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Electronic Supplementary Information

Cucurbit[7]uril-functionalized Magnetic Nanoparticles for Imaging-guided Cancer Therapy

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1. Experimental Section

Materials and Characterization

Ferric acetylacetonate ($Fe(acac)_3$), ethanol, paraformaldehyde, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), folic acid, amantadine,1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) were purchased from Aladin and used as received. Oleic acid and oleic amine were purchased from TCI (tcichemicals, Shanghai, China). Oxaliplatin was purchased from Yuanye biological company (China).

Milli-Q Integral system (Merck) was used to supply Milli-Q water in this work. The morphology of NPs was observed by transmission electron microscopy (TEM, JEOL 2100F, Japan) at 200KV. The Zeta potential of nanomaterials was measured on a Zetasizer (Malvern). NMR spectra were obtained using a Bruker Ultra Shield 600 PLUS NMR spectrometer. Fluorescence spectra were obtained on fluorescence spectrometer (Thermo Scientific Lumina). Fluorescence images were acquired by confocal laser scanning microscopy (CLSM, Leica TCS SP8, German) and inverted fluorescence microscope (Olympus IX73). Fourier transform infrared (FT-IR) spectra and UV-vis-NIR absorbance spectra were measured on IFS-66V/S and Shimadzu UV-1800 spectrophotometers. MTT assays were measured using a microplate reader (Infinite F200 Pro, TECAN). Intracellular uptake and cell apoptosis were both quantified by a flow cytometer (Beckman coulter).

Preparation of Fe_3O_4 NPs

Fe_3O_4 NPs were synthesized following a previously reported method[1]. Briefly, 2 mmol $Fe(acac)_3$, 10 mmol 1,2-hexadecanediol, 6 mmol oleic acid and 6 mmol oleic amine were dissolved in benzylether under argon. The mixture was stirred and heated to 300 °C at the rate of 4 °C/min followed by refluxing for another 30 min. After cooling to room temperature, the obtained black magnetic nanoparticles were washed by excessive ethanol.

Preparation of CB[7]-Fe_3O_4 NPs

2 mL DMSO with 20 mg DMSA was added to 2 mL toluene containing 20 mg of the Fe_3O_4 NPs and stirred for 12 hours. The obtained precipitant was then washed with ethyl acetate and finally stored in 2 mL Milli-Q.

$AO_1CB[7]$ was synthesized following Kim's method[2, 3] and confirmed by NMR. The $AO_1CB[7]$ was conjugated with Fe_3O_4 NPs via a "click" reaction between $AO_1CB[7]$ and DMSA under UV irradiation. The excessive $AO_1CB[7]$ was washed by Milli-Q.

The amount of CB[7] on the surface was calculated by a fluorescence assay. Briefly, an excessive amount of the fluorescent guest molecular AO was used to bind with the CB[7] to form CB[7]·AO. A known quantity of CB[7]-Fe_3O_4 NPs was mixed with an excessive amount (amount A) of ADA in water and shake for 5 min before ultra-centrifugation. The ADA (amount B) remain in the supernatants was measured by adding CB[7]·AO and the fluorescence was corresponded in the CB[7]·AO standard curve(y=-2049.1+2529, Fig. S3). A minus B is the amount of CB[7].

TGA of CB[7]-Fe_3O_4 NPs

20 mg CB[7]-Fe_3O_4 NPs was used for TGA under nitrogen protection. The weight loss was recorded from room temperature to 700 °C. Fe_3O_4 NPs, $AO_1CB[7]$ and the mixture of CB[7] and Fe_3O_4 NPs were analyzed as the control.

Preparation of OX/FA/CB[7]-Fe_3O_4 NPs

CB[7]-Fe_3O_4 NPs were surface-functionalized by simple mixing the NPs with functional tags, such as FA-Ada, FITC-Ada and OX, and subsequent centrifugation to remove excess ligands. The drug loading content was measured according to Pt content of OX by ICP-MS.

FITC/FA/CB[7]-Fe_3O_4 NPs was produced with the same method for studying the cellular uptake

Release profile of OX in Vitro

The OX release profile of OX/FA/CB[7]-Fe_3O_4 NPs was assessed in vitro by using a dialysis method. Phosphate-buffered saline (PBS) solution containing 0, 0.1 and 1 mM concentrations of spermine (at room temperature) was used as the release medium to study the OX release kinetics. 100 μL dialysate was suck out and replenished at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 5, 10, 20, 30, 48, 72h. The OX release was quantified by ICP-MS.

Cell Culture and in Vitro Cytotoxicity Assays.

The 4T1 cell line (mice breast cancer cell line) and L02 cell line (human fetal hepatocyte line) were cultured in 25 cm^2 flasks using Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin & streptomycin (PS) solution at 37 °C with 5% CO_2.

The biocompatibility of NPs was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays using 4T1 and L02 cell lines (1×10^4 cells per well). The cellular viability was calculated following a previously published procedure.

Cellular uptake

The MCF-7, 4T1 and L02 cells were incubated by NPs and free FITC at an equal concentration of FITC for 12 h, respectively.

The internalized fluorescence images were acquired by a confocal laser scanning microscope (CLSM) on the cells after washing with PBS. The cellular fluorescence signal was obtained by flow cytometry assay.

Cellular Apoptosis Evaluation.

The apoptosis rate of MCF-7, 4T1 and L02 cells after incubation with NPs was evaluated by flow cytometry assay. Briefly, the cells after incubation were processed by an Annexin/V-FITC Kit. The fluorescence signal of annexin V-FITC and PI obtained by flow cytometer reflected the apoptosis rate.

Biosafety and Cytotoxicity

The biosafety of CB[7]-Fe_3O_4 NPs was studied via MTT assays. Briefly, the MCF-7, 4T1 and L02 cell line were seeded in 96-well plates with 10^4 cells/well density in 100 μL of DMEM (with 10% FBS and 1% PS), respectively, and subsequently incubated with CB[7]-Fe_3O_4 NPs (50, 100, 150, 200, 250 μg/mL Fe). The absorbance value was measured by a microplate reader.

The cytotoxicity of CB[7]-Fe_3O_4 NPs was studied via MTT assays. Briefly, the MCF-7, 4T1 and L02 cell line were seeded in 96-well plates with 10^4 cells/well density in 100 μL of DMEM (with 10% FBS and 1% PS), respectively, and subsequently incubated with OX/FA/CB[7]-Fe_3O_4 NPs and free OX (at Fe concentration of 0.5, 2,5, 5, 25, 50 μg/mL and Pt concentration of 0.342, 1.71, 3.42, 17.12, 34.23 μM) for 24 and 48 h, respectively. The absorbance value was measured by a microplate reader.

In Vivo Antitumor Study.

In vivo antitumor effect of OX/FA/CB[7]-Fe_3O_4 NPs were studied by 4T1 tumor-bearing balb/c mice. 12 balb/c mice were subcutaneously injected with 10^6 4T1 cells(10^7 cells per mL PBS solution). When the average volume of the tumor reached about 50 mm^3, the mice were random grouped into 4 groups according to the disposal condition: PBS, free OX, FA/CB[7]-Fe_3O_4 NPs, OX/FA/CB[7]-Fe_3O_4 NPs.(at Fe concentration of 100 μg/mL and Pt concentration of 68.45 μM for 200 μL). The PBS and all NPs were injected intravenously into the tumor-bearing mice on day 0. Tumor size and bodyweight of mice were measured and recorded every 2 days.

Histological Staining.

On the14th day after administration, the mice were sacrificed and their major tissues, including heart, liver, spleen, lung, and kidney, were excised to perform the histological assay.

The organs and tumors were fixed in 4% paraformaldehyde and the tissue section stained with hematoxylin−eosin (H&E) was observed under an IX73 microscope (OLYMPUS, Japan). All animals used in the procedures were handled in strict accordance with the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the University Committee in the Use of Animals of University of Macau.

2. Supplementary Results

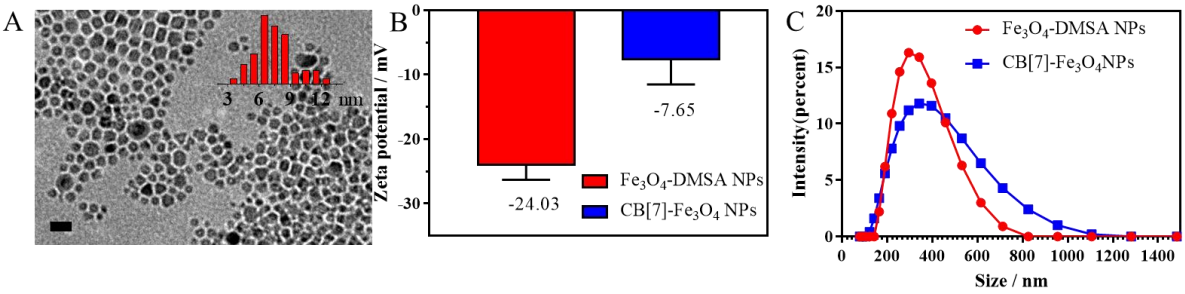

Fig. S1. Materials Characterization. (A) TEM image and size distribution of Fe_3O_4-DMSA NPs (Scale bar: 20 nm). (B) Zeta potentials of Fe_3O_4-DMSA NPs, and CB[7]-Fe_3O_4 NPs. (C) DLS analysis of Fe_3O_4-DMSA NPs and CB[7]-Fe_3O_4 NPs.

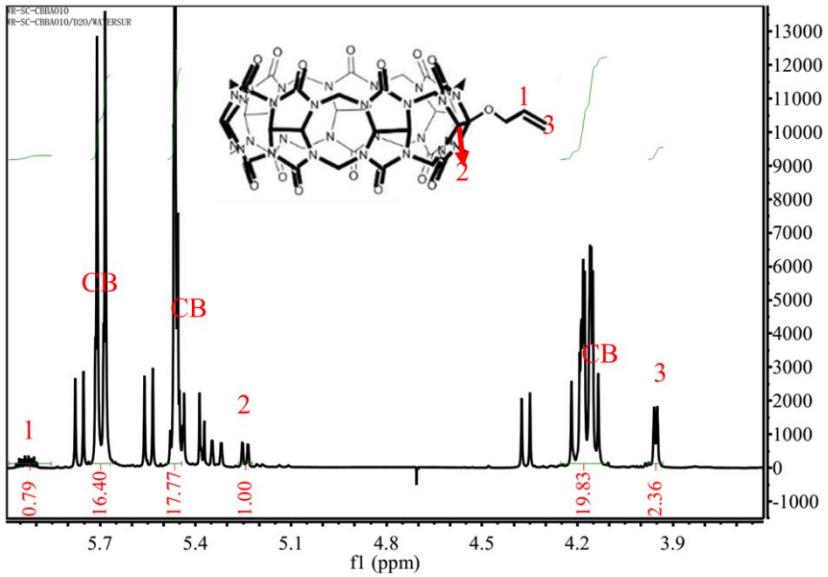

Fig. S2. The ^{1}H NMR spectrum of AO_1CB[7] in D_2O.

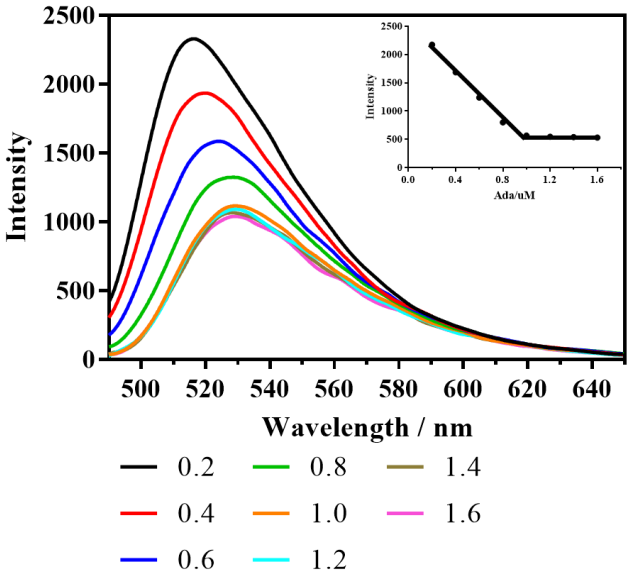

Fig. S3. Fluorescence intensity (λ_{ex} = 450 nm) and standard curve of CB[7]·AO with different amount of ADA. (2 mM AO, 1.6 mM CB[7]).

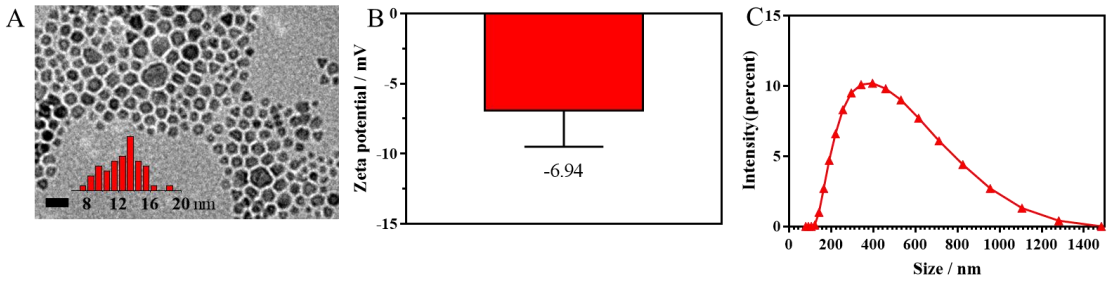

Fig. S4. Materials Characterization. (A) TEM image and size distribution of OX/FA/CB[7]-Fe_3O_4 NPs (Scale bar: 20 nm). (B) Zeta potential of OX/FA/CB[7]-Fe_3O_4 NPs. (C) DLS analysis of OX/FA/CB[7]-Fe_3O_4 NPs.

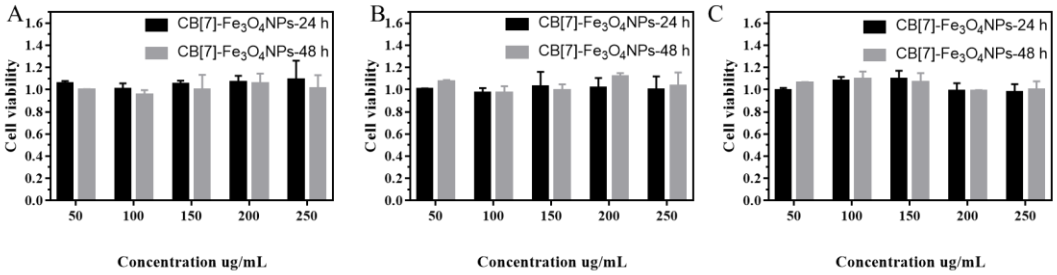

Fig. S5. MTT assays of CB[7]-Fe_3O_4 NPs with MCF-7, 4T1 and L02 cell lines upon incubation of CB[7]-Fe_3O_4 NPs at Fe concentration of 50, 100, 150, 200, 250 μg/mL for 24 and 48 h, respectively.

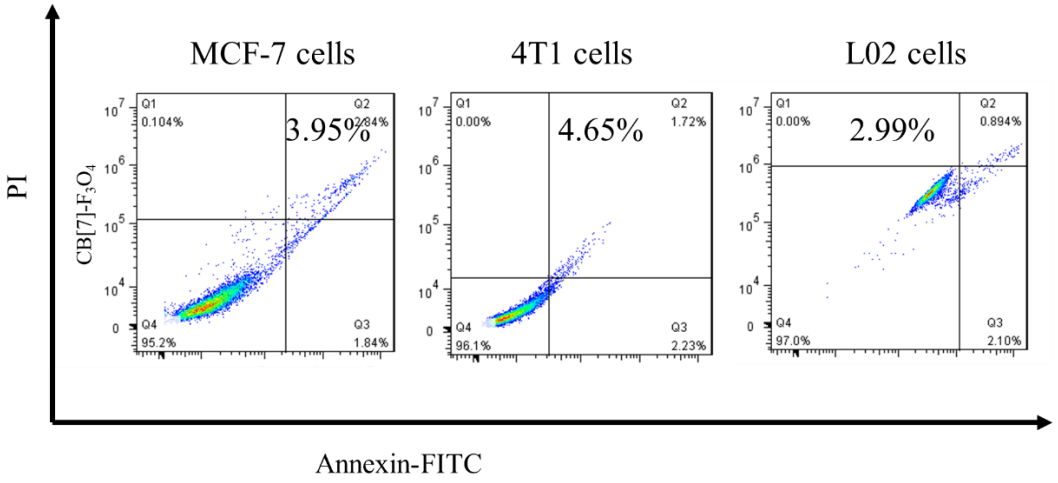

Fig. S6. Flow cytometry assay of MCF-7, 4T1 and L02 cells after 24 h incubation of CB[7]-Fe_3O_4 NPs at Fe concentration of 250 μg/mL.

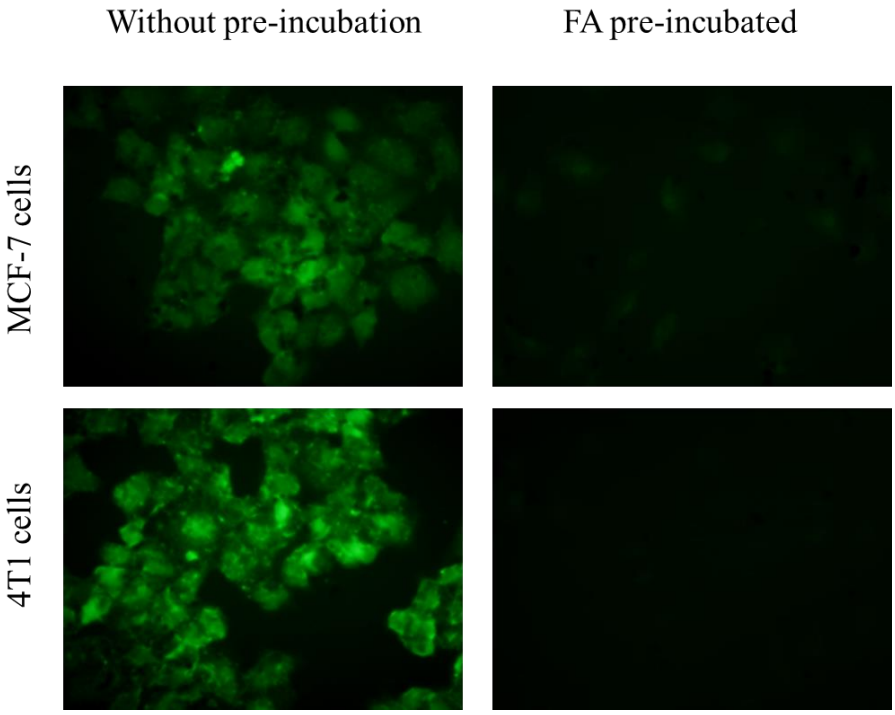

Fig. S7. Fluorescence images of MCF-7 and 4T1 cells with (or without) 4 h pre-incubation of excessive FA before 24 h incubation of FITC/FA/CB[7]-Fe_3O_4 NPs.

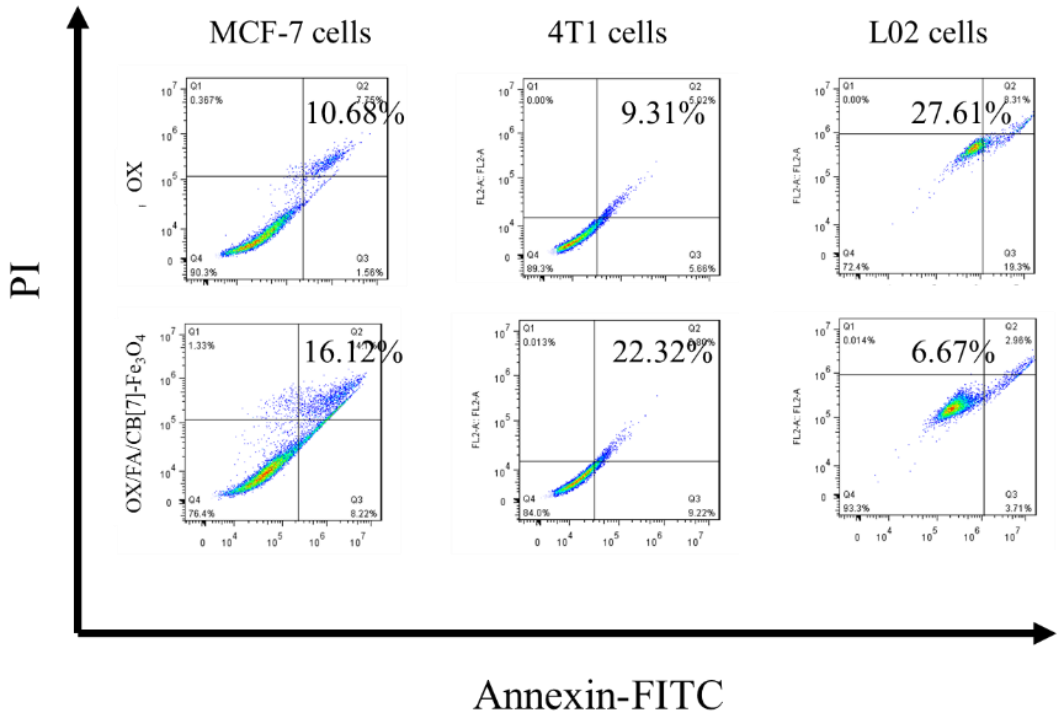

Fig. S8. The apoptosis rate of MCF-7 cells(A and D), 4T1 cells(B and E) and L02 cells(C and F) after 24 incubation of OX/FA/CB[7]-Fe_3O_4 NPs and OX at Fe concentration of 50 μg/mL and Pt concentration of 34.23 μM.

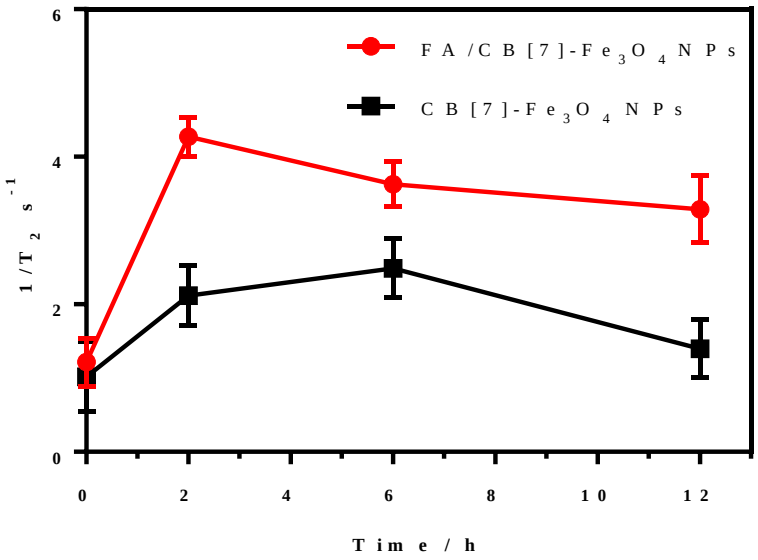

Fig. S9. The corresponding $1/T_2$ value of tumor site of 4T1 tumor bearing mice post intravenous injection of FA/CB[7]-Fe_3O_4 NPs and CB[7]-Fe_3O_4 NPs (at 1 mg/mL Fe concentration) for 0, 2, 6 and 12 h, respectively.

Reference

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2. Y. Ahn, Y. Jang, N. Selvapalam, G. Yun and K. Kim, *Angew. Chem. Int. Ed. Engl.*, 2013, **52**, 3140-3144.
3. D. Shetty, J. K. Khedkar, K. M. Park and K. Kim, *Chem. Soc. Rev.*, 2015, **44**, 8747-8761.