Supporting Information

for

Facile formation of hierarchical mesoporous silica nanocarriers for tumor selective multi-model theranostics

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Materials and reagents

$NH_3 \cdot H_2O$ (AR, 25% ~ 28%) was purchased from Xi'an Sanpu Chemical Reagent Co., Ltd. (Xi'an, China). Aminopropyltriethoxysilane (APTES) was purchased from Energy Chemical (Shanghai). Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) was purchased from Tianjin Tianli Chemical Reagent Co., Ltd. Absolute ethanol (AR, 99.7%) and formamide were purchased from Tianjin HengXing Chemical Reagent Co., Ltd. (Tianjin, China). Tetraethylorthosilicate (TEOS, 99.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd (Xi'an, China). Fluorescein sodium and hexadecyl trimethyl ammonium bromide (CTAB) were purchased from Tianjin Kemiou Chemical Reagent Co.,Ltd (Tianjin, China). Glutathione (GSH), glyceryl dimethacrylate, ammonium persulfate (APS), tetramethylethylenediamine (TEMAD), Folic acid (FA), paclitaxel (PTX), β-Cyclodextrin (β-CD) and 1,3,5-trimethylbenzene (TMB) were obtained from Shanghai Macklin Biochemical Co. Dulbecco's modified

Eagle's medium (DMEM), RPMI 1640 medium and PBS were purchased from HyClone (Logan, UT, USA). Fetal bovine serum (FBS) was from ExCell Bio Co., Ltd (Shanghai, China). 3-(4, 5-Dimethylthiazol-2-yl)-2.5-diphenyl-2H-tetrazolium bromide (MTT), trypsin and antibiotics (penicillin and streptomycin) were obtained from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). Bovine serum albumin (BSA) was obtained from Servicebio (Wuhan, China). Dimethyl sulfoxide (DMSO) was from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). RNase and propidium iodide were from Sigma-Aldrich (St. Louis, MO, USA). Anti-Ki67 rabbit mAb was obtained from ABclonal (Boston, MA, USA).

Preparation of Amino Group-Functionalized Mesoporous Silica Nanospherez through Disulfide Linkage (MSN-NH_2)

MSNs were prepared by the classical CTAB-templated, base-catalyzed sol-gel method. The pH value of 1000 mL deionized water was adjusted to approximately 11 with 52.8 mL ammonium hydroxide (25~28 wt% $NH_3 \cdot H_2O$). The temperature was raised to 323 K, and then 1.12 g CTAB was added. After the CTAB was completely dissolved, 5.8 mL TEOS was added dropwise with rapid stirring. After 2 h, the mixture was incubated overnight, centrifuged and washed thoroughly with distilled water and ethanol. As-synthesized silica nanoparticles were dispersed in ethanol by sonication for 30 min, followed by the addition of 20 mL of 1:1 mixture (v/v) of water and 1,3,5-trimethylbenzene (TMB). The mixture was placed in the autoclave, and kept at 140 °C for 4 days without stirring. The resulting white powder was washed with ethanol and water for five times each. Then, the surfactant templates were removed by extraction using acidic methanol (9 mL of HCl/400 mL of methanol, 36 h) at 70°C, and then the MSNs were centrifuged, washed several times with ethanol and dried under a vacuum for 20 h. For this step, the as-prepared MSNs (1 g) were dispersed in 50 mL toluene and sonicated for 20 min (100 W), and then APTES (1 mL) was added into the suspension. The toluene solution was refluxed for 6 h for amino-functionalization. Then the solution was filtered. The obtained nanoparticles (MSN-NH_2) were washed with ethanol and dried in a vacuum oven at 100 °C overnight.

Preparation of Fe_3O_4/CDs-MSN

50mg MSN-NH_2 and 100mg $FeCl_2 \cdot 4H_2O$ were dispersed in 10ml deionized water, stirred overnight and washed with water. Next, disperse the previous particles and 200mg glutathione (GSH) in 10ml water, stir overnight, wash with water and freeze dry. 10 mg of MSN grafted with GSH was added to the 20 ml of formamide solution of 25 mg of $FeCl_2 \cdot 4H_2O$ and 1 ml of $NH_3 \cdot H_2O$. Then transfer the solution into a Teflon-lined autoclave and heated at 90 °C for 2h and then at 160 °C for 1 hour. After the autoclave cooled to room temperature naturally, the reaction mixture was washed with deionized water and freeze-dry.

Preparation of Fe_3O_4/CDs-MSN-FA

Fe_3O_4/CDs-MSN-FA were prepared by self-assembly of β-CD, 60 mg β-CD was added into 100 mL Fe_3O_4/CDs-MSN (0.1 mg/mL) at 55 °C for 3.5 h under mild stirring. After cooling down, add 10mg Folic acid (FA) and stir overnight in a dark environment under nitrogen protection. The resulting powder was dried under vacuum after completely washed with deionized water.

Preparation of PTX@Fe_3O_4/CDs-MSN-FA

Add 10 mg Fe_3O_4/CDs-MSN-FA and10 μl PTX (0.05 mg/μl) to10 μl glyceryl dimethacrylate containing10 μl deionized water, and disperse the particles evenly. The mixture was evacuated three times with a 10 min interval of 20 min each. Add 365 mg ammonium persulfate (APS) to 30 mL deionized water with nitrogen 20 minutes. Add 360 μl of tetramethylethylenediamine (TEMAD) to 240 μl deionized water for later use. After the particles were evacuated, 850 μl of APS solution and 240 μl of TEMAD solution were added and reacted for 1h under the protection of nitrogen. In vitro simulated responsive drug release and presence and loading efficiency of PTX uses FITC grafted PTX instead of pure PTX. Fluorescent samples used in cell endocytosis experiments, replacing PTX with sodium fluorescein.

The PTX loading efficiency and capacity were calculated by the following equation.

$$\text{Loading capacity} = \frac{\text{Weight of drug in particles}}{\text{Weight of drug loaded particles}}$$

$$\text{Loading efficiency} = \frac{\text{Weight of drug in particles}}{\text{Weight of addition drug}}$$

Cell culture

Human colon cancer cell line LoVo and Human normal colon epithelial cell line NCM460 were purchased from Shanghai Institute of Cell Biology in the Chinese Academy of Sciences. LoVo cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin), and NCM460 was cultured in Roswell Park Memorial Institute 1640 medium supplemented with the same FBS and antibiotics. Both of the cell lines were incubated in a humidified atmosphere of 5% CO_2 at 37 °C.

Cell viability assay

The effects of PTX@Fe_3O_4/CDs-MSN-FA on cell viability were determined using the MTT assay. Growing cells were collected and seeded in 96-well plates at a cell density of 2×10^4 cells/well. After 24 h of incubation, cells were treated with various concentrations of PTX@Fe_3O_4/CDs-MSN-FA, followed by 24 h or 48 h cell culture, respectively. MTT solution (5 mg/mL and 10 μL) was then added to each well 4 h before the end of incubation. After removing the medium, 150 μl DMSO was added to each well. The absorbance was measured by the micro-plate reader at a wavelength of 490 nm and the inhibition ratio (I%) was calculated.

Cellular uptake

For fluorescent microscope, growing cells were seeded in 24-well plate overnight, followed by treated with FITC-PTX@Fe_3O_4/CDs-MSN-FA for 1 h, 3 h, 6 h, and 12 h, respectively. Cells were rinsed three times in PBS and stained with DAPI for 5

minutes at room temperature to visualize the nuclei. Images were acquired by fluorescent microscope.

For flow cytometry, growing cells were plated in 6-well plate overnight, followed by incubated with FITC-PTX@Fe_3O_4/CDs-MSN-FA for 1 h, 3 h, 6 h, and 12 h, respectively. After rinsed by PBS 3×, all stained cells were analyzed by NovoCyte 2040R Flow Cytometer (ACEA, San Diego, CA, USA).

Animals and Tumor Model

Male BALB/C nude mice were housed in Laboratory Animal Center of Xi'an Jiaotong University, Xi'an, China. All the animal studies were conducted following the principles of the Institutional Animal Care and Use Committee and approved by the Biomedical ethics committee of Xi'an Jiaotong University Health Science Center. Each nude mice was subcutaneously inoculated with 0.2 mL LoVo cell suspension (2 × 107 cells/mL). When the tumors reached approximately 100 mm3, the nude mice were randomly divided into 5 groups (n=4). Group 1-5 mice received tumor injection of 200 μL PBS, PTX@Fe_3O_4/CDs-MSN-FA and Fe_3O_4/CDs-MSN-FA, respectively. The tumor site of group 1, 2 and 4 mice were put under an NIR light (5 min, 808 nm). The thermal images were obtained using an infrared thermal imaging camera. The body weights and tumor volumes of the mice were monitored and recorded every 2 days. Tumor volume was measured as $(A\times B^2)/2$, where A is the larger and B is the smaller dimension of the tumor. On the last day, the mice were sacrificed, and their spleen and tumor were weighed. Tumor tissues were collected for HE staining and immunohistochemical staining.

For NIRFL imaging, tumor-bearing mice were injected with PTX@Fe_3O_4/CDs-MSN-FA (0.2 mL). The animal imaging system was used to collect in vivo fluorescence images and the NIRFL images of the main organs were also collected.

H&E staining and immunohistochemistry assay

Tumor tissue was embedded in paraffin and cut into 5 μm sections. The sections were stained with hematoxylin and eosin for H&E assay. For immunohistochemical assay, the sections were deparaffinized and hydrated. Following treating with 0.01 mol/L

citrate-buffered saline and quenching of endogenous peroxidase, sections were incubated with 5% bovine serum albumin (BSA) to block nonspecific sites, and the slides were then incubated with primary antibody against Ki67 (1:200 dilution) overnight at 4°C, After washing in PBS, slides were incubated with secondary antibody secondary antibody for 1 h at room temperature, followed by rinsing in PBS, incubation with the HRP-streptavidin working solution and staining with diaminobenzidine (DAB). Finally, slides were re-dyed with hematoxylin for imaging.

Table S1. Loading capacity and loading efficiency of PTX in PTX@Fe_3O_4/CDs-MSNs-FA at different weight ratio of PTX and Fe_3O_4/CDs-MSNs-FA

PTX:Fe_3O_4/CDs-MSNs-FA	Loading Capacity	Loading Efficiency
0.05	3.6 wt%	74.6 wt%
0.1	7.2 wt%	77.6 wt%
0.2	13.9 wt%	80.7 wt%
0.5	28.8 wt%	80.8 wt%
1	28.5 wt%	39.8 wt%

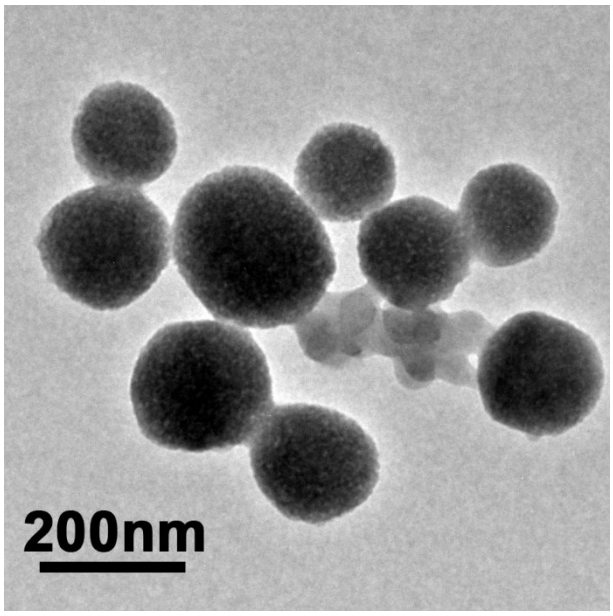

Figure S1. TEM image of Fe_3O_4/CDs-MSNs.

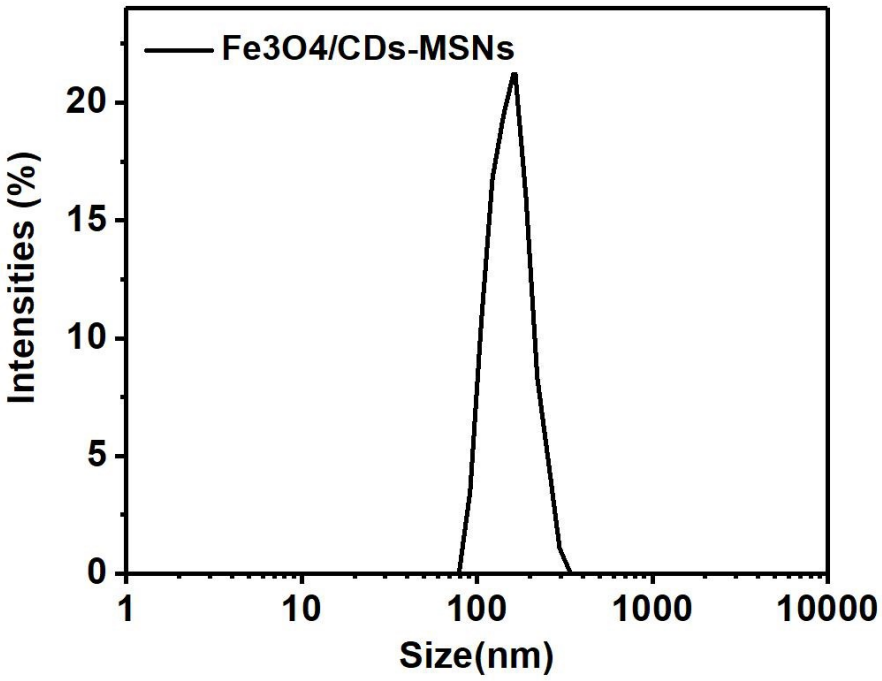

Figure S2. Size distribution of Fe_3O_4/CDs-MSNs-FA measured by dynamic light scattering.

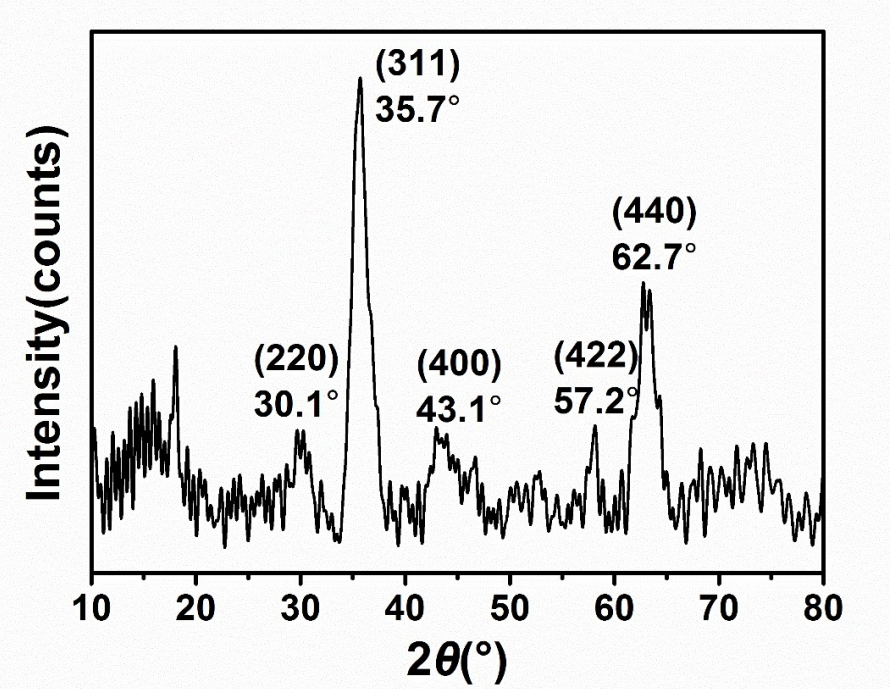

Figure S3. The XRD spectra of Fe_3O_4/CDs-MSNs

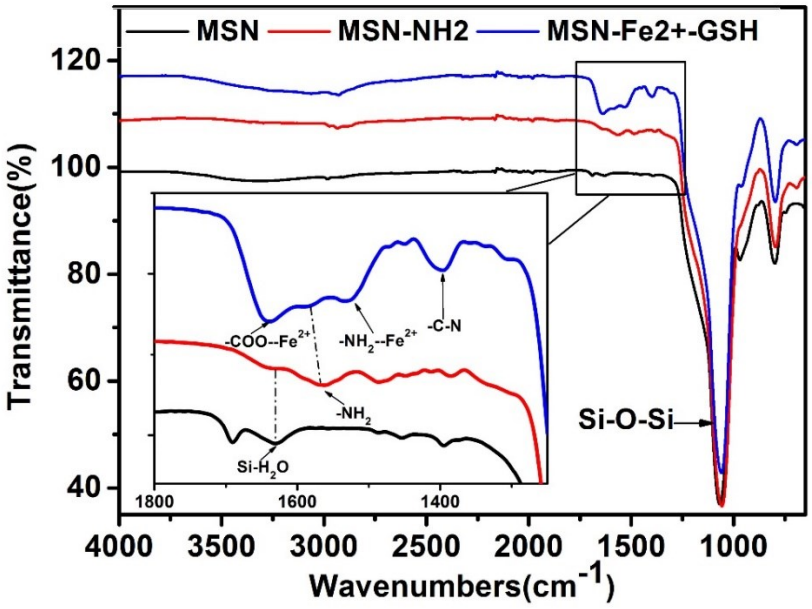

Figure S4. Infrared (IR) spectrum of MSN, MSN-NH_2 and MSN-Fe^{2+}-GSH.

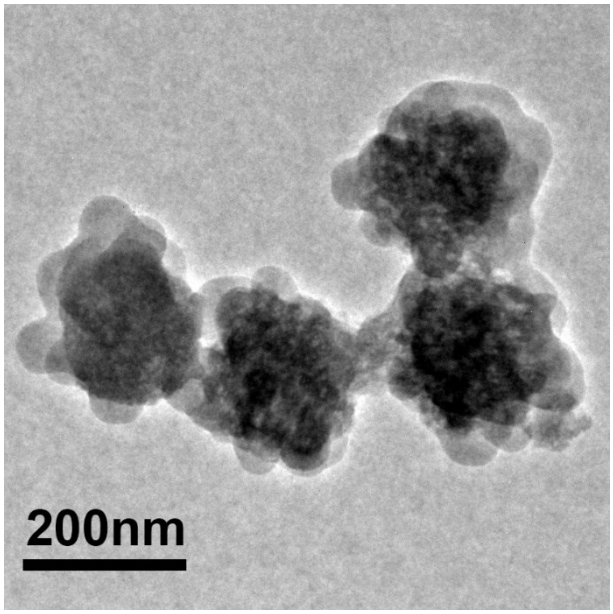

Figure S5. TEM image of Fe_3O_4/CDs-MSNs-FA.

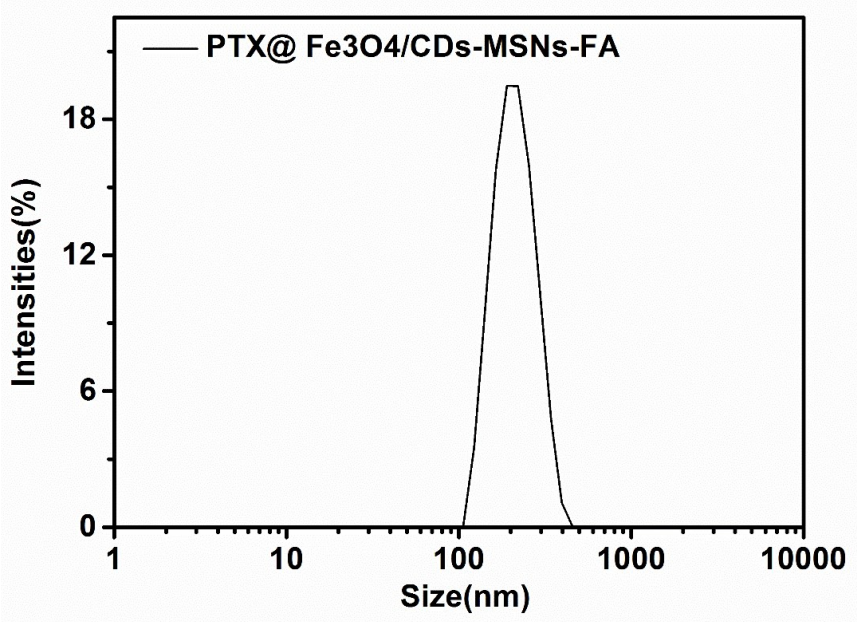

Figure S6. Size distribution of PTX@Fe_3O_4/CDs-MSNs-FA measured by dynamic light scattering.

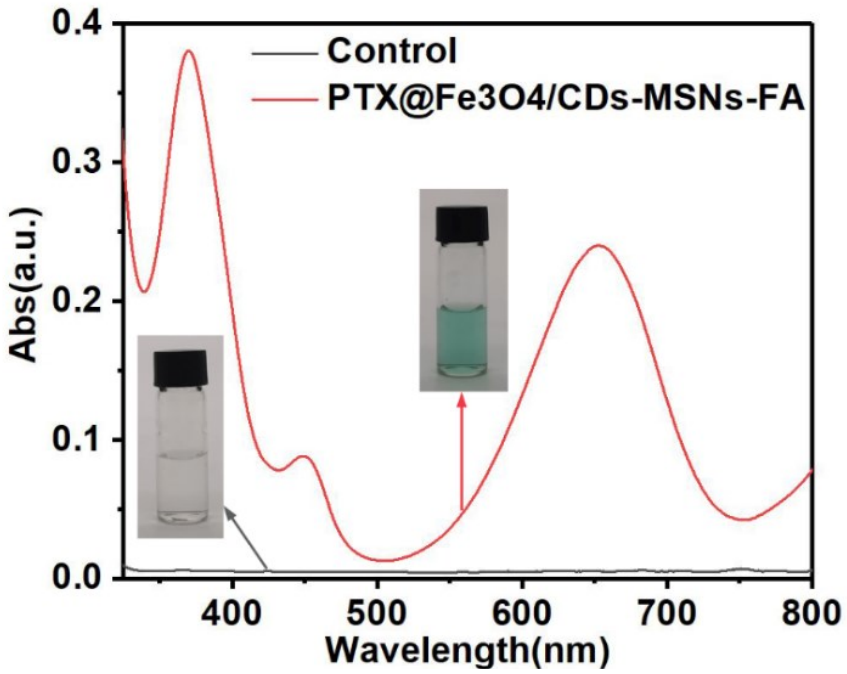

Figure S7. UV-vis spectrum and photos of the mixture of TMB and H_2O_2 with or without PTX@ Fe_3O_4/CDs-MSNs-FA

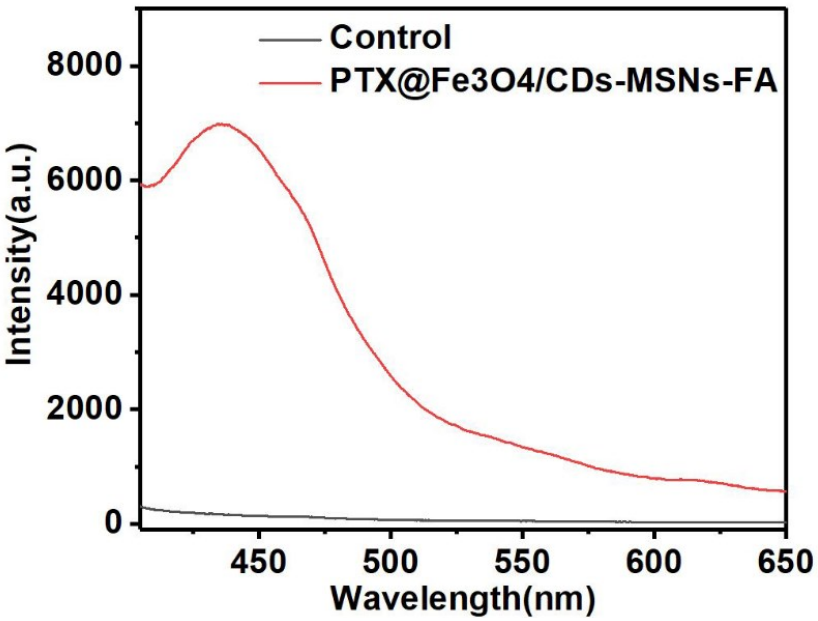

Figure S8. Fluorescence spectrum of the mixture of TA and H_2O_2 with or without PTX@ Fe_3O_4/CDs-MSNs-FA

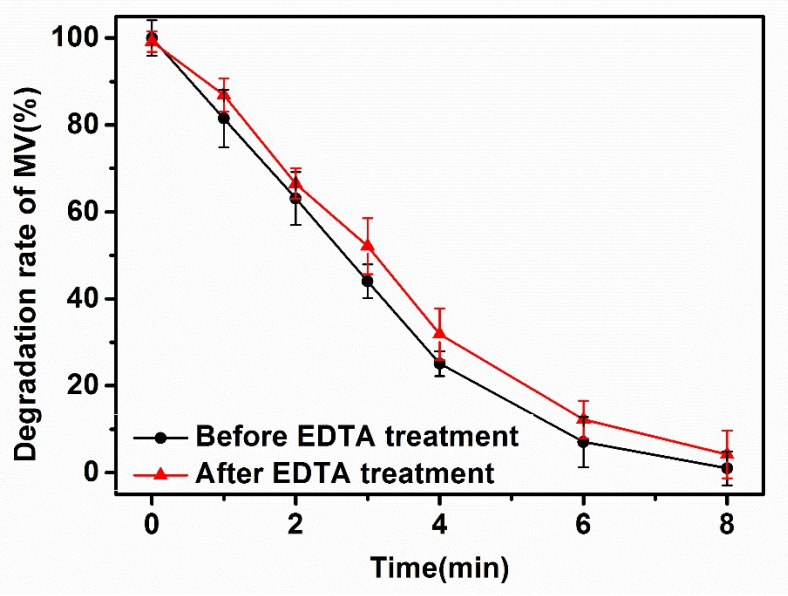

Figure S9. H_2O_2-dependent hydroxyl radical generation of Fe_3O_4/CDs-MSNs-FA before and after EDTA treatment, which was determined by the degradation of methyl violet.

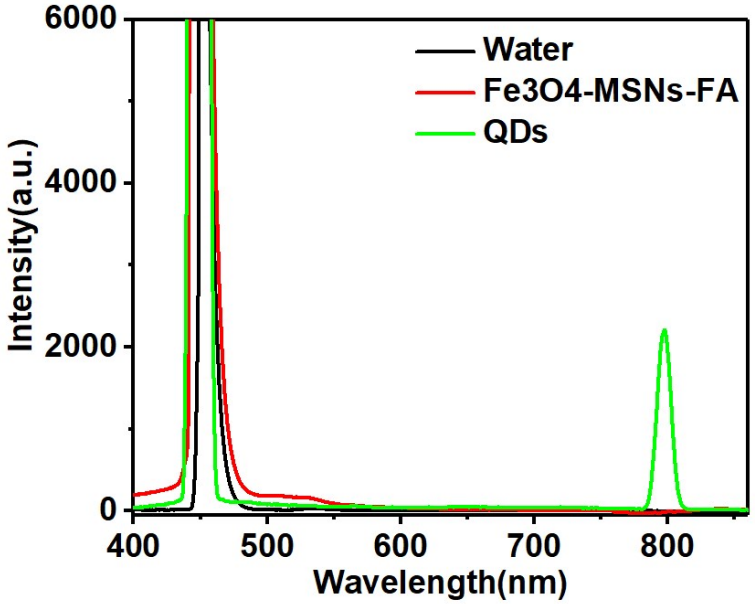

Figure S10. Fluorescence spectrum of water, Fe_3O_4/CDs-MSNs-FA and carbon dots obtained from Fe_3O_4/CDs-MSNs-FA nanoparticles.

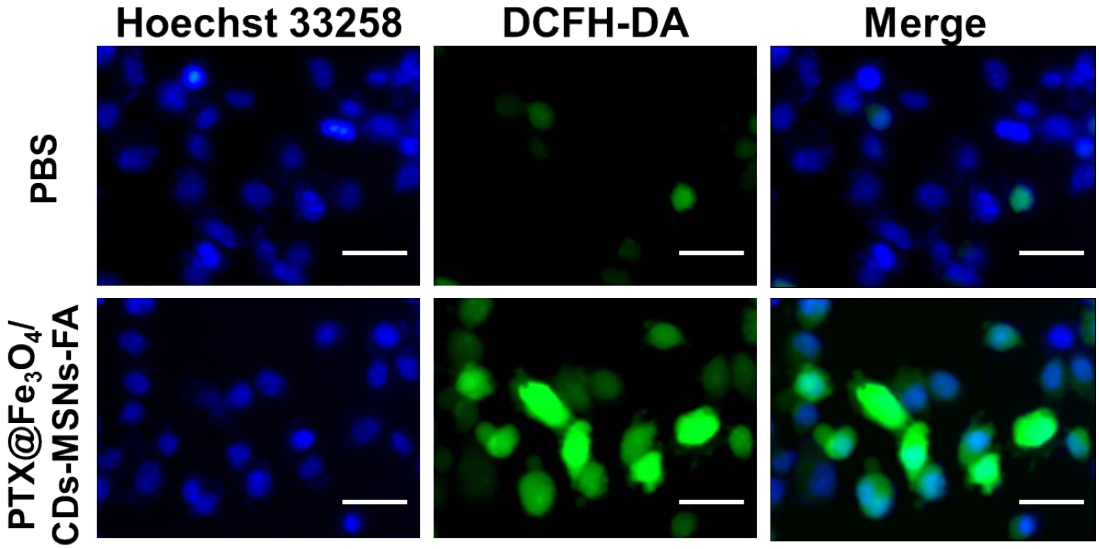

Figure S11. Generation of ROS (Green fluorescence) in LoVo cells after treatment by PTX@Fe_3O_4/CDs-MSNs-FA and PBS, respectively. (Scale bar = 20 μm).

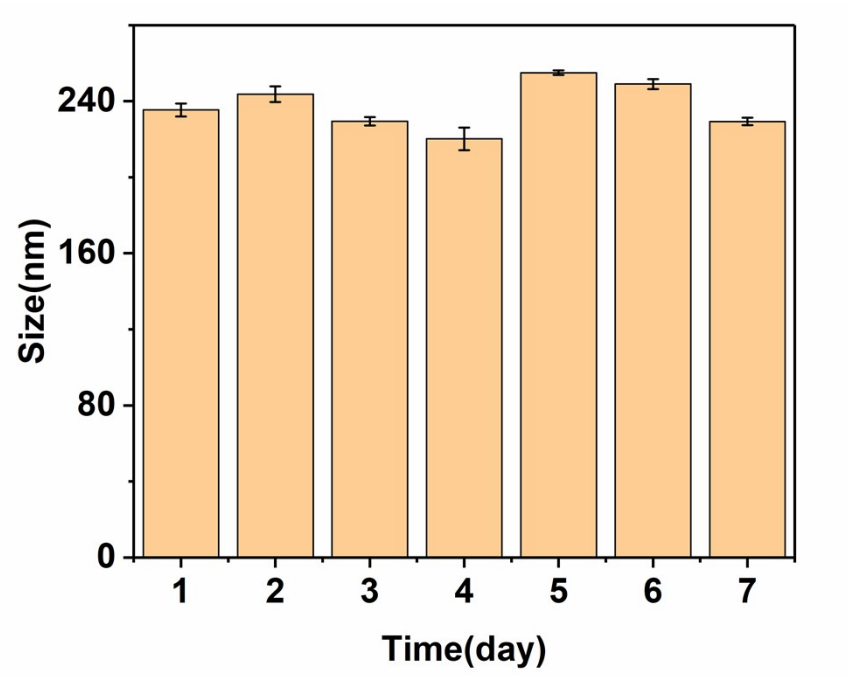

Figure S12. Average size of PTX@ Fe_3O_4/CDs-MSNs-FA after different time of incubation in PBS, which were measured by dynamic light scattering

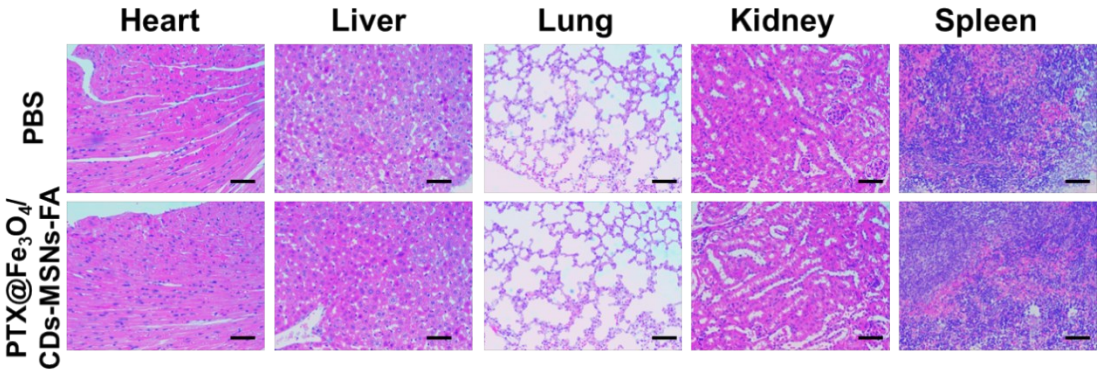

Figure S13. H&E staining of heart, liver, lung, kidney and spleen after treatment by PBS and PTX@Fe_3O_4/CDs-MSNs-FA. The scale bar is 100 μm