

Electronic Supplementary Information

Preparation of Magnetic Rattle-type Silica through a General and Facile Pre-shell/post-core Process for Simultaneous Cancer Imaging and Therapy

Li Qiang,^{a,c} Xianwei Meng,^a Linlin Li,^a Dong Chen,*^b Xiangling Ren,^a Huiyu Liu,^a Jun Ren,^a Changhui Fu,^a Tianlong Liu,^a Fuping Gao,^a Yanqi Zhang,^a and Fangqiong Tang*^a*

^a Laboratory of Controllable Preparation and Application of Nanomaterials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, People's Republic of China. E-mail: tangfq@mail.ipc.ac.cn, mengxw@mail.ipc.ac.cn; Fax: +86-10-62554670;

Tel: +86-10-82543521

^b Beijing Creative Nanophase Hi-Tech Company, Limited, Beijing 100086, People's Republic of China. E-mail: creative.nanophase@gmail.com

^c University of Chinese Academy of Sciences
Beijing 100049, People's Republic of China.

Experimental Section

Materials

All the reagents used in this work are products of analytical purity and used without further purification. Ammonium ferrous sulfate and ammonium ferric sulfate are purchased from Xilong Chemical corp. Ethanol and ammonia and hydrochloric acid are obtained from Beijing Chemical works. Various kinds of rattle-type silica are synthesized through previous method.

Synthesis of the MRSs

A typical synthesis of MRSs described as follows. Firstly, different kinds of rattle-type silica particles were prepared by a previously described method. Then 50 mg of the silica was dispersed in 10 mL water and 3 g ammonium ferrous sulfate and 3.69 g ammonium ferric sulfate were dissolved. After stirring for 6 h, the particles were isolated by centrifugation. We decanted the supernatant, then quickly poured ethanol to redisperse the particles and used ethanol to wash the particles for two times. Secondly, the particles were dispersed in 50 ml ethanol at a nitrogen atmosphere and 0.2 M ammonia was added to adjust pH to 8 with stirring and further kept at 70 °C for 2 h. The final particles were isolated by a magnet and washed with ethanol for several times. Finally, 0.1 M HCl was used to remove the residue iron oxide outside the silica shell.

Characterization

The yielded nanocomposites were characterized by transmission electron microscope (TEM), scanning electron microscope (SEM), X-ray diffraction (XRD), Energy Dispersive Spectroscopy (EDS), Fourier Transform infrared spectroscopy (FTIR) and hysteresis loop. TEM (JEM-2100F JEOL) and SEM (Hitachi 4300 and Hitachi S4800) were used to characterize the surface morphology. EDS result was also made by JEM-2100F JEOL. XRD measurements were performed on a D8 Focus X-ray diffractometer with a scanning rate of 0.02 deg/s. FTIR was taken on Varian Excalibur 3100. The magnetic hysteresis loops of the samples were recorded on a physics property measurement system (PPMS-9 Quantum Design Co.). Inductively

coupled plasma (ICP) measurement was performed on an inductive coupled plasma-optical emission spectrometer (ICP-OES, VISTA-MPX).

Drug release loading and release

To load Docetaxel (Sanwei Pharmaceutical., Shanghai) into the cavities of the MRSs, MRS was dispersed in 20 mg mL⁻¹ Docetaxel in ethanol and stirred for 24 h, following washing extensively with Phosphate buffered saline (PBS) to get Docetaxel loaded MRS (MRS-Dtxl). For drug release assay, the MRS-Dtxl samples were put into dialysis tubing (10,000 Da) and immersed in release medium, stirring at 25 °C. 4 mL release medium was taken at given time intervals. The concentration of Docetaxel was determined by UV/vis spectroscopy measurements at a wavelength of 230 nm.

The *in vitro* MRI

5×10⁵ of HepG-2 cells were incubated with different concentration of MRS for 4 h. Then the cells were trypsinized and sufficiently washed with PBS for 2 times. The cells were dispersed in 50 μL low-melting 1% agarose gel at 40 °C. Samples were then quickly chilled at -20 °C until gelation. The *in vitro* MR imaging experiment was performed on a 3.0 T clinical Philips magnetic resonance system at room temperature. For T₂ relaxivity measurement, firstly, the Fe concentration of the MRS in water was determined by inductive coupled plasma atomic emission spectrometry (ICP-OES). Then, different Fe concentrations of MRSs were prepared in pure water. The T₂ relaxation time was performed with the following parameters: Relaxivity values of R₂ were calculated through the curve fitting of 1/T₂ relaxation time versus the Fe concentration (mM).

The *in vivo* tumor imaging

All experiments involving animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee. Balb/c nude mice (female, 4-6 week old, 18–22 g; Vital River Laboratory Animal Technology Co. Ltd., Beijing) were injected subcutaneously into the thighs of the mice with 0.1 mL cell suspension containing 5×10⁶ HepG2 cells. When the tumor size reached to about 400 mm³, 400 μg MRS in 200 μL PBS were intravenously injected into the mice via the tail vein. MR images were taken prior to injection and one hour after intravenous

injection. The mouse was subjected to the T₂-weighted MRI test using the same 3.0 T clinical Philips magnetic resonance system at room temperature. Signal intensities (SIs) were measured in defined regions of interest (ROI), which were in comparable locations within tumor sites.

Cell viability test

The cytotoxicity of nanoparticles was evaluated by WST-1 assay. Human liver carcinoma cells of Hep-G2 cells (ATCC) were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco), supplemented with 10% FBS (Hyclone) at 37 °C in a humidified atmosphere of 5% CO₂. The cell culture media were supplemented with 100 units/mL penicillin and 100 µg MI⁻¹ streptomycin. The HepG-2 cells were plated in 96-well plates at 8000 cell/well for 24 h detection or 3000 cell/well for 48 h detection and incubated at 37 °C. Cell viability was assessed by adding WST-1 reagent (Roche) to the culture medium at 1:10 dilution. After 4 h of incubation at 37 °C, colorimetric measurements were performed at 450 nm on a scanning multiwell spectrometer (Multiskan MK3 Thermo labsystems). Data were expressed as mean ± standard deviation (SD) of at least six independent experiments.

Animals viability experiments

The MRSs were dispersed in sterile 5% glucose and sonicated for 15 minutes before use. The starting dose administered intravenously with MRSs was 200 mg kg⁻¹. If major adverse reactions were not examine in the MRSs-treated group within 10 days, Mice in the next group were received a higher dose (400 mg kg⁻¹). The control group were injected with equal-volume 5% glucose. Mortality and clinical feature of the mice were carefully monitored during the experiment period. At day 10, a part of the heart, liver, spleen, lung and kidney was collected and fixed in 10% formalin. The tissues were stained with hematoxylin and eosin (H&E). Histological analysis was performed using an optical microscope.

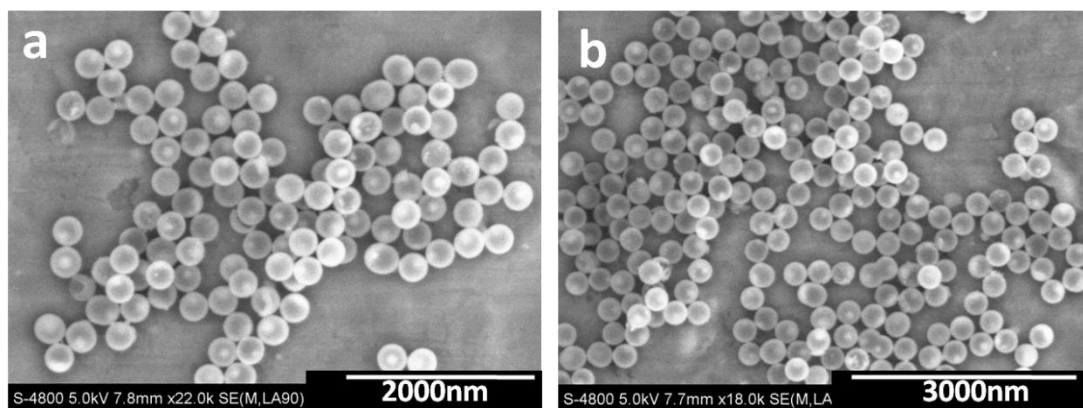


Figure S1. SEM images of the magnetic rattle-type silica nanocomposites

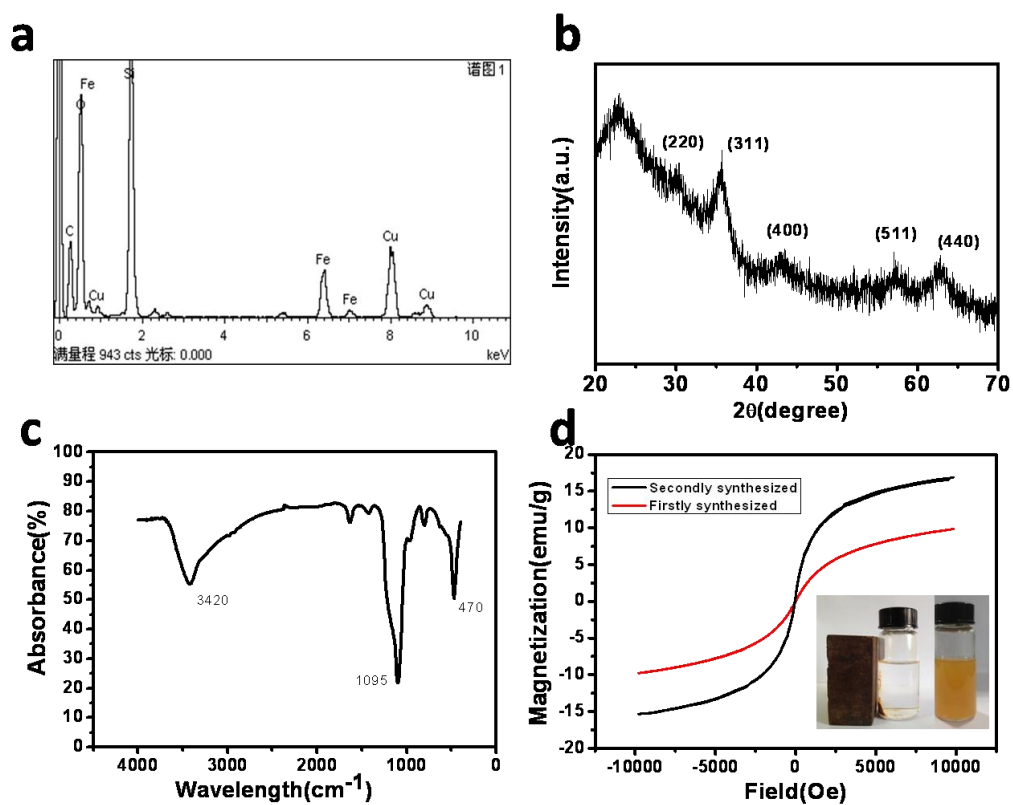


Figure S2. (a) EDX spectrum, (b) XRD patterns, (c) FTIR absorbance spectrum, and (d) Room temperature magnetization curve of the MRSs; Inset of (d) the photo of the MRS attracted by a magnet.

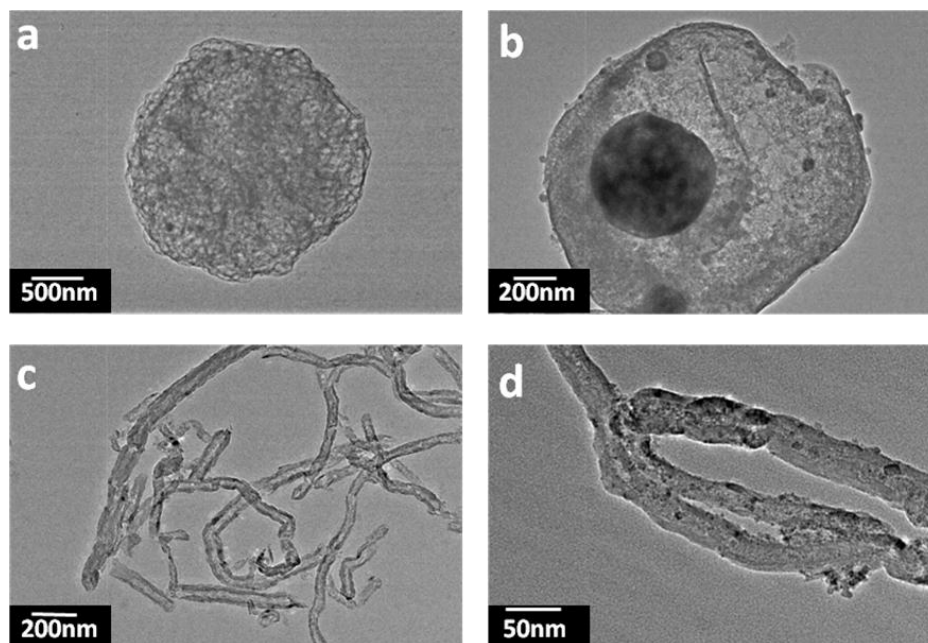


Figure S3. The magnetic nanoparticles produced in different cavity structures. (a) Hollow carbon sphere and (b) the as-prepared magnetic composite; (c) carbon nanotube and (d) the as-prepared magnetic carbon nanotube.

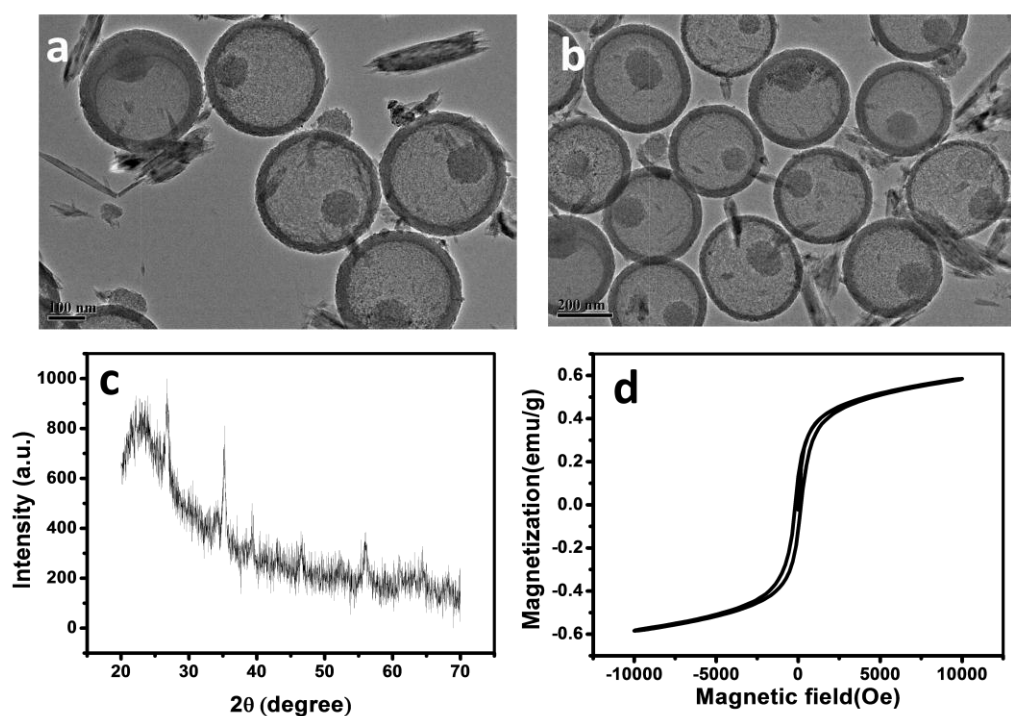


Figure S4. (a)(b)The TEM images of the product synthesized by chloride at different scale; (c) XRD pattern and (d) Hysteresis loop measured at room temperature of the composites.

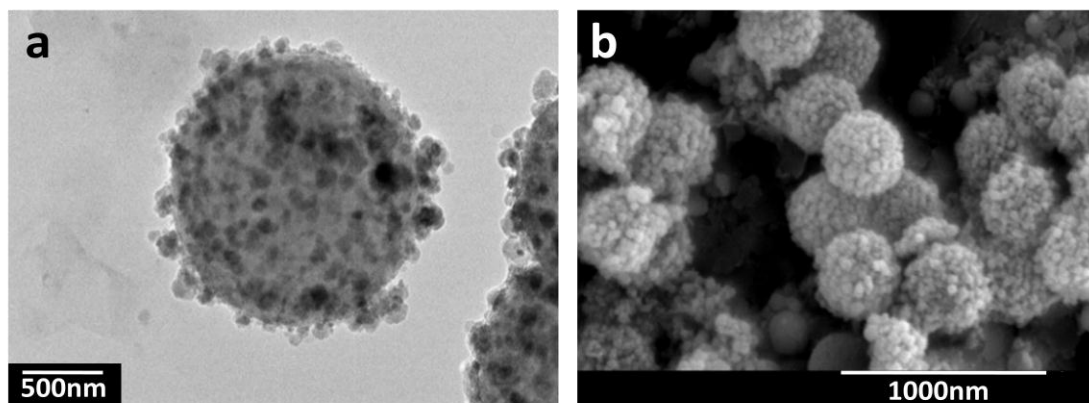


Figure S5. TEM and SEM micrographs of the composite produced in the water phase as contrast.

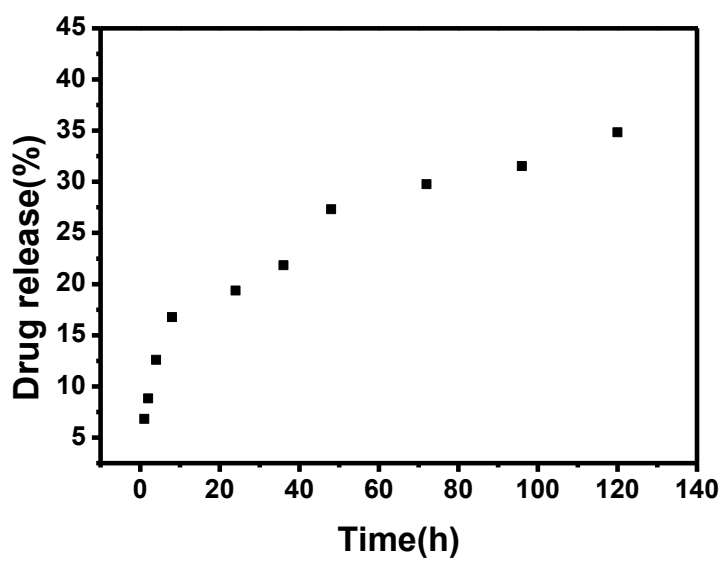


Figure S6. Docetaxel release from MRSs versus release time.

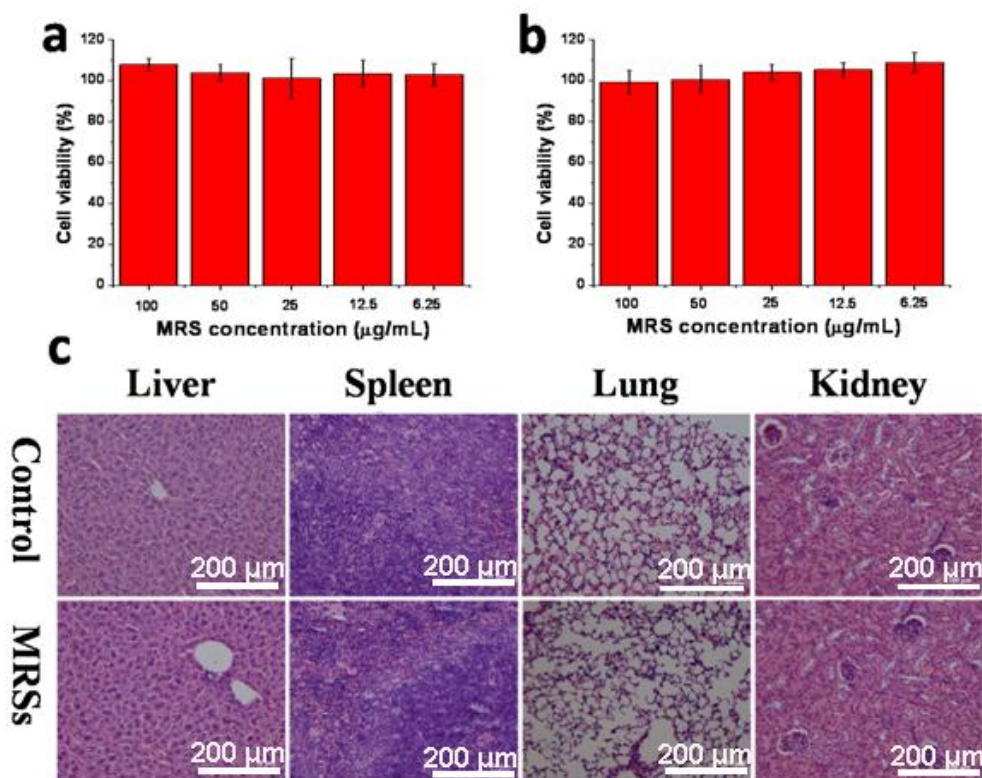


Figure S7. (a) and (b) the cell viability at different MRSs concentration at 24 hours and 48 hours. (c) Histological analyses of liver, spleen, lung and kidney from mice injected with MRSs at 400 mg kg^{-1} and the control group. Scale bar is $200 \mu\text{m}$.

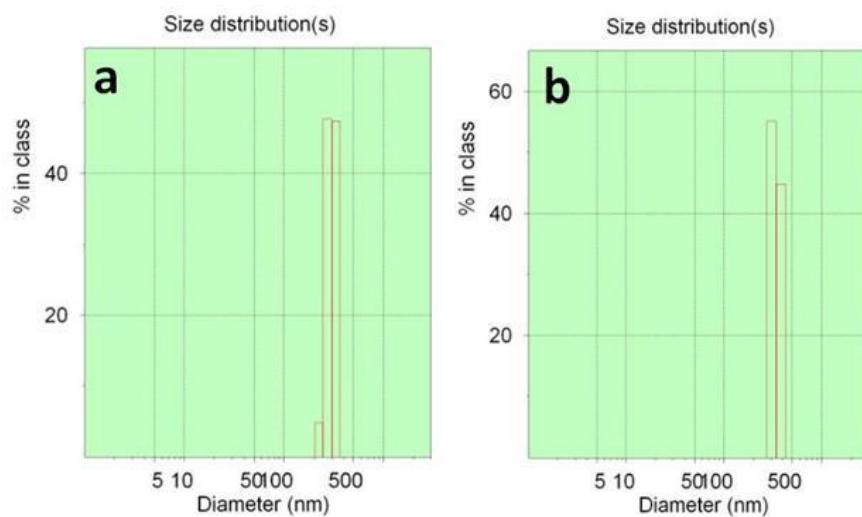


Figure S8 MRS diameter distribution in water(a) and glucose solution (b) obtained from DLS technique.