Supporting Information

Smart theranostic agent based on Fe-HPPy@Au/DOX for CT imaging and PTT/chemotherapy/CDT combined anticancer therapy

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Experimental section

1.1 Reagents and chemicals

Pyrrole, ferric chloride ($FeCl_3 \cdot 6H_2O$) and sodium citrate were purchased from Aladdin Chemistry Co. Ltd., Hydrochloric acid, nitric acid, ethyl alcohol, ammonium hydroxide, ammonium acetate, ethylene glycol and chloroauric acid were supplied by Sinopharm Chemical Reagent Co., Ltd. Doxorubicin hydrochloride (DOX) was gained from Huafeng United Technology Co., Ltd, Beijing. Water used was obtained by a Milli-Q water purification system from Millipore.

1.2 Synthesis of porous Fe_3O_4 nanospheres

The porous Fe_3O_4 nanospheres were synthesised by a solvothermal method according to previous references.[1, 2] In a typical process: 1.35 g $FeCl_3 \cdot 6H_2O$ and 3.85 g ammonium acetate were dissolved in the 70 mL ethylene glycol. The responsive solution was treated with ultrasound and transferred to a 100 mL Teflonlined

autoclave. After reaction at 200 ^{o}C for 8 h, the black Fe_3O_4 nanospheres were collected by magnetic separation and washing with ethyl alcohol. Finally, the Fe_3O_4 nanospheres were dried at 60 ^{o}C under vacuum and stored for further use.

1.3 Preparation of Fe-HPPy nanospheres

The Fe-doped hollow polypyrrole (Fe-HPPy) nanospheres were prepared via in situ polymerisation according to previous references with some modifications[1]. 0.15 g Fe_3O_4 nanospheres were dispersed in 15 mL of alcohol under sonication for 1 h. 2 mL pyrrole was dissolved in 15 mL alcohol, and the solution was added to above dispersion. After the mixture was treated with ultrasound for 2 h, 60 mL deionized water and 10 mL HCl solution (6 mol/L) were then added into the above solution. Then the mixture was kept under sonication for 2 h. Finally, the black Fe-HPPy nanospheres were collected by centrifugation and washed with ethanol.

1.4 Synthesis of Au nanoparticles

All the glass vessels were soaked by aqua regia solution at first. The Au nanoparticles were synthesised according to previous method.[3] 100 mL deionized water containing 0.0243 mmol chloroauric acid was boiled under strong stirring, then 2.5 mL sodium citrate (1 wt%) was dropwise added immediately. After reaction for 10 min, the mixture was cooled to room temperature rapidly. The obtained Au nanoparticles solution was stored in 4 ^{o}C for further use.

1.5 Preparation of Fe-HPPy@Au nanocomposites

The Fe-HPPy@Au nanocomposites were prepared by facile electrostatic adsorption. 50 mg Fe-HPPy nanospheres were slowly added to the above Au nanoparticles solution, then the mixture was stirred for 12 h. Finally, the Fe-HPPy@Au nanocomposites were collected by centrifugation and washed with water.

1.6 The photothermal performance of Fe-HPPy@Au nanocomposites

The dispersions containing Fe-HPPy@Au nanocomposites with different concentrations (0 to 600 μg/mL) were irradiated with NIR laser (808 nm, 1.0 W/cm^2,-Sfolt，Shanghai，China). The temperature of the dispersions was recorded by infrared camera（Fotric 225， Shanghai， China）.

1.7 The drug loading and releasing

2.5 mg Fe-HPPy@Au nanocomposites mixed with 2 mL DOX aqueous solution（0.5 mg/mL）, and the mixture was shaken for 24 h in a water-bathing constant temperature shaking incubator. The Fe-HPPy@Au/DOX nanocomposites were collected by centrifugation.

The release of DOX was studied in pH 5.0 and pH 7.4 with or without NIR irradiation. 2.5 mg Fe-HPPy@Au/DOX nanocomposites were dispersed in 5 mL PBS solution (pH 5.0 and pH 7.4), and the mixture was shaken in a shaking incubator. At the predetermined time, the supernatants were collected by centrifugation to determine the release and then the fresh PBS was replaced. For the NIR irradiation group, Fe-HPPy@Au/DOX nanocomposites were additionally irradiated for 5 min by 808 nm light (1 W/cm^2).

1.8 The ROS generation properties of Fe-HPPy@Au nanocomposites

5 mg Fe-HPPy@Au nanocomposites were dispersed in 10 mL PBS at different pH values (7.4, 6.5, 5.0), respectively. After stirring for certain time, the mixtures were centrifuged to collect the supernatant liquid for measurement of Fe by inductively coupled plasma atomic-emission spectroscopy (ICP-AES, Leeman Prodigy, NH). For the measurement of •OH radicals, DPBF was used to investigate the •OH radicals generation capability of Fe-HPPy@Au nanocomposites. The H_2O_2 (200 μM) group, Fe-HPPy@Au (200 μg/mL) group and Fe-HPPy@Au (200 μg/mL) + H_2O_2 (200 μM) group were added with 100 μL of DPBF solution, respectively. Then the samples

were stirring, and supernatant liquid was collected by centrifugation to measure the absorbance at 410 nm. For cellular ROS experiment, HeLa cells were pre-treated with DMEM or Fe-HPPy@Au NCs (200 μg/mL) for 24 h, followed by H_2O_2 solution (50 μM) treated cells for 30 min. The intracellular ROS generation HeLa cells were detected via the H_2DCFDA probe according to the manufacturer's protocol. The green ROS fluorescence was captured by a confocal microscope.

1.9 Cytotoxicity assay of Fe-HPPy@Au nanocomposites

HUVECs cells were seeded in 96-well plates and incubated with Fe-HPPy@Au nanocomposites at different concentrations for 24 h. Then, the cell viability was measured according to the standard procedure of the manufacturer.

1.10 The performance of Fe-HPPy@Au nanocomposites for CT imaging

The Fe-HPPy@Au nanocomposites with Au different concentrations (Au: 0, 8, 16, 32, 64 mM) in 200 μL Eppendorf tubes were scanned by high-resolution micro-CT system (SkyScan 1176, Bruker, Belgium) to acquire the Hounsfield unit (HU) values and CT images. For *in vivo* CT imaging, HeLa tumor-bearing nude mouse was scanned by CT system before and post-injection with dispersion of Fe-HPPy@Au nanocomposites ([Au] = 60 mmol/L, 0.1 mL).

1.11 PTT *in vivo* by Fe-HPPy@Au/DOX nanocomposites and H&E analysis

HeLa tumor-bearing mice (weight about 22 g) were randomly divided into six groups. When the tumors reached approximately 60 mm^3, each mouse was intravenously injected with 200 μL of the corresponding solution: (1) Control group (no treatment), (2) PBS+NIR group, (3) Fe-HPPy@Au group (2 mg/mL), (4) DOX group (0.2 mg/mL), (5) Fe-HPPy@Au + NIR (2 mg/mL), (6) Fe-HPPy@Au/DOX + NIR group (2 mg/mL, DOX loading efficiency was 10%). At 12 h of post-injection, the mice of group 2, 5 and 6 were irradiated with NIR light (808 nm, 1 W/cm^2) for 10 min. The

temperature changes were recorded by an infrared camera （Fotric 225，Shanghai）. The weights of the mice and volumes of tumor were recorded every two days. After post-treatment of 14 days, the mice were sacrificed, and the tumors and the major organs were collected. Tissue samples of group 1 and 6 were embedded in paraffin, sliced and stained using hematoxylin and eosin (H&E). Tissue sections were observed by a light microscope. All animal procedures were performed in strict accordance with National Regulation of China for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Shanghai Jiaotong University.

Supplementary Figures

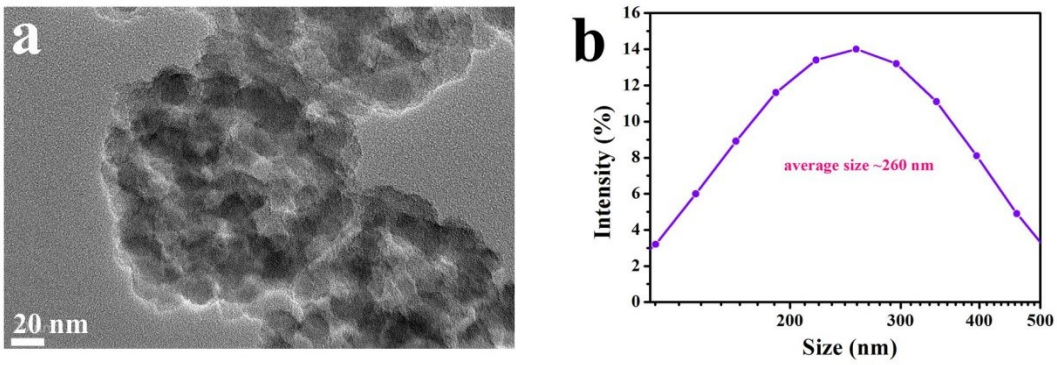

Fig. S1. (a) The high magnified TEM image of Fe-HPPy nanospheres. (b) The size distribution of Fe-HPPy@Au nanocomposites by DLS method.

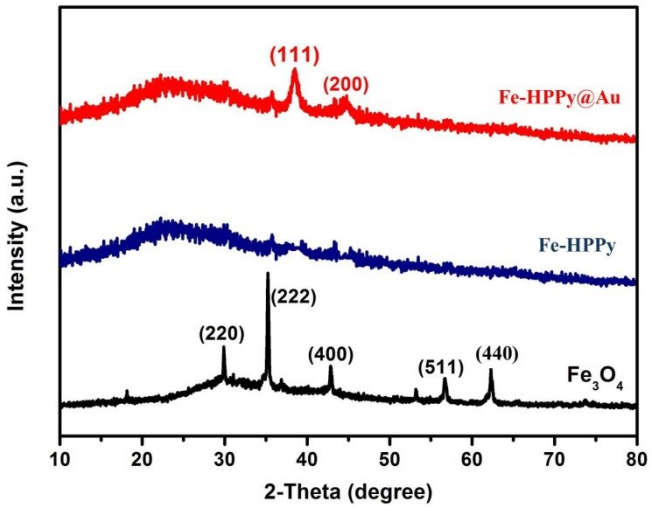

Fig. S2. The XRD patterns of Fe_3O_4 nanopheres, Fe-HPPy nanospheres and Fe-HPPy@Au nanocomposites.

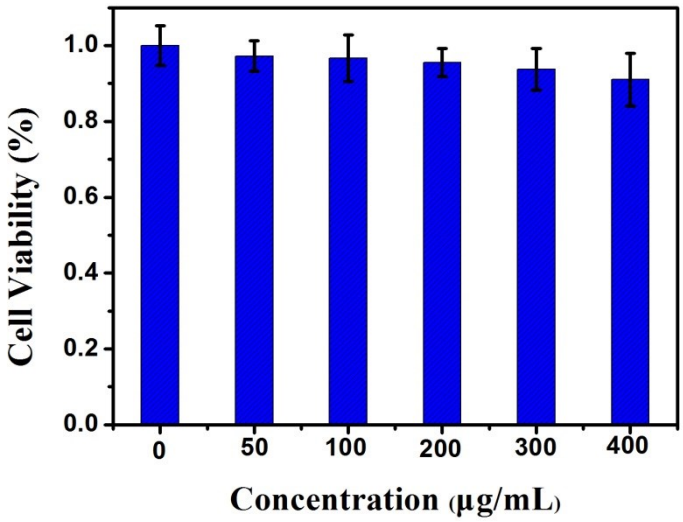

Fig. S3. The cell viabilities of Fe-HPPy@Au nanocomposites incubated with HUVECs cells (normal cells) for 24 h.

References

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3. G. FRENS, *Nat. Physic. Sci.*, 1973, **241**, 20-22.