Electronic Supplementary Information (ESI) for

A Smart Tumor-microenvironment Responsive Nanoprobe for Highly Selective and Efficient Combination Therapy

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1. Materials

Cerium nitrate hexahydrate ($Ce(NO_3)_3$), polyvinylpyrrolidone, iron(II) sulfate heptahydrate ($FeSO_4•7H_2O$), N-hydroxysuccinimide (NHS), 1-ethyl-3-[3-dimethylamin-opropyl]-carbodiimide hydrochloride (EDC), 1,3-diphenylisobenzofura (DPBF), chlorine e6 (Ce6), doxorubicin and $Ti(SO_4)_2$ were bought from J&K Scientific Ltd (Beijing, China). 1,2-Distearoyl*sn*-glycero-3-Phophoethanolamine-N-[amino(polyethyleneglycol)-2000] (DSPE-PEG-NH_2) were bought from Ruixi Biological Technology Co, Ltd (Xi'an China). Calcein AM and propidium iodide (PI), MTS and DCFH-DA were obtained from Promega. All drugs were used directly without further treatment.

2. Instruments

The photos of TEM and EDX were taken on the RTEM (Tecnai G2F30; Fei Company) with a working voltage of 300 KV. SEM images were obtained by S-3400N (Hitachi, Japan). Zeta Potential was detected by Malvern Instruments. Fluorescence spectra were determined by F-4500 spectrophotometer (Hitachi, Japan). The ultraviolet-visible absorption spectra were determined by Cary 5000 UV-VIS-NIR of Agilent Company (China). DLS were carried out on a BI-200SM (USA). Laser at 660 nm was used as the excitation source. Dissolved oxygen was detected by dissolved oxygen meter (Mettler Toledo, China). XRD spectrum was obtained with D/Max-2400 (Japan). XPS spectrum was analyzed by AXIS Ultra DLD (Japan).

3. Experimental Methods

3.1. Synthesis of 25 nm CeO_x nanocubes.

Small-sized CeO_x NCs with a size of 25 nm were prepared from the hydrolysis of Ce $(NO_3)_3$ in an acetate–acetic acid buffer system under hydrothermal conditions. In a typical procedure, 10 g sodium acetate and 10 mL pure acetic acid were dissolved in 50 mL water to form a buffer solution. 5 mmol Ce $(NO_3)_3$ was dissolved in the buffer solution and stirred for about 10 min. The solution was then diluted to 85 mL and

transferred into a 100 mL Teflon-lined stainless-steel autoclave and heated to 220 °C for 24 h. After being cooled to room temperature, the precipitates were collected by centrifugation, and washed with distilled water five times. Then the sample is freeze-dried overnight.

3.2. Synthesis of flower Fe_2O_3.

The flower Fe_2O_3 were synthesized by hydrothermal method. 0.1 g polyvinylpyrrolidone, 0.12 g iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$) were dissolved in 35 mL deionized water and 5mL glycerol were added into the mixed solution. After stirring at room temperature for 1h, the mixed solution was poured into stainless steel autoclave at 170 ℃ for 7 hours. After the reaction was cooled to room temperature, red precipitate is isolated by centrifugation, next the red Fe_2O_3 were washed several times with deionized water and ethanol, respectively. Then the sample were freeze-dried overnight.

3.3. Synthesis of sphere Fe_2O_3.

0.27 g of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was dissolved in 50 mL of ultrapure water to form a clear yellowish solution. The solution was poured into stainless steel autoclave at 105 ℃ for 50 h. After the reaction was cooled to room temperature, red precipitate is isolated by centrifugation and washed several times and freeze-dried overnight.

3.4. Analyze the decomposition products of Fe_2O_3

After treating Fe_2O_3 with pH 6 PBS for 3 days, potassium thiocyanate and H_2O_2 was added dropwise to the supernatant successively, the color of the solution was unchanged.

3.5. Cell lines and cell culture.

HepG2 cells were provided by life of science, Lanzhou University. Normoxic HepG2 cells were cultured in MEM supplemented with 10% FBS at 37℃ in a humidified environment containing 5% CO_2. Hypoxia HepG2 cells were cultured at 37 ℃ under

4% CO_2，1% O_2，95% N_2. Cells exhibited different cells states and morphologies under different condition, because living cells were adhered to culture dishes and the dead cells would shrink into globules compared to living cells in a flat state.

3.6. In vitro ROS detecting.

To determine the ROS of CeO_x/Fe_2O_3-C&D, HepG2 cells were cultured for 24 h at 37 °C, then the CeO_x/Fe_2O_3-C&D and CeO_x were added to the cells at 37 °C for 6 h, respectively. Next, DCFH-DA was appended to the cell culture medium and incubated for 30 minutes at 37 °C. Then the cells culture medium was washed with PBS buffer solution. Besides 660 nm light irradiation for 5 minutes before being analyzed by CLSM. CLSM images were obtained with 448 nm excitation and 525 nm detection.

3.7. MTS determination of cytotoxicity.

HepG2 cells were inoculated on 96-well plate with a density of ~10^4/well and cultured at 37 ℃ for 24 hours. After the cell medium was removed, different concentration of the nanoprobe and 100 μL cell growth medium were supplied to the cells. Then, washing cells with cold PBS and irradiated with 660 nm laser for 10 min. Then, 100 ml fresh medium was cultured with 0.5 mg/ml MTS for 2 hours. MTS analyzed Cell viability and Bio-Telex 800 was used to monitor the absorbance at 490 nm. Besides, the cytotoxicity of nanoprobe without light were also measured as a control. In addition, the cytotoxicity of CeO_x/Fe_2O_3-C&D and CeO_x/Fe_2O_3 in L02 cells were measured through the above methods.

4. Supporting Figures

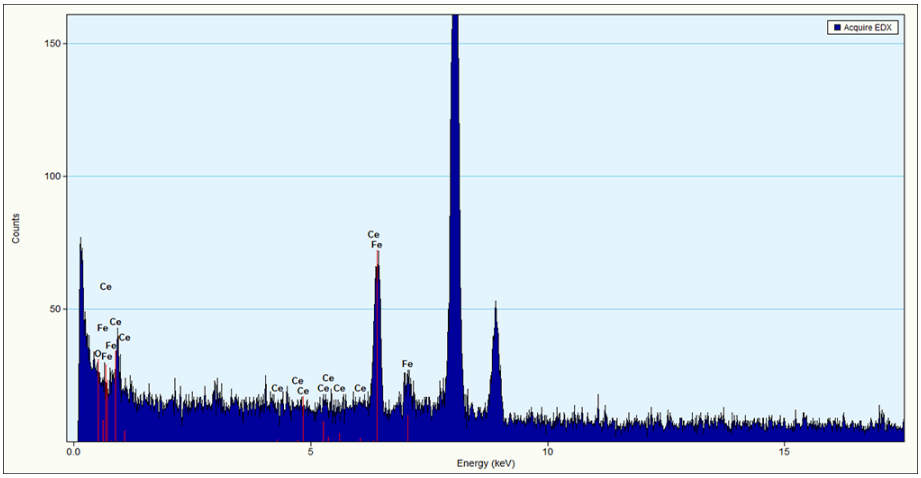


Fig. S1 Energy dispersive X-ray (EDX) spectrum of CeO_x/Fe_2O_3.

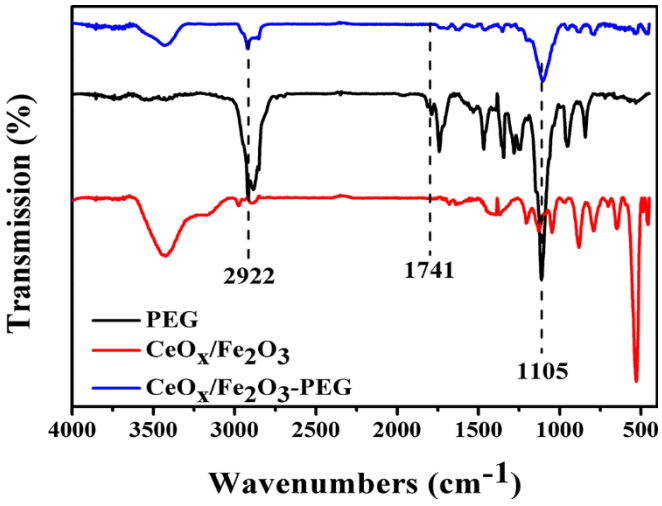


Fig. S2 FT-IR spectra of PEG, CeO_x/Fe_2O_3 and CeO_x/Fe_2O_3-PEG, respectively.

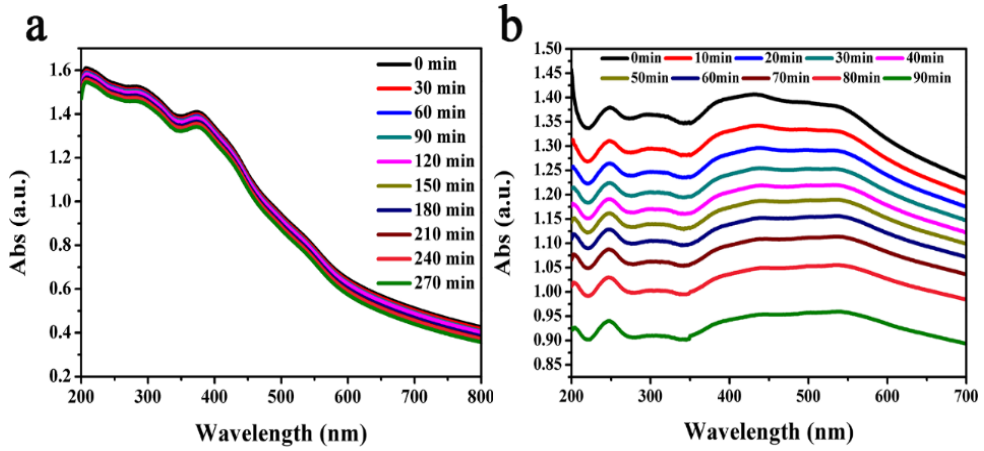


Fig. S3 The stability of CeO_x/Fe_2O_3-PEG (a) and CeO_x/Fe_2O_3 (b) was detected by UV-vis absorption spectrum for 270 and 90 min, respectively.

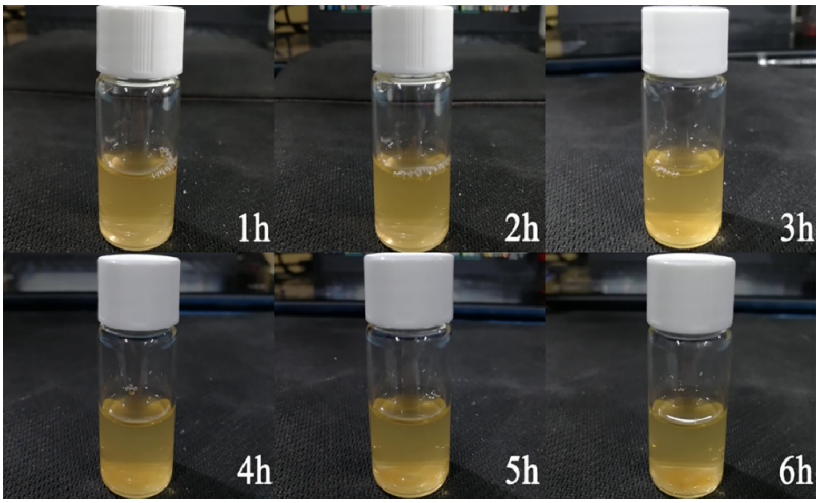


Fig. S4 Digital photos of 250 μg/mL CeO_x/Fe_2O_3-PEG stability analysis in different time.

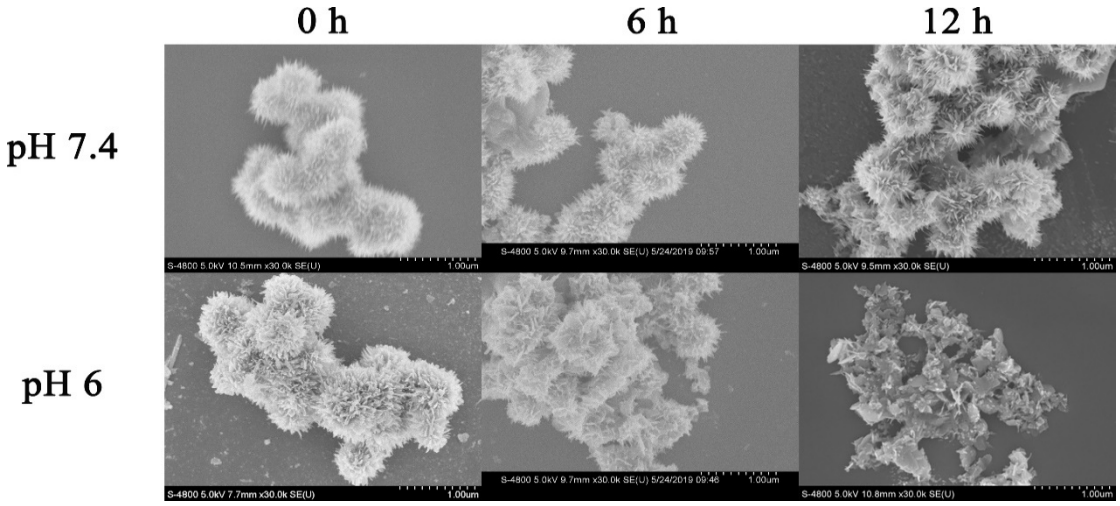


Fig. S5 SEM images of as-synthesized CeO_x/Fe_2O_3. CeO_x/Fe_2O_3 was treated in PBS with different pH values (7.4 and 6) for 6 and 12 hours, respectively.

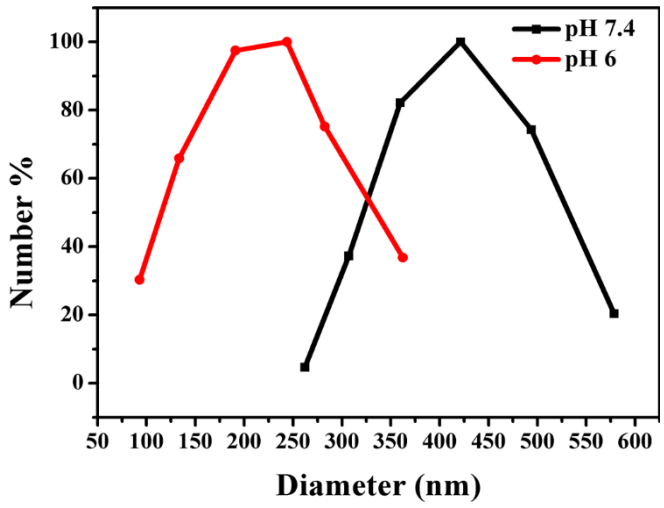


Fig. S6 Dynamic light scattering (DLS) of CeO_x/Fe_2O_3 was treated in PBS with pH at 7.4 and 6.

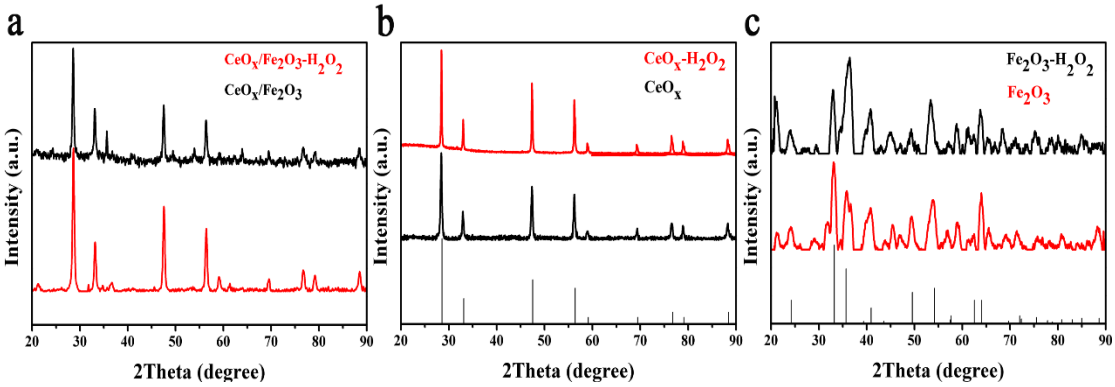


Fig. S7 X-ray Diffraction (XRD) patterns of CeO_x/Fe_2O_3 (a), CeO_x (b), Fe_2O_3 (c) were treated with and without H_2O_2.

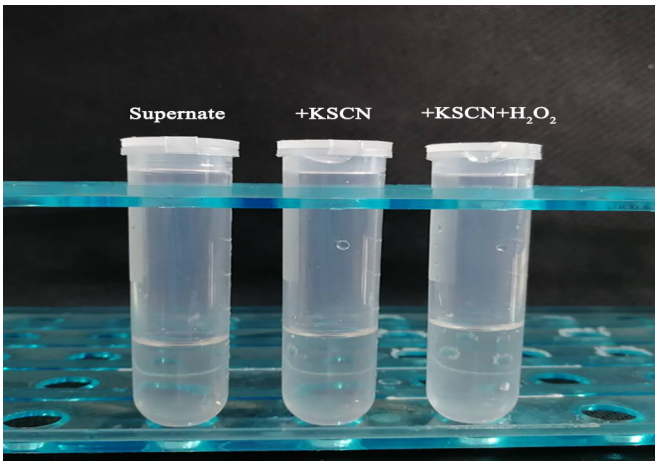


Fig. S8 Digital photo of the iron ion test with potassium thiocyanate.

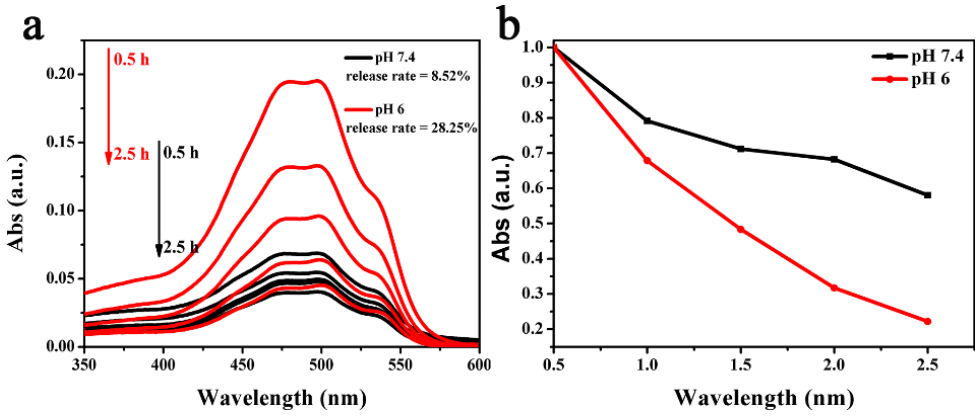


Fig. S9 UV-vis absorption spectra of DOX release behaviors at pH 7.4 and 6 PBS (a) and linear at the maximum absorption (b).

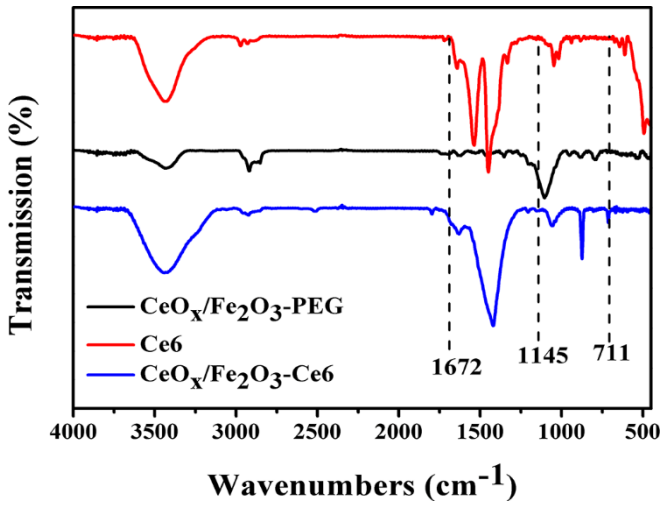


Fig. S10 FT-IR spectra of CeO_x/Fe_2O_3-PEG, Ce6 and CeO_x/Fe_2O_3-Ce6, respectively.

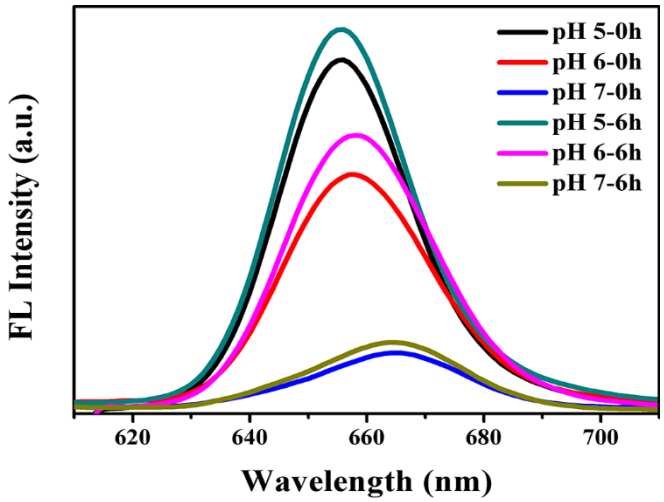


Fig. S11 The fluorescence property of CeO_x/Fe_2O_3-Ce6 at different pH were detected by fluorescence method with 405 nm excitation and 600~750 nm detection.

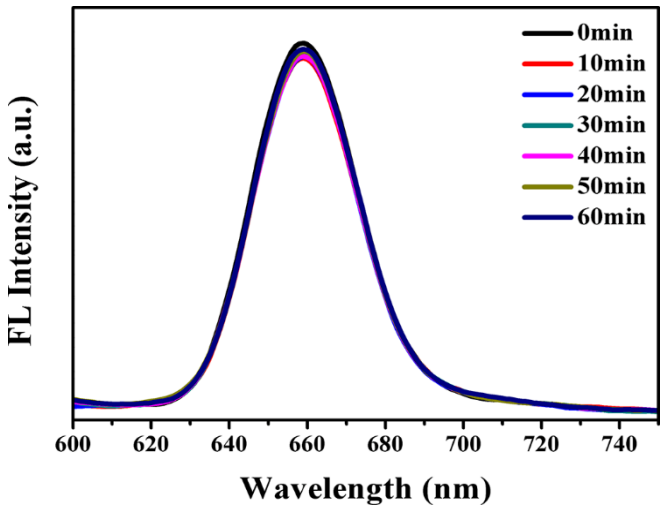


Fig. S12 The optical stability of the CeO_x/Fe_2O_3-Ce6 at pH 6 PBS was detected by fluorescence method for 60 min.

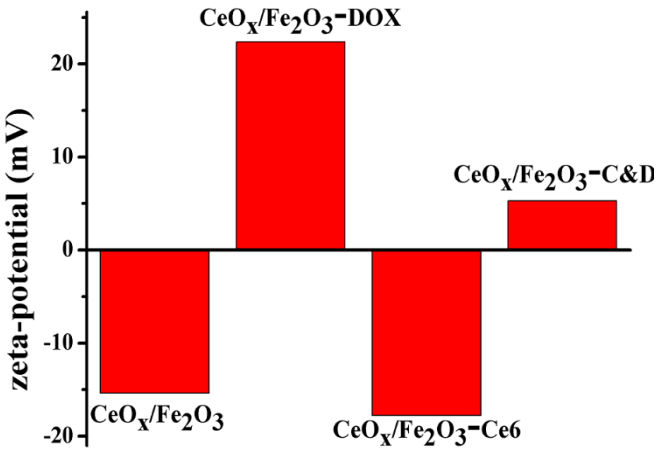


Fig. S13 The zeta potential of CeO_x/Fe_2O_3, CeO_x/Fe_2O_3-DOX, CeO_x/Fe_2O_3-Ce6 and CeO_x/Fe_2O_3-C&D.

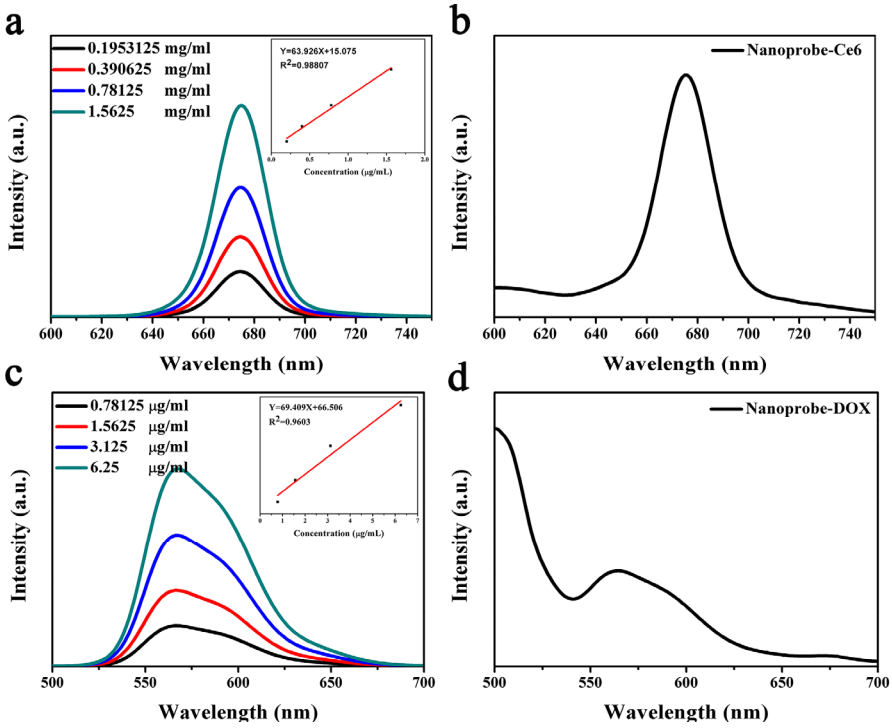
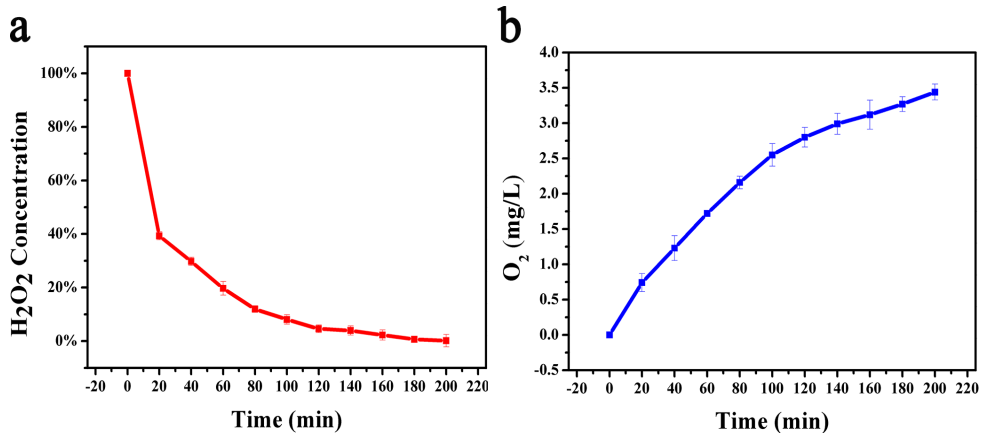


Fig. S14 Loading of Ce6 and DOX in the nanoprobes by fluorescence spectra. Standard curve of Ce6 (a) and DOX (c), and fluorescence spectra of nanoprobes with 405 nm excitation (b) and 480 nm excitation (d).



The reaction equations are represented as follows:[S1]

$$H_2O_2 + 2Ce^{4+} \rightarrow 2H^+ + O_2 + 2Ce^{3+}$$

$$H_2O_2 + 2Ce^{3+} + 2H^+ \rightarrow 2H_2O + 2Ce^{4+}$$

Fig. S15 Decomposition of H_2O_2 (a) and production of O_2 (b) by CeO_x (100 μg/mL) with 1 mM H_2O_2.

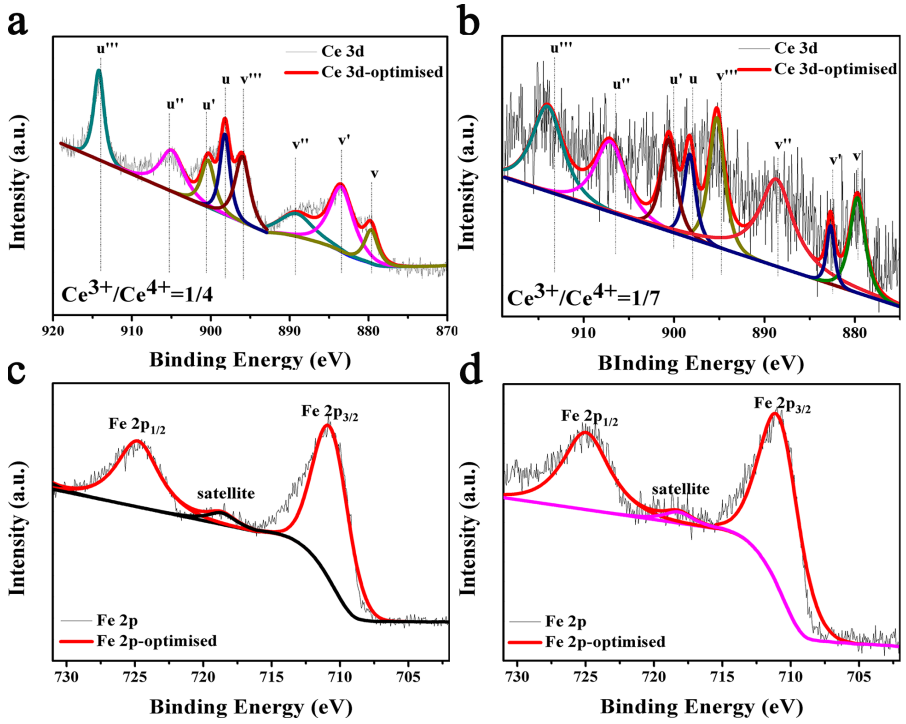


Fig. S16 XPS analysis to show the chemical valence of Ce on the surface of CeO_x (a) and CeO_x/Fe_2O_3-C&D (b), and the chemical valence of Fe on the surface of Fe_2O_3 (c)and CeO_x/Fe_2O_3-C&D (d)

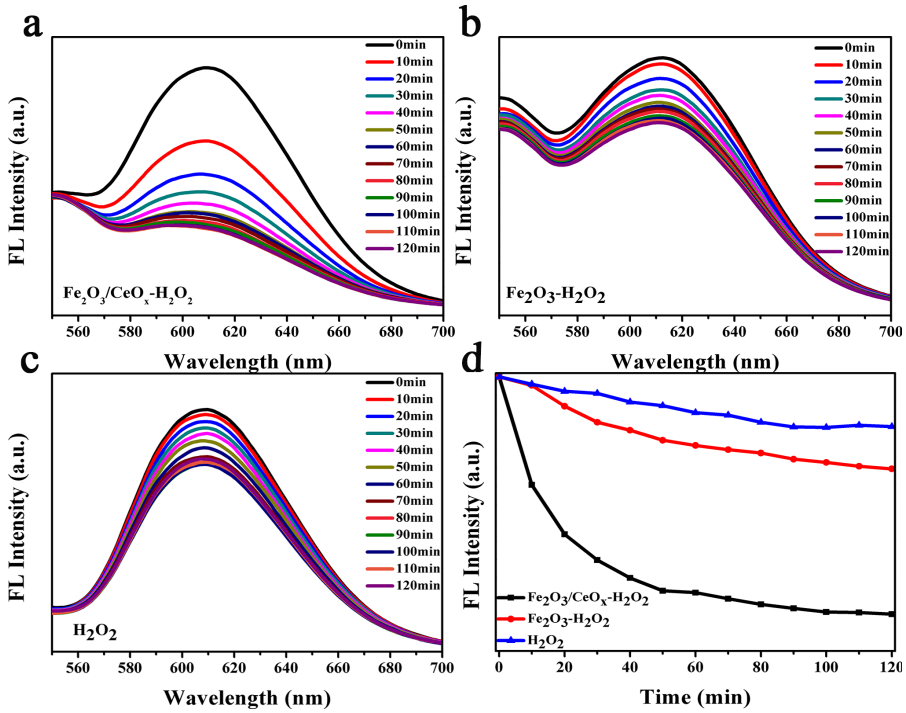


Fig. S17 $[Ru(dpp)_3]^{2+}Cl_2$ was used to detect O_2 by fluorescence method. Image of fluorescence of CeO_x/Fe_2O_3 (a), Fe_2O_3 (b), H_2O_2 (c) and linear at the maximum absorption (d) in PBS (pH 6) for 120 min.

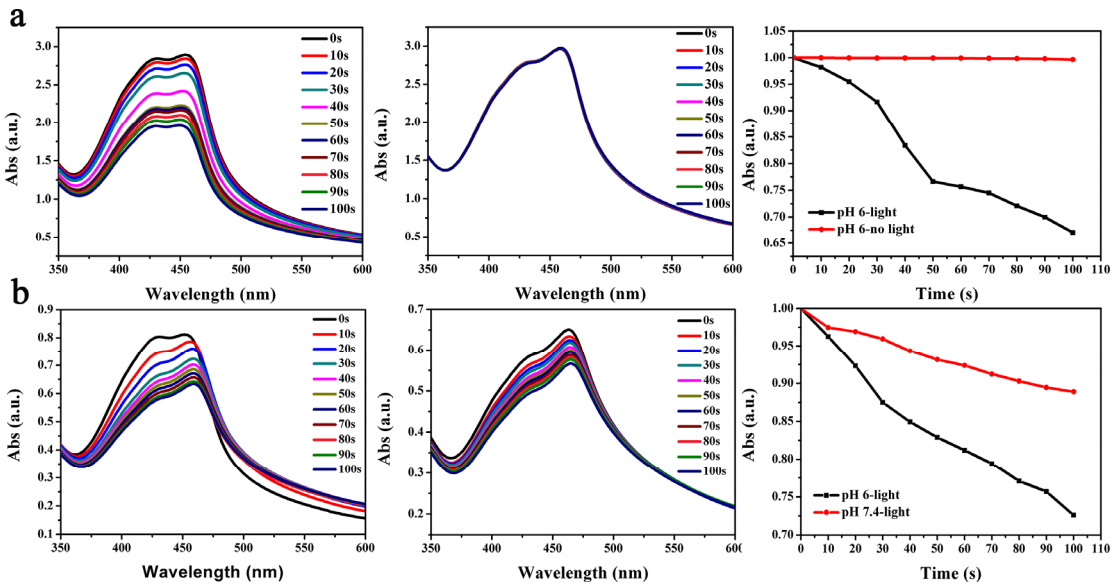


Fig. S18 Absorption of DPBF at 410 nm as a function of $^1O_2$ production. Under the pH 6 buffer solution by controlling the presence (left) and absence (middle) 660-nm light (a) and under 660-nm light irradiation by controlling different pH (pH 6 (left) and 7.4 (middle)) (b).

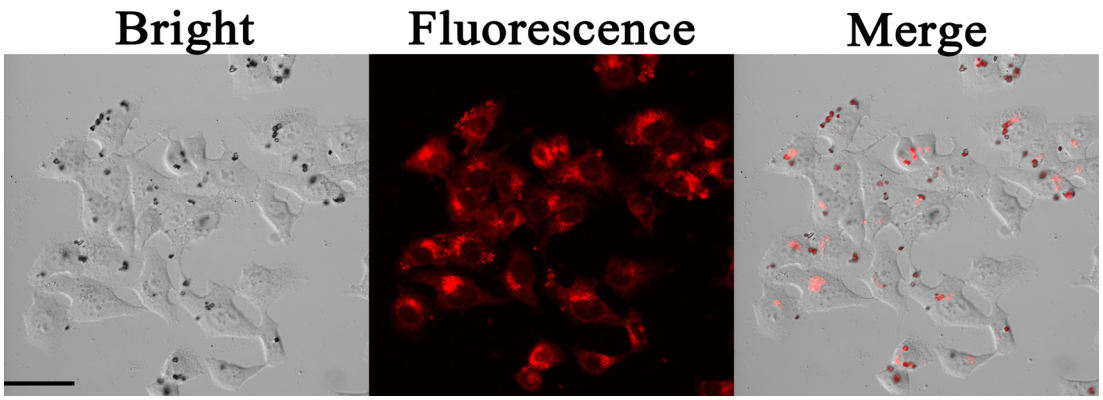


Fig. S19 CLSM images of the cell uptake capacity of CeO_x/Fe_2O_3-C&D with 405 nm excitation and 670 nm detection. Scale Bars = 32.4 μm.

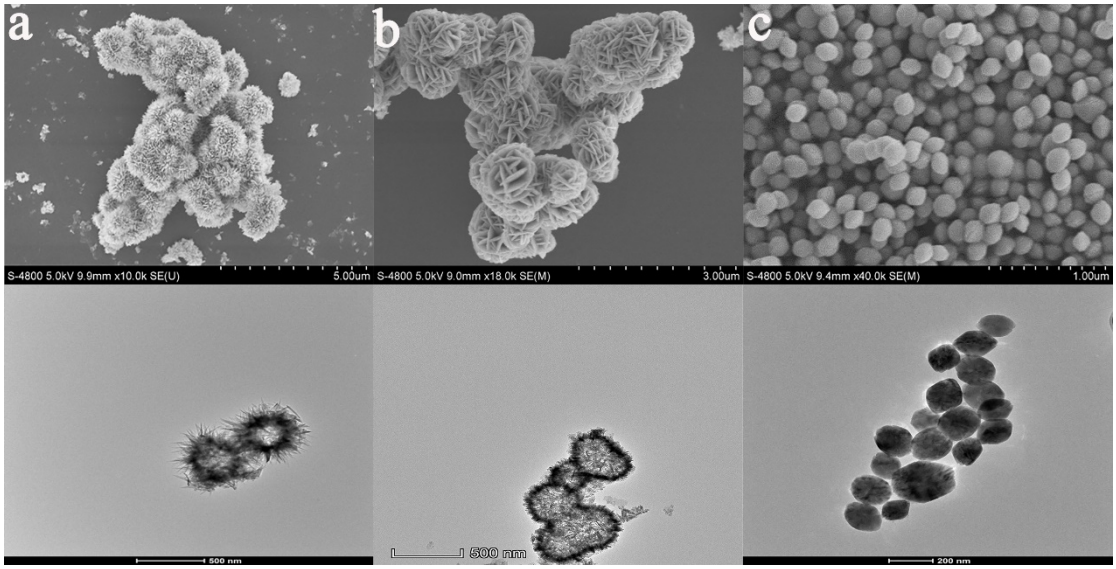


Fig. S20 SEM and TEM images of as-synthesized Fe_2O_3 with urchin (a), flower (b) and sphere (c) morphologies.

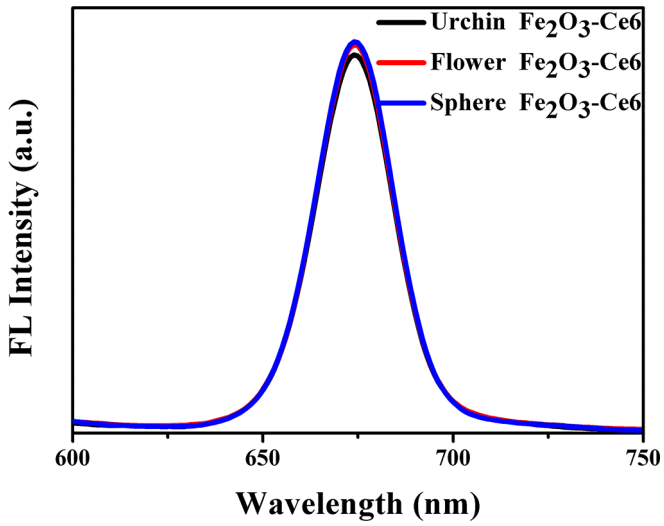


Fig. S21 The fluorescence property of Fe_2O_3-Ce6 with different morphologies (405 nm excitation and 600~750 nm detection).

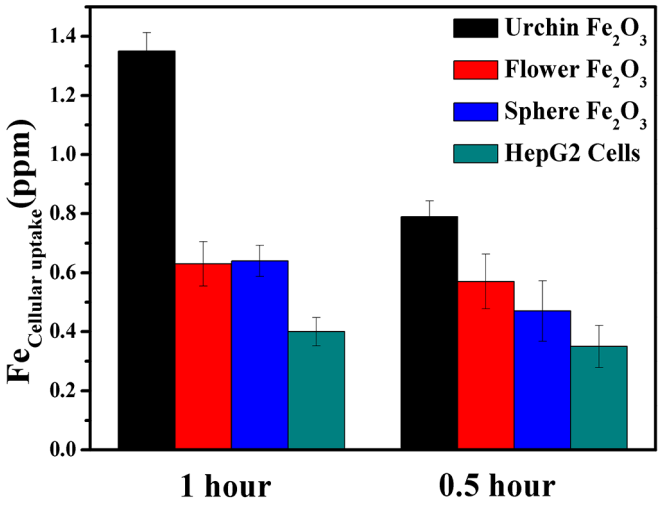


Fig. S22 The cell uptake capacity with different morphologies were studied by inductively coupled plasma mass spectrometry (ICP-MS) by detecting Fe element in cells and error bars showed the standard deviation of three separate experiments. Mean ± SD.

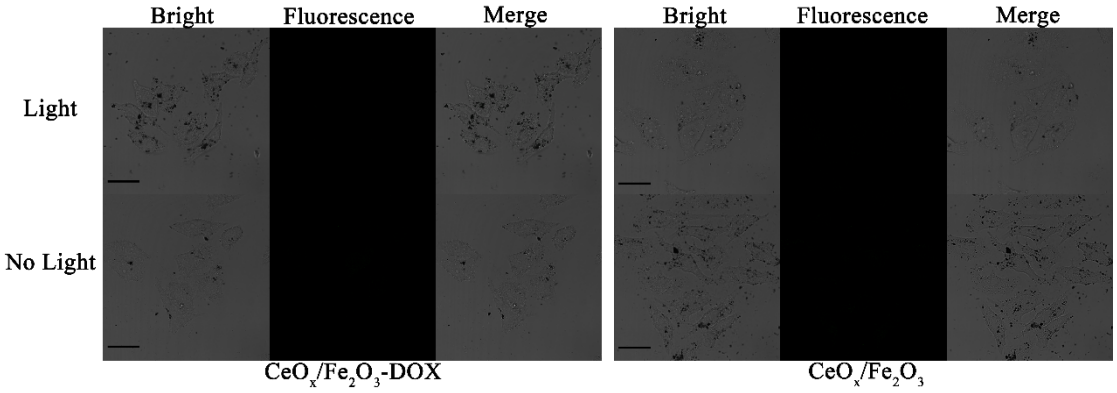


Fig. S23 CLSM images of intracellular ROS generation of HepG2 cells co-incubated with CeO_x/Fe_2O_3-DOX and CeO_x/Fe_2O_3 by normoxic environment. Scale Bars = 32.4 μm.

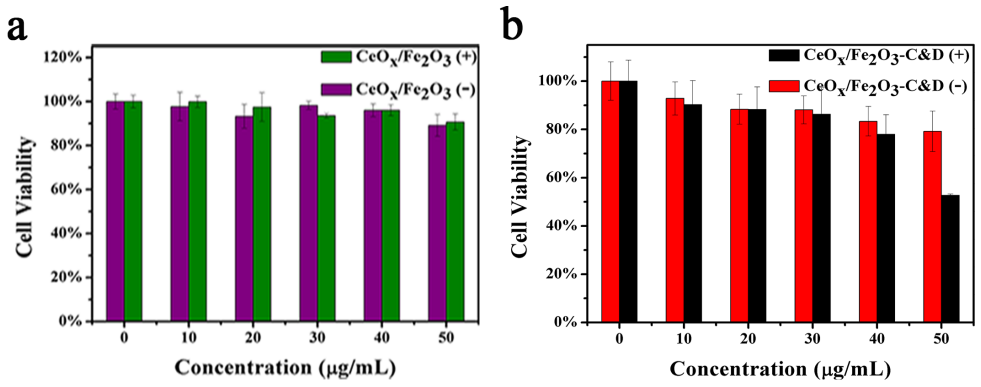


Fig. S24 The cell viabilities of CeO_x/Fe_2O_3-C&D and CeO_x/Fe_2O_3 co-incubated with L02 cells with (+) and without (-) 660 nm light irradiation.

5. Supporting References

(S1) Fan, Y. F.; Li, P. Y.; Hu, B. B.; Liu, T.; Huang, Z.; Shan, C. F.; Cao, J.; Cheng, B.; Liu, W. S.; Tang, Y. A Smart Photosensitizer–Cerium Oxide Nanoprobe for Highly Selective and Efficient Photodynamic Therapy. *Inorg. Chem.,* **2019**, *58*, 7295-7302.