Electronic Supplementary Information

Detecting and delivering platinum anticancer drug by fluorescent maghemite nanoparticles

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1 Experimental

1.1 Reagents and materials

Cisplatin was obtained from Shandong Boyuan Chemical Co., Ltd. Supercoiled pUC19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian). Tris-(hydroxymethyl)aminomethane and ethidium bromide (EB) were purchased from Sigma. (3-Aminopropyl)-triethoxy-silane, R6G and tetraethylorthosilicate were purchased from Alfa-aesar. $FeCl_3 \cdot 6H_2O$, NaOH, succinic anhydride，ethylene glycol (EG) and other chemical reagents were of analytical grade and used as received without further purification. Doubly deionized water (18.2 MΩ*cm at 25 °C) prepared on a Milli-Q (MQ) water system was used throughout all experiments.

1.2 Methods

Images of scanning electron microscopy (SEM) were taken using Hitachi S-4800 field emission electron microscope at an accelerating voltage of 5 kV; and those of TEM were obtained using a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. Samples for TEM were prepared by dripping a droplet of dilute phosphate-buffered saline (PBS) sample solution (5 μL) onto a carbon-coated copper grid and drying at room temperature. X-ray photoelectron spectra (XPS) were acquired on a Thermo ESCALAB 250 electron spectrometer with 150 W monochromatized Al Kα radiation (1486.6 eV), where all peaks were referred to the signature C1s peak for adventitious carbon at 284.8 eV. FTIR spectra were recorded on a Nicolet 6700 Fourier transform infrared spectrograph in the range of 4000–400 cm^{-1}. Field-dependent magnetization measurements were carried out on the superconducting quantum interference device (SQUID, Quantum Design) magnetometer by MPMS-XL-7T instrument at 300 K. The inductively coupled plasma mass spectrometry (ICP-MS) data were determined using a standard Plasma-Quad II instrument (VG Elemental, Thermo Optek Corp.). MRI experiments were performed at gradient concentrations in terms of iron in the PBS buffer on a 3.0 T MR scanner (3 T Siemens Magnetom Trio, Siemens, Germany) with a spin-echo pulse sequence. The transverse relaxivity (r_2) was calculated according to the measured T_2. The fast spin-echo was performed under the following conditions:

matrix size = 256 × 256, field of view (FOV) = 160 mm × 160 mm, slice thickness (SL) = 3 mm, echo time (TE) = 12.3 ms, repetition time (TR) = 1500 ms, and number of acquisitions = 1. Confocal fluorescence microscopic images of the cells and zebrafish were obtained using fluorescence microscope (Olympus TH4-200) and ZEISS Laser Scanning Microscope (LSM 710). All experiments with live animals were performed in compliance with the relevant laws in China and approved by Nanjing University.

1.3 Synthesis and characterization of the CMDP-OTPBA-R6G-SPION

Fe_3O_4 NPs were prepared by a modified literature method.[1] γ-Fe_2O_3 NPs were obtained by oxidizing Fe_3O_4 NPs in HNO_3 (0.01 M) at 95 °C for 1 h. OTPBA were obtained according to the reported procedures.[2] γ-Fe_2O_3 NPs (30 mg) and R6G (0.5 g) were dispersed into the mixture solution of isopropanol (200 mL), H_2O (2 mL) and $NH_3 \cdot H_2O$ (1 mL) under ultrasonic wave for 30 min. Ethanol (10 mL) solution of tetraethylorthosilicate (TEOS, 500 μL) was added slowly into the reaction system within 1 h and the temperature was kept at 50 °C for 3 h. The coated magnetic nanoparticles (R6G-SPION) were collected by magnetic separation, washed with water and ethanol for at least five times to remove the unreacted TEOS and R6G. R6G-SPION was again dispersed into the mixture solution as described above. OTPBA (0.5 g) was added slowly into the reaction system within 1 h and kept reacting for 3 h at 50 °C. OTPBA-R6G-SPION was gained by magnetic separation. The unreacted OTPBA was removed by washing with water and ethanol for at least five times respectively.

Cisplatin (90 mg) and $AgNO_3$ (51 mg) were dissolved in *N*,*N*-dimethylformamide (DMF, 9 mL) and stirred in the dark at room temperature for 12 h. The resultant suspension was centrifuged to remove the precipitate and CMDP was acquired in the supernatant. OTPBA-R6G-SPION was dispersed into the solution of CMDP and sonicated to give a suspension, which was stirred vigorously for 48 h. CMDP-OTPBA-R6G-SPION was obtained with magnetic separation. The redundant CMDP was removed by washing with DMF and ethanol. Exhaustive separation was checked by detecting platinum content in the supernatant with ICP-MS. The loading ratio of Pt to the conjugate was also determined by ICP-MS. The sample for ICP-MS

was treated with concentrated HNO_3 at 95 °C for 2 h. The average of three replicates was taken as the final result.

1.4 Agarose gel electrophoresis

Supercoiled pUC19 DNA (20 ng μL^{-1}) was treated with gradient concentrations of CMDP-OTPBA-R6G-SPION in the buffer of pH 7.4 (50 mM Tris-HCl/50 mM NaCl) and pH 5.2 (50 mM HAc-NaAc, 50 mM NaCl,), respectively. The mixtures (10 μL) were incubated at 37 °C for 24 h. The resulting solutions were loaded onto the agarose gel (1%) and subjected to electrophoresis in a TAE buffer (40 mM Tris acetate and 1 mM EDTA). DNA bands were stained by EB, visualized under UV light, and photographed.

1.5 Cytotoxicity assay

The cytotoxicity of CMDP-OTPBA-R6G-SPION was investigated by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with cisplatin as the reference. Briefly, A549 and A549R cell lines were seeded respectively in 96-well plates at 5000 cells per well in Dulbecco's modified Eagle's medium and incubated at 37 °C in the humidified atmosphere with 5% CO_2 for 24 h. The cells were then treated in triplicate with fresh medium containing grade concentrations of CMDP-OTPBA-R6G-SPION and cisplatin (in terms of Pt), respectively. Aliquot of MTT (20 μL, 5 mg mL^{-1}) PBS solution was added to each of the wells after the cells were incubated at 37 °C for 48 and 72 h, respectively. The supernatant was taken off after 4 h of incubation and DMSO (200 mL) was added to each well. The remanent nanocomposite was attracted to the bottom of 96-well plate by an external magnet under the well for 20 min, and the supernatant (100 mL) was removed to another 96-well plate for the enzyme-linked immunosorbent assay (ELISA). The absorbance at 570 nm was determined using an ELISA plate reader. The cytotoxicity was calculated based on the data of three replicate tests.

1.6 In vitro and in vivo fluorescence imaging

A549 and A549R cells (2.5×10^5) were cultured in 6 cm plates containing Dulbucco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (10%), penicillin (100 units mL^{-1}), streptomycin (100 μg mL^{-1}), and $NaHCO_3$ (3.7

mg mL^{-1}). After incubation for 24 h, CMDP-OTPBA-R6G-SPION (120 μg mL^{-1}) was added to the dishes and incubated for 24 and 48 h at 37 °C, respectively. Before imaging, the culture medium was removed and the cells were rinsed three times with 1 × PBS. The fluorenscence image was recorded by a confocal fluorescence microscope. The band path is from 550 to 600 nm, and the excitation wavelength is 530 nm.

The 5-day-old zebrafish larva was incubated with CMDP-OTPBA-R6G-SPION (24 μg mL^{-1}) in distilled water for 10 h at 28 °C. After washing with distilled water to remove the nanocomposite, the zebrafish larva was imaged by both fluorescence microscope and laser scanning confocal fluorescence microscope.

1.7 Cellular uptake

A549 and A549R cells (2.5×10^5) were seeded in 6 cm plates containing Dulbecco's modified Eagle's medium and incubated overnight at 37 °C in a humidified atmosphere of 5 % CO_2 for 24 h. The medium was then replaced with medium containing CMDP-OTPBA-R6G-SPION (120 μg mL^{-1}) and incubated for 12, 24 and 48 h. The medium was removed, and the cells were then washed with ice-cold PBS and digested by addition of trypsin (200 μL) to each plate. The cells were then collected and digested by HNO_3 (100 μL) and H_2O_2 (25 μL) for 2 h at 95 °C respectively, and HCl (40 μL) was continually added for 30 min at room temperature. The samples were then diluted to 1 mL. The content of platinum and iron in the samples was determined by ICP-MS.

2 Supplementary Figures

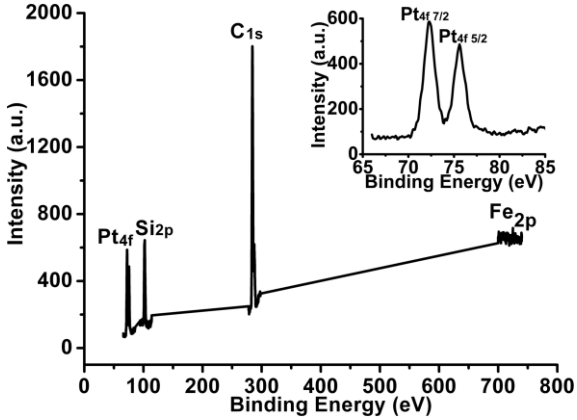

Fig. S1 XPS spectrum of CMDP-OTPBA-R6G-SPION. Inset shows the high

resolution XPS spectrum of Pt4f.

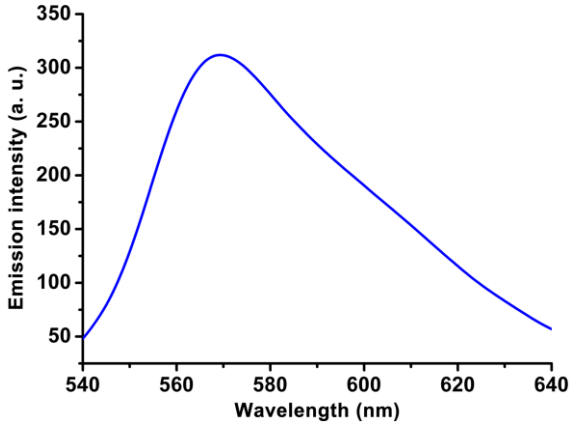

Fig. S2 Emission spectrum (λ_{ex} = 530 nm) of CMDP-OTPBA-R6G-SPION in PBS (pH 7.4).

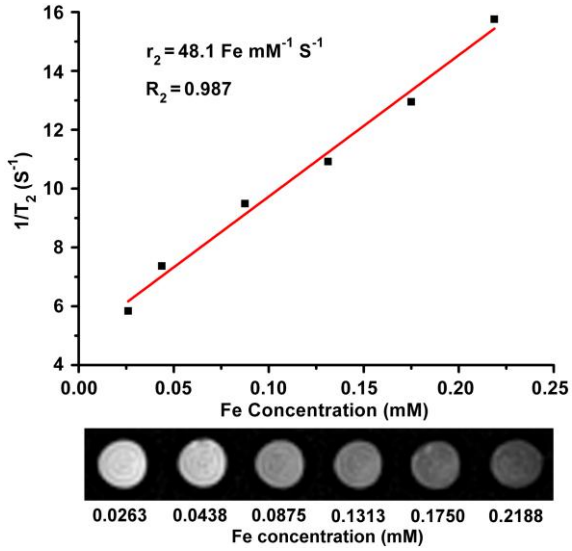

Fig. S3 T_2 relaxation rate ($1/T_2$) versus the Fe concentration (top) and T_2-weighted MR images of CMDP-OTPBA-R6G-SPION (bottom) in the PBS buffer (pH 7.4) solutions at different Fe concentrations. The transverse relaxivity (r_2), was calculated according to the equation: $1/T_2 = 1/T^0 + r_2 \cdot [\text{Fe}]$, where $1/T_2$ is the observed relaxation rate in the presence of the nanocomposite, $1/T^0$ is the relaxation rate of pure water, and [Fe] is the concentration of CMDP-OTPBA-R6G-SPION.[2]

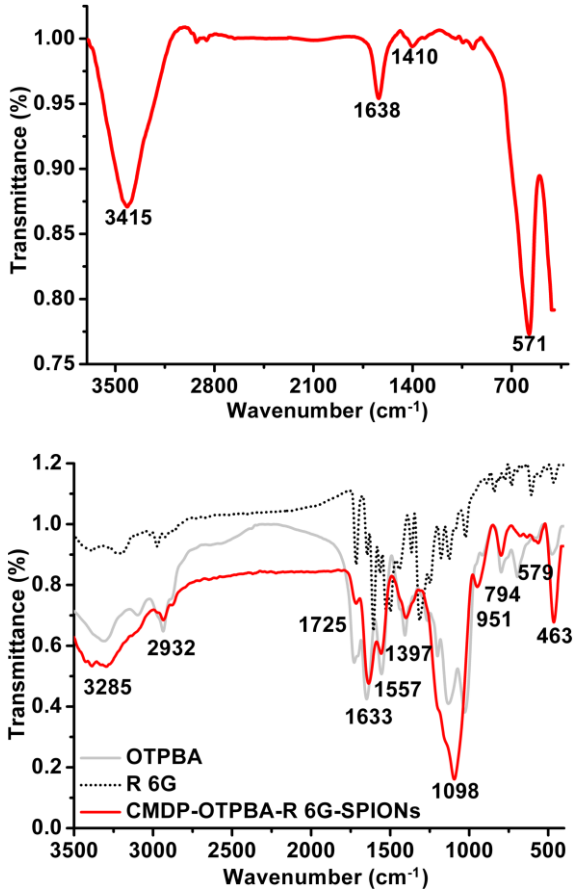

Fig. S4 FTIR spectra (KBr) of γ-Fe_2O_3 nanoparticles (top), OTPBA, R6G and CMDP-OTPBA-R6G-SPION (bottom). Attributions (cm^{-1}): 3285, ν_{O-H}; 2932, ν_{CH2}; 1725, $\nu_{C=O}$ carboxylic acid; 1633, $\nu_{C=O}$ amide; 1557, δ_{N-H}; 1397, 1098, δ_{Si-O}; 579, ν_{Fe-O}.

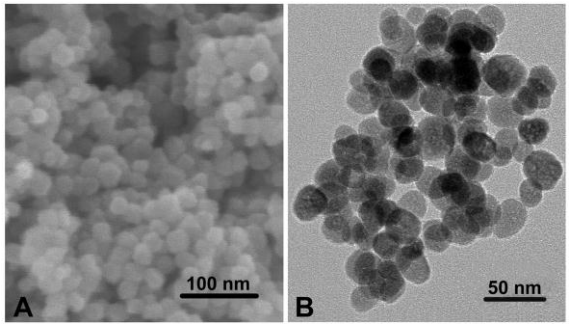

Fig. S5 SEM (A) and TEM (B) images of γ-Fe_2O_3 nanoparticles.

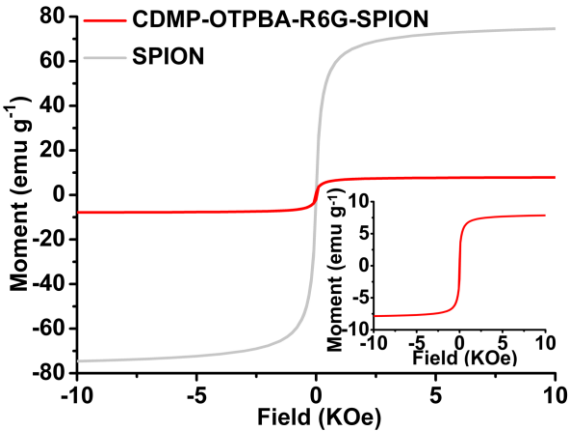

Fig. S6 Magnetization curve of CMDP-OTPBA-R6G-SPION and SPION at room temperature.

3 References

1. L. Y. Wang, J. Bao, L. Wang, F. Zhang and Y. D. Li, *Chem. Eur. J.*, 2006, **12**, 6341.
2. J. Aburto, M. Ayala, I. Bustos-Jaimes, C. Montiel, E. Terrés, J. M. Domínguez and E. Torres, *Microporous Mesoporous Mat.*, 2005, **83**, 193–200.
3. Hong Yang, Cuixia Zhang, Xiangyang Shi, He Hu, Xiaoxia Du, Yong Fang,Yanbin Ma, Huixia Wu and Shiping Yang, *Biomaterials*, 2010, **31**, 3667–3673.