Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting Information

Designed synthesis of multifunctional Fe_3O_4@carbon/zinc phosphate nanoparticles for simultaneous imaging and synergic chemo-photothermal cancer therapy

Chunyan Wang,[a] Lingyu Zhang,[a] Shengnan Li,[a] Manjie Zhang,[a] Tingting Wang,[b] Lu Li,*[a] Chungang Wang*[a] and Zhongmin Su.[a]

[a]Faculty of Chemistry, Northeast Normal University, Changchun, 130024, P. R. China

[b]School of Chemistry & Environmental Engineering, Changchun University of Science and Technology, Changchun, 130022, P. R. China

E-mail: wangcg925@nenu.edu.cn, lil106@nenu.edu.cn

Experimental Section

Materials: Anhydrous ferric chloride ($FeCl_3$, 98%), zinc oxide (ZnO), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), potassium dihydrogen phosphate (KH_2PO_4), and isopropyl alcohol (IPA) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Poly(acrylic acid) (PAA, $M_w \approx 1800$) and DOX hydrochloride were obtained from Sigma-Aldrich (USA). Deionized water was used in all experiments. All chemicals were used without any further purification.

Characterization: Transmission electron micrographs (TEM) were taken by JEOLJEM-2100F transmission electron microscope under a 200 kV accelerating voltage. Scanning electron microscopy (SEM) images and the energy dispersive X-ray (EDX) spectrum were performed with an XL30 ESEM-FEG field-emission scanning electron microscope (FEI Co.). High-resolution TEM (HRTEM) characterizations were recorded by a TECNAI G2 F20 transmission electron microscope under 200 kV accelerating voltage. X-ray diffraction (XRD) patterns were obtained on a D8 Focus diffractometer with $Cu_{K\alpha}$ radiation. Fourier transform infrared (FTIR) spectra were recorded on a Magna 560 FTIR spectrometer (Nicolet, USA). The magnetic properties of the samples were measured by using a superconducting quantum interference device magnetometer (SQUIDMPMS XL-7) with fields up to 1.5 T. The absorption spectra were obtained by UV-Vis absorption spectroscopy on U-3010 spectrophotometer (Hitachi, Japan). X-Ray photoelectron spectra (XPS) were measured on an ECSALAB 250 using non-mono-chromatized Al-Kα radiation. A CWdiode laser (LSR808H) with wavelength of 808 nm was used for the laser irradiation experiment. N_2 adsorption-desorption measurements were measured using an intelligent gravimetric analyser Autosorb-iQ (Quantachrome). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was measured with a Leeman ICP-AES Prodigy instrument. UV-Vis-NIR absorption spectra were measured with a Cary 500 UV/Vis spectrophotometer (Varian, USA). Fluorescence spectra were recorded using an Eclipse fluorescence spectrophotometer (Varian, USA).

Synthesis of Fe_2O_3 NPs and nanospindles

In a typical synthesis, 100 mL of 2.0× 10^{-2} M $FeCl_3$ (or 2.0× 10^{-2} M $FeCl_3$ and 4.0 × 10^{-4} M KH_2PO_4) aqueous solution were aged at 100 °C for 72 h. And the solution equipped with a magnetic stirrer and a condenser was in an oil bath. Then the excess reagent $FeCl_3$ was removed after centrifugation and the precipitate was washed for three times with anhydrous ethanol and deionized water. The Fe_2O_3 NPs (or Fe_2O_3 nanospindles) were concentrated at 50 °C for 10 h in a vacuum for further experiment.

Synthesis of Fe_2O_3@PAA/ZnP NPs and nanospindles

In a 100 mL of flask, 5 mg ZnO and 100 μL of PAA aqueous solution (0.2 g mL^{-1}) were firstly added to 10 mL deionized water under magnetic stirring. In succession, 5, 3, 1 mg of Fe_2O_3 NPs (or Fe_2O_3 nanospindles) were dispersed ultrasonically into the solution to form a suspension. Then, 20 mL of IPA was dripped into the suspension under magnetic stirring. Afterwards, 5 mg $NH_4H_2PO_4$ were added to the above mixed suspension under magnetic stirring for more than 1 h. Finally, Fe_2O_3@PAA/ZnP NPs (or nanospindles) with different shell thickness were obtained. After being centrifuged and washed with deionized water and anhydrous ethanol repeatedly to remove the excess precursors, the obtained NPs were dried at 50 °C for 24 h for further use.

Synthesis of Fe_3O_4@C/ZnP NPs and nanospindles

Monodisperse Fe_3O_4@C/ZnP NPs (or nanospindles) were produced according to the calcination of as-synthesized Fe_2O_3@PAA/ZnP NPs. The Fe_2O_3@PAA/ZnP NPs were calcinated from room temperature to 300 °C at a heating rate of 2 °C min^{-1} and maintained at 300 °C for 1 h, then up to 400 °C and maintained at 400 °C for 4 h in a furnace under a high-purity argon atmosphere. Then the NPs were cooled to room temperature.

Loading DOX into Fe_3O_4@C/ZnP NPs

UV-Vis spectroscopy was used to determine the amount of DOX loaded into the Fe_3O_4@C/ZnP NPs. After mixing Fe_3O_4@C/ZnP NPs (1 mg mL^{-1}, 1 mL) with DOX aqueous solution (10 mg mL^{-1}, 20 μL) for 48 h, we got the contents of original DOX and residual DOX in supernatant after magnetic separation by measuring the absorbance at 490 nm in a UV-Vis spectrophotometer. The DOX-loading efficiency (LE) was calculated by Equation 1:

$$LE\ (\%) = [m_{(total\ DOX)} - m_{(DOX\ in\ supernatant)}]/m_{(total\ DOX)} \times 100\% \quad (1)$$

Drug release from DOX-loaded NPs

Two portions of the prepared DOX-loaded Fe_3O_4@C/ZnP NPs at equal amount were redispersed in pH 7.4 and pH 5.1 PBS (0.5 mL) and then transferred in pretreated semipermeable dialysis bags at room temperature, respectively. After the two bags were immersed into 3 mL of PBS buffer (pH 7.4 and pH 5.1) at 37 ^oC, the amount of released DOX moving into the solution was determined by fluorescence spectrophotometer with emission at 591 nm and excitation at 479 nm at selected time intervals. To confirm that the laser irradiation can induce the drug release, another experiment was also carried out under the same procedures. The sample was immersed in PBS buffer at pH 5.1 with NIR irradiation (808 nm, 2 W cm^{-2}) at selected time interval. DOX concentration in the supernatant was determined by UV-Vis spectrophotometer as well. The samples (1 mg) were put into pH 5.1 PBS (3 mL). The supernatants were collected by centrifugation at selected time intervals and analyzed by ICP-AES to measure the Zn^{2+} content.

Cell culture

Human hepatocellular carcinoma (HepG-2) cells were grown as a monolayer in a humidified incubator at 37 ^oC in a 95% air 5% CO_2 in dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum.

***In vitro* MR Imaging and cellular uptake of the Fe_3O_4@C/ZnP NPs**

The MR imaging experiment *in vitro* was performed on a 1.5 T MRI instrument. The Fe_3O_4@C/ZnP NPs with series concentrations (0, 0.24, 0.48, 0.72, 0.96, 1.2 mg mL^{-1}) were prepared. The specific proton relaxivity value (r_2) was ascertained by a linear fitting of the inverse relaxation times as a function of Fe_3O_4@C/ZnP NPs concentration. 1.0×10^5 mL^{-1} HepG-2 cells were seeded in a 24-well plate in DMEM. Then the cells were incubated with Fe_3O_4@C/ZnP NPs at different NP concentrations for 24 h at 37 ^oC. After being washed with PBS three times, the cells were suspended in 0.2 mL PBS for MR imaging. The cells were washed with PBS buffer three times, trypsinized, and harvested by centrifugation. The digestion of the cells was performed in aqua regia, and the amount of iron uptake in the HepG-2 cells was then quantified

using ICP-AES.

.The photothermal therapy of Fe_3O_4@C/ZnP NPs

The photothermal conversion performance of the Fe_3O_4@C/ZnP NPs was measured by exposing the NPs suspensions with certain concentration (0, 0.1, 0.25, 0.50 and 1.00 mg mL^{-1}) and pure Fe_3O_4 NPs (1.00 mg mL^{-1}) to an 808 nm NIR laser (power density = 2 W cm^{-2}). The temperature was recorded by a thermometer and monitored in 30 s intervals for a total of 5 min. Then, the photothermal effect in the cell level was measured by using calcein AM staining method. Calcein AM can only penetrate in live cells and emit green fluorescence. HepG-2 cells were cultured in 96-well plates with DMEM and maintained in an incubator under a 5% CO_2 atmosphere at 37 ^oC. After overnight incubation, the medium was carefully aspirated and individually replaced with 25 μL of Fe_3O_4@C/ZnP NPs (50 μg mL^{-1}) in serum-free DMEM. After incubation for 24 h, the marked cells were irradiated by a NIR laser (808 nm, 2 W cm^{-2}) for 5 min. Finally, the marked cells stained with calcein AM and imaged by a light microscope (Olympus, DP73). In control groups, the only cells and cells treated with only NPs or laser irradiation were investigated, respectively.

In vitro cytotoxicity evaluation against HepG-2 cells

HepG-2 cancer cells were seeded in a 96-well plate at a density of 2.5×10^4 per well and cultured in 5% CO_2 at 37 ^oC for 24 h. Then serial concentrations of empty Fe_3O_4@C/ZnP NPs, DOX-loaded Fe_3O_4@C/ZnP NPs and free DOX in serum-free DMEM with 100 μL were respectively added to the cells and the cells were incubated for another 24 h in 5% CO_2. Afterward, the NIR laser irradiation (2 W cm^{-2}, 5 min) was applied to the cells for studying the NIR-guided cytotoxicity effect. One row of the 96-well plate was used as a blank control with culture medium only. Then, the MTT assay was performed to evaluate the cell viability. Cell viability was determined by Equation 2:

$$\text{Cell viability (\%)} = [Abs_{(\text{test cells})} / Abs_{(\text{reference cells})}] \times 100\% \quad (2)$$

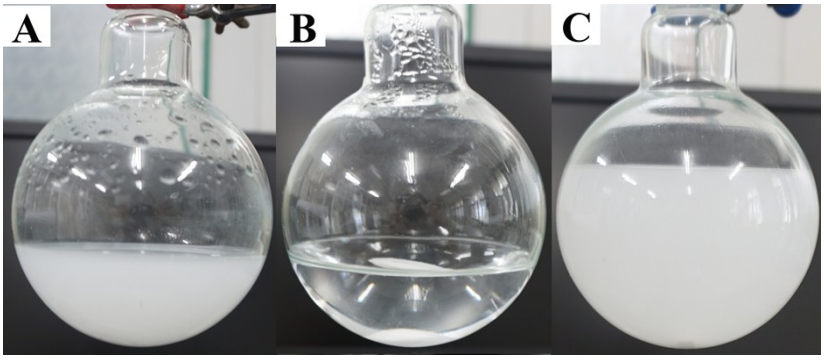

Fig. S1 Photographs of (A) ZnO suspension, (B) PAA-Zn aqueous solution and (C) PAA-Zn suspension after the addition of IPA.

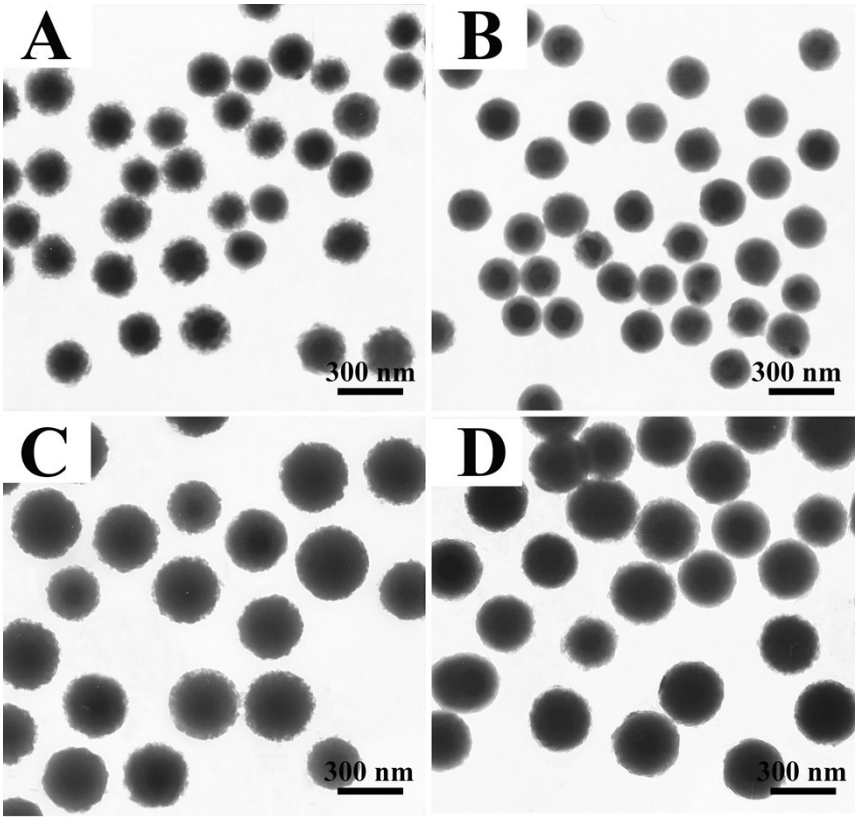

Fig. S2 TEM images of Fe_2O_3@PAA/ZnP NPs with (A) 45 nm and (C) 85 nm PAA/ZnP shell thickness, and Fe_3O_4@C/ZnP NPs with (B) 45 nm and (D) 85 nm C/ZnP shell thickness.

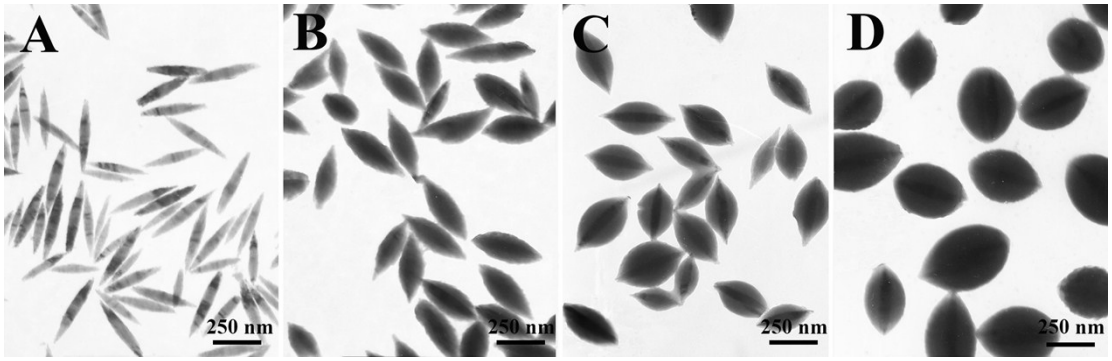

Fig. S3 TEM images of (A) Fe_2O_3 nanospindles, (B-D) Fe_3O_4@C/ZnP nanospindles with 30, 60, 105 nm C/ZnP shell thickness.

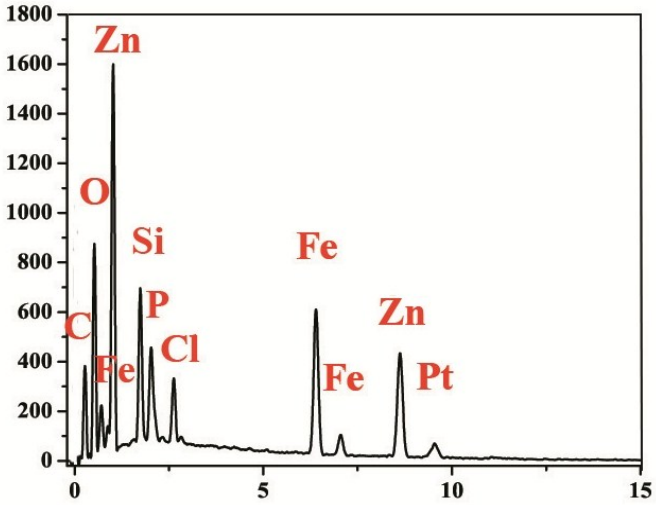

Fig. S4 EDX spectrum of Fe_3O_4@C/ZnP NPs.

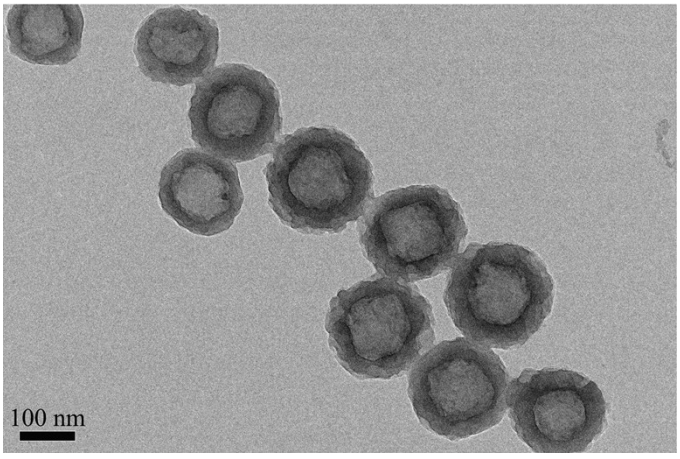

Fig. S5 TEM image of hollow C nanospheres by etching Fe_3O_4@C/ZnP NPs using the diluted acid solution.

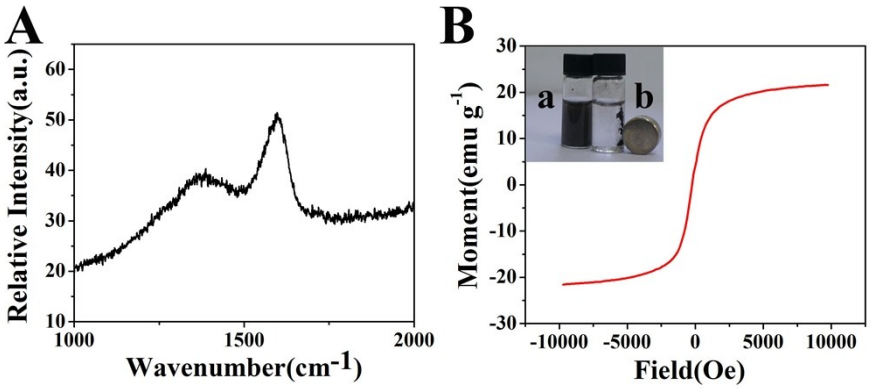

Fig. S6 (A) Raman spectrum of the Fe_3O_4@C/ZnP NPs. (B) Hysteresis loop measurement of Fe_3O_4@C/ZnP NPs measured at a temperature of 300 K. Inset: photographs of Fe_3O_4@C/ZnP NPs solution (a) before and (b) after magnetic separation by an external magnetic field.

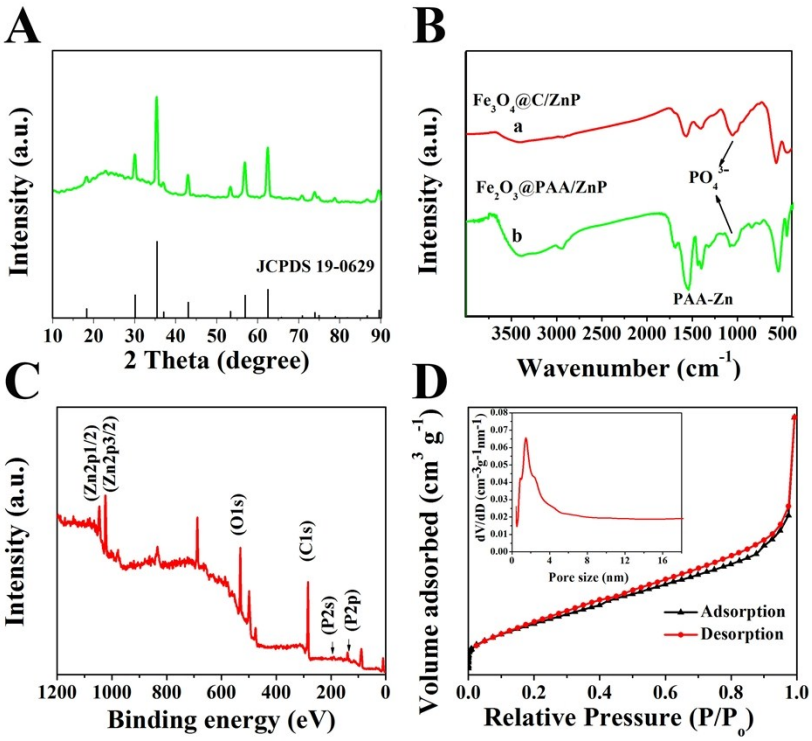

Fig. S7 (A) XRD patterns of Fe_3O_4 NPs, JCPDS 19-0629, and Fe_3O_4@C/ZnP NPs. (B) FTIR spectra of Fe_2O_3@PAA/ZnP NPs and Fe_3O_4@C/ZnP NPs. (C) Fully scanned XPS spectrum of Fe_3O_4@C/ZnP NPs. (D) N_2 adsorption-desorption isotherm and pore size distribution curve (inset) of Fe_3O_4@C/ZnP NPs.

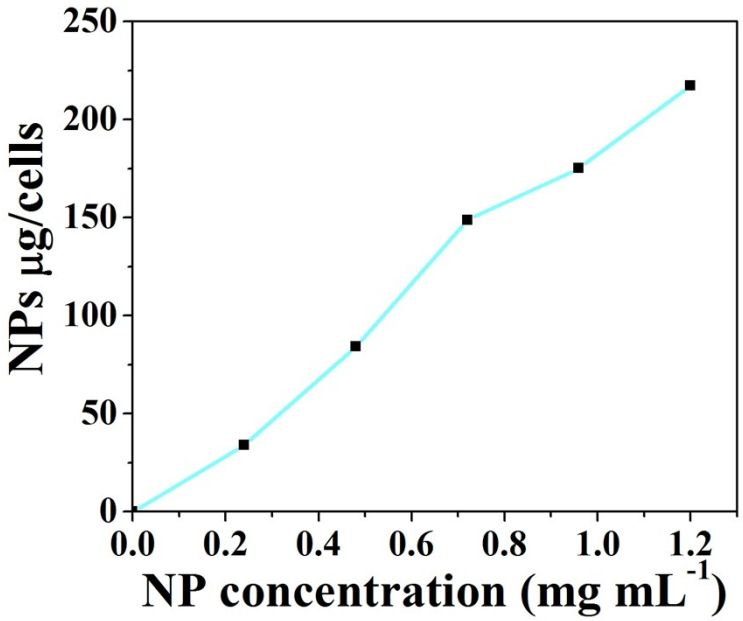

Fig. S8 The cellular uptake of Fe_3O_4@C/ZnP NPs based on the content of Fe measured by ICP-AES.

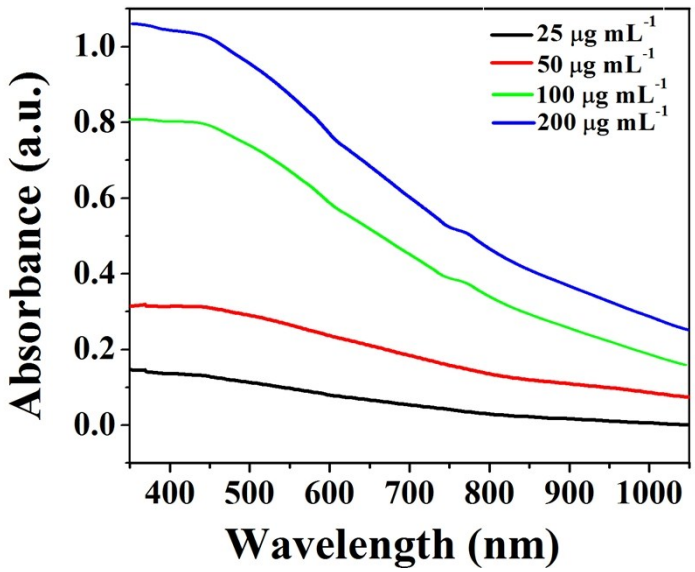

Fig. S9 UV-Vis-NIR absorption spectra of the Fe_3O_4@C/ZnP NPs with different concentrations.

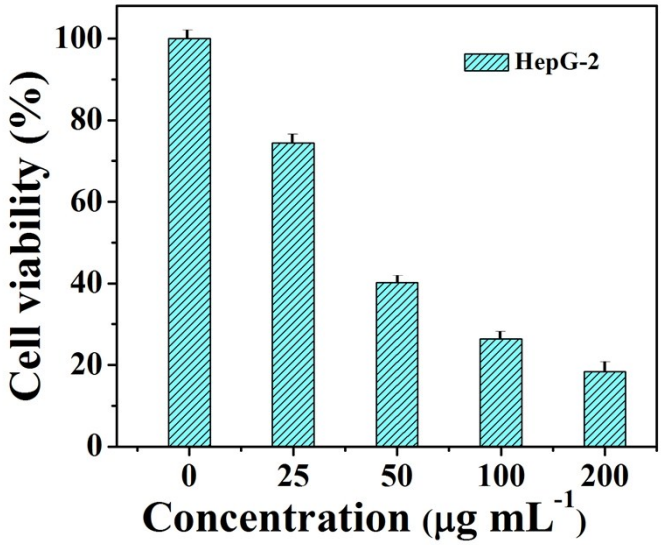

Fig. S10 Cell viability of HepG-2 cells treated with different concentrations of Fe_3O_4@C/ZnP NPs and laser irradiation (808 nm, 2 W cm^{-2}, 5 min).

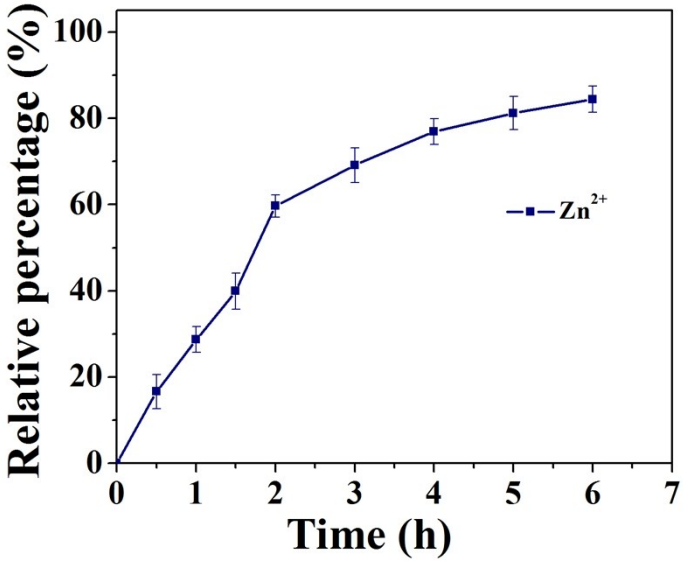

Fig. S11 The Zn^{2+} content profile for Fe_3O_4@C/ZnP NPs measured at pH 5.1 PBS.