@SPIONs-DOX-HA with free HA, SNT@SPIONs-DOX-HA, SNT@SPIONs-DOX-HA with magnetic field and equivalent free DOX (5 μg/ml), respectively. After incubated for another 1h, the medium was removed and the cells were washed with PBS for three times and fixed with 10% formalin (0.5 mL/well) for 10 min. After washing the cells with PBS, Hoechest 33258 was added to stain the nuclei for 10 min. The cells were washed with PBS followed by confocal microscopy fluorescent imaging.

Determination of Cellular Uptake by Flow Cytometry

Cells with a seeding density of 2×10^5/well were seeded in 24-well plate in DMEM supplemented with 10% FBS overnight at 37 °C. The medium was removed and then added with SNT@SPIONs-DOX-HA with free HA, SNT@SPIONs-DOX-HA, SNT@SPIONs-DOX-HA with magnetic field and equivalent free DOX (5 μg/ml), respectively. The cells were incubated for 1 h and then washed three times with PBS, trypsinized, centrifugated and resuspended in PBS. The uptake of the nanoparticles were measured using a BD Accuri C6 flow cytometer.

In Vivo MR Imaging

Kunming mice were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and used under protocols approved by Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences Animal Care and Use Committee. To setup 4T1 tumors, 4T1 cells (5×10^6) suspended in serum-free cell medium were injected on the flanks of each mouse. After one week, SNT@SPIONs-DOX-HA (200 μL, 1 mg/mL) was administrated to mouse through tail-vein injection for in vivo imaging. Magnetically targeting was conducted by attaching a small magnet to the tumor on the left flank. MR imaging was performed using a 3-T clinical MRI scanner (Siemens) equipped with a special coil designed for small animal imaging.

Biodistribution study

The 4T1 tumor-bearing mice were intravenously injected with 200 μL of SNT@SPIONs-DOX-HA (2 mg/mL). After 24 h, the mice were sacrificed and the heart, liver, spleen, lung, kidney and tumor were collected. The organs were weighted and digested with aqua regia for one day. The Fe content was measured by ICP-OES, and the background Fe signals of the organs in untreated mice were also determined and subtracted.

Histology examination

The mice treated with SNT@SPIONs-DOX-HA were sacrificed at 24 h post-injection. The organs were collected, fixed with 10% formalin and sectioned at 8 μm. After washing, the sections were stained with hematoxylin for 3 min, washed with water and stained with eosin for another 1 min. The observation was performed on a Nikon Eclipse 90i microscope.

References

1 C. Gao, Z. Lu and Y. Yin, *Langmuir*, 2011, **27**, 12201–12208.

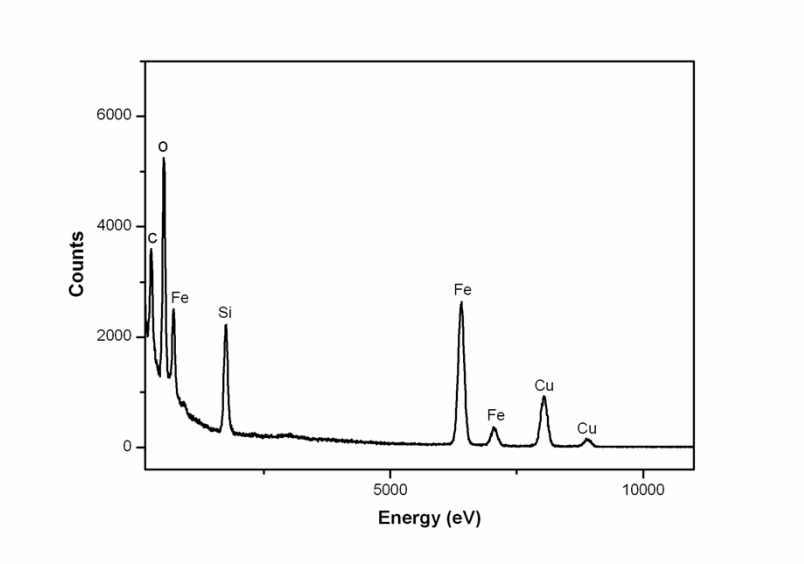

Fig.S1 EDX spectrum of SNT@SPIONs nanocomposites.

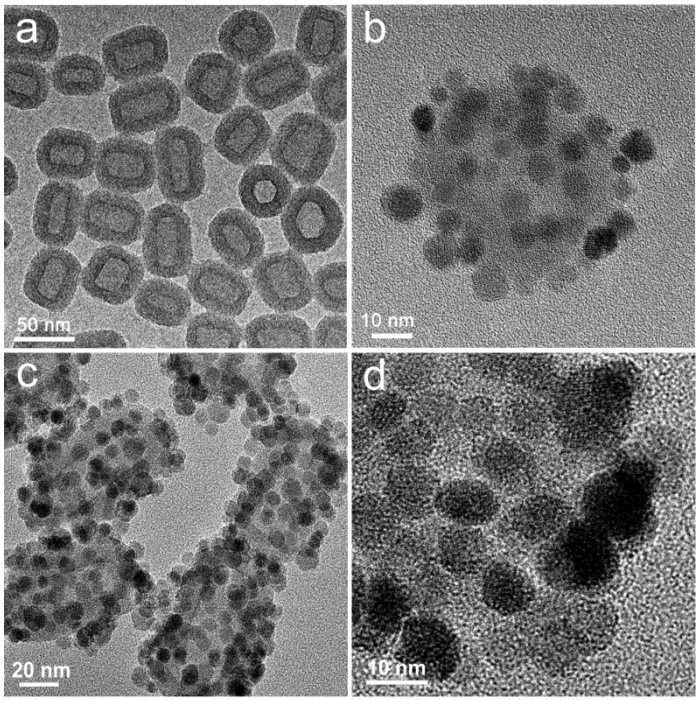

Fig. S2 (a-b) TEM images of SNTs with a dimension of 38×50 nm (a) and the resulting SNT@SPIONs nanocomposites (b). (c) TEM image of SNT@SPIONs nanocomposites derived from 45×75 nm templates with higher SPIONs packing density. (d) An enlarged image of single SNT@SPIONs in (c).

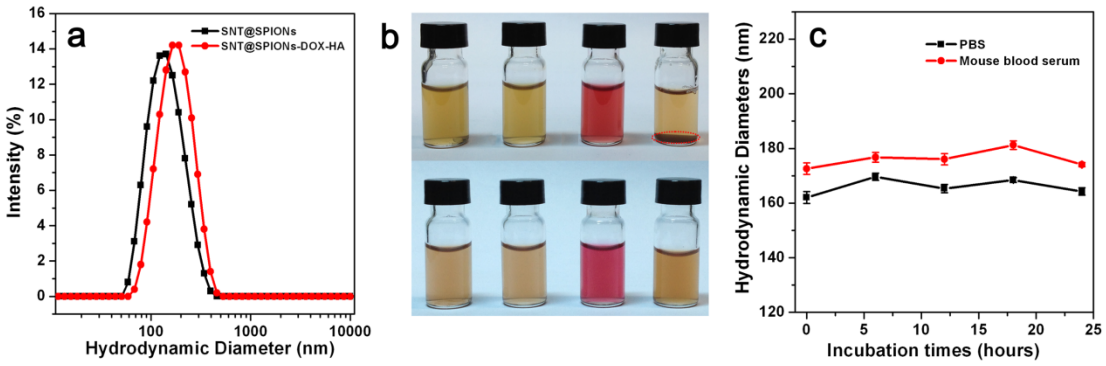

Fig. S3 (a) Hydrodynamic diameter distributions of SNT@SPIONs and SNT@SPIONs-DOX-HA water dispersions by DLS. (b) Photographs of SNT@SPIONs (upper panel) and SNT@SPIONs-DOX-HA (lower panel) dispersed in water, PBS, DMEM and mouse blood serum (from left to right) after 24 h incubation. Red circle indicates the precipitates observed in SNT@SPIONs serum dispersion. (c) Hydrodynamic diameters of SNT@SPIONs-DOX-HA in PBS and mouse blood serum during 24 h incubation at 37 ºC.

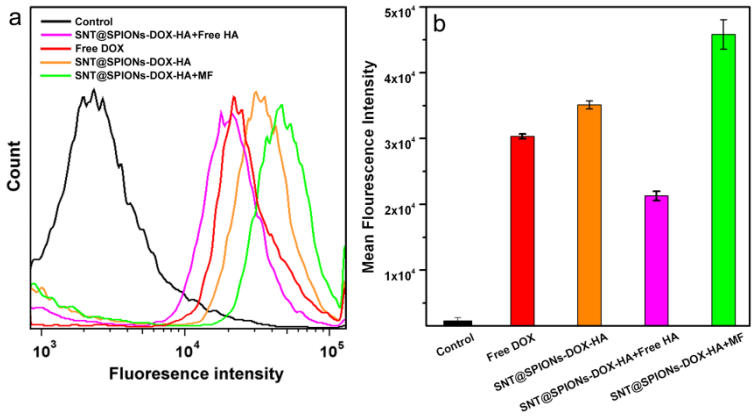

Fig. S4 (a-b) Flow cytometric analysis of control 4T1 cells, and 4T1 cells treated with free DOX, SNT@SPIONs-DOX-HA, SNT@SPIONs-DOX-HA with free HA and SNT@SPIONs-DOX-HA under magnetic field.

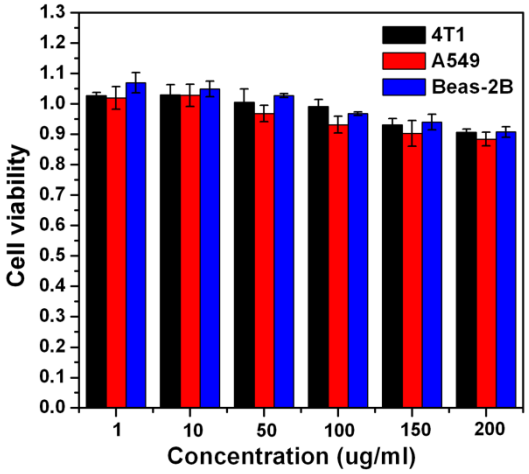

Fig. S5 Viabilities of 4T1, A549 and Beas-2B cells after incubation with SNT@SPIONs-HA for 48 h.

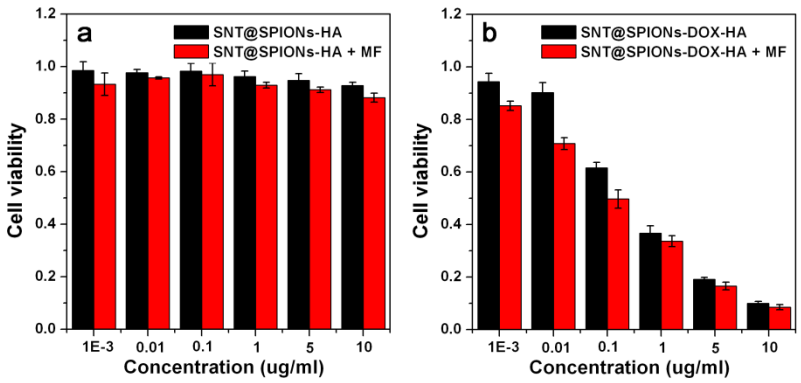

Fig. S6 Viability of 4T1 cells after incubation with SNT@SPIONs-HA (a) and SNT@SPIONs-DOX-HA (b) in absence and presence of magnetic field for 48 h, respectively.

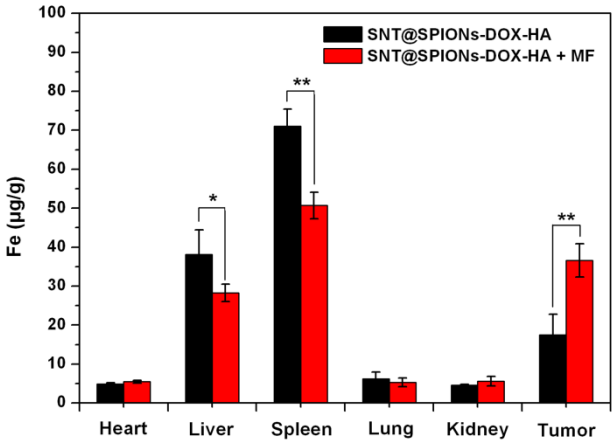

Fig. S7 Biodistribution of Fe in the major organs of the 4T1 tumor-bearing mice at 24 h post-injection of SNT@SPIONs-DOX-HA. Error bars are based on SD of three mice per group. (*, $p < 0.05$; **, $p < 0.01$). The Fe content was determined by ICP-OES, with the Fe background signal from untreated mice subtracted.

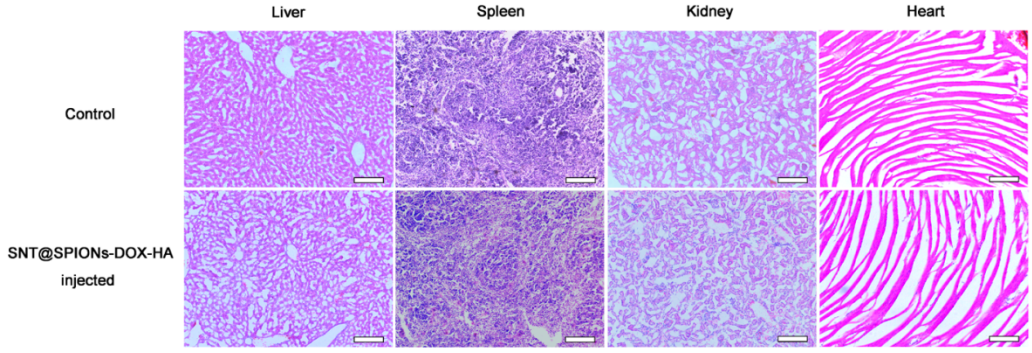

Fig. S8 H&E stained images of major organs collected from the mice treated with SNT@SPIONs-DOX-HA at 24 h post-injection. Scale bars = 200 μm.