Biodegradable Hollow Manganese/Cobalt Oxide Nanoparticles for Tumor Theranostics

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Supplemental Experimental Section

Drug loading and release: For Dox loading, the solution of MCO NPs (0.1 mg mL^{-1}) was incubated with different concentrations of Dox (from 0 to 0.4 mg mL^{-1}) for 24 h followed by centrifugation for two times to remove free Dox to yield Dox-loaded MCO NPs (MCO-Dox NPs). The Dox to MCO NPs (L_{DOX}) ratio can be calculated by the following equation, in which the mass of MCO NPs was measured

$$L_{DOX} = \frac{Mass\ of\ Dox\ in\ MCO\ NPs}{Mass\ of\ MCO\ NPs} * 100\%$$

by an inductively coupled plasma- optical emission spectrometry (ICP-OES) and the mass of Dox in MCO NPs was measured by a UV-vis spectrometer. For the drug release experiment, the solution of MCO-Dox NPs was incubated with GSH solutions with predetermined concentrations (0.1, 0.5, 1.0 and 2.0 mM GSH) followed by high-speed centrifugation. The concentration of Dox in supernatant was determined by a UV-vis spectrometer.

GSH-activated MRI contrast performance.

The longitudinal (r_1) and transverse (r_2) relaxivity of individual MCO NPs were measured using a a Bruker MRI scanner (7.0 T, B-C 70/16, Bruker, US). The Mn concentrations were determined using an ICP-OES. To activate the MRI functions, MCO NPs were incubated 10 mM GSH for 60 min. Their r_1 and r_2 values were compared with original MCO NPs. The r_1 and r_2 values were obtained by plotting the T_1 and T_2 relaxivity times as a function of Mn concentrations

***In vitro* cell experiments.**

The human malignant glioma cell line (U87MG) was grown in a Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ^{0}C in a humidified 5% CO_2 atmosphere. For cell toxicity assay, cells were seeded into 96-well plates (1×10^4 per well) until adhesion and then incubated with MCO NPs with different concentrations. The standard thiazolyl tetrazolium (MTT, Sigma-Aldrich) test was used to measure cell viability of cells. For confocal fluorescence imaging, 5000 U87MG cells were cultured in 8-well cell culture slide for 24 h. Then the cells were mixed with MCO-70/Dox for 1 h. After washing with PBS for three times, the cells were fixed in 4% paraformaldehyde at room temperature for 15 min and rinsed with PBS. Then, cell culture slide was mounted with labeled with 4′, 6-diamidino-2-phenylindole (DAPI) containing mounting medium and the cells were analyzed using a laser scanning confocal fluorescence microscope.

***In vivo* MRI study.**

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of " the National Institutes of Health " and approved by the Animal Ethics Committee of " Clinical Center Animal Care and Use (NIH CC/ACUC)". 3×10^6 U87MG cells were injected at right hind leg to build the subcutaneous tumor model. The MRI study was performed on a Bruker MRI scanner (7.0 T, B-C 70/16, Bruker, US). Both T_1 and T_2-weighted MRI of tumors were acquired before and at 2, 15, and 24 h after the injection of MCO-70-Dox NPs. The inject dose for Mn in MCO NPs was 2.5 mg/kg. For T_1-weighed MRI, multi-slice

multi-echo sequence was employed to acquire images using parameters as follows: repetition time (TR) = 400 ms, echo time (TE) = 8 ms, flip angle = 180^{o}, matrix size = 256 × 256. For T_2-weighted MRI, multi-slice multi-echo sequence was employed to acquire images using parameters as follows: repetition time (TR) = 2000 ms, echo time (TE) = 50 ms, flip angle = 180^{o} , matrix size = 256 × 256.

***In vivo* tumor inhibition.**

The U87MG tumor-bearing mice were randomly divided into 4 groups (4 mice each group) including (1) PBS group, (2) Dox group, (3) MCO NP group, (4) MCO-Dox group. The treatment was performed when the tumor volume exceeded 50 mm^3. 100 uL PBS, 2.5 mg/kg Dox, 2.5 mg/kg MCP-70 NP, and MCO-70/Dox NPs with 2.5 mg/kg Dox and MCO NPs were intravenously injected for each group on 0, 2 and 4 day of treatment, respectively. The volume of tumors was measured every other day and calculated by the following equation: Volume = Length × Width^2/2. The body weight of the mice and survival curves were also monitored.

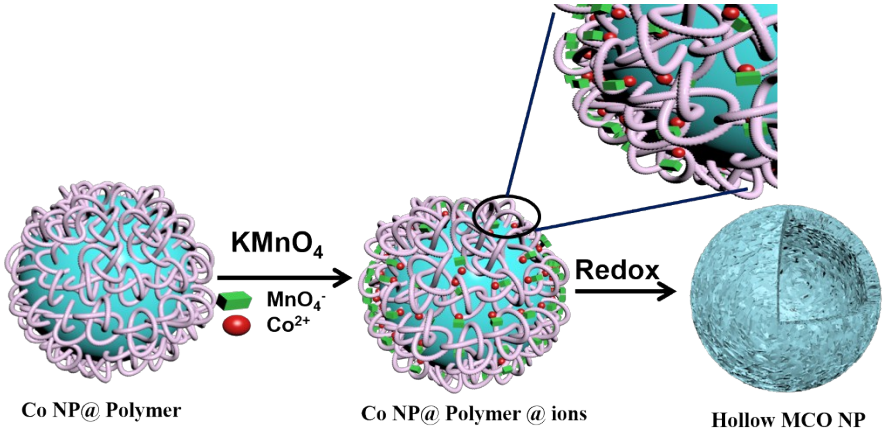

Figure S1. Schematics illustration of the formation mechanism of hollow manganese/cobalt oxide (MCO NPs).

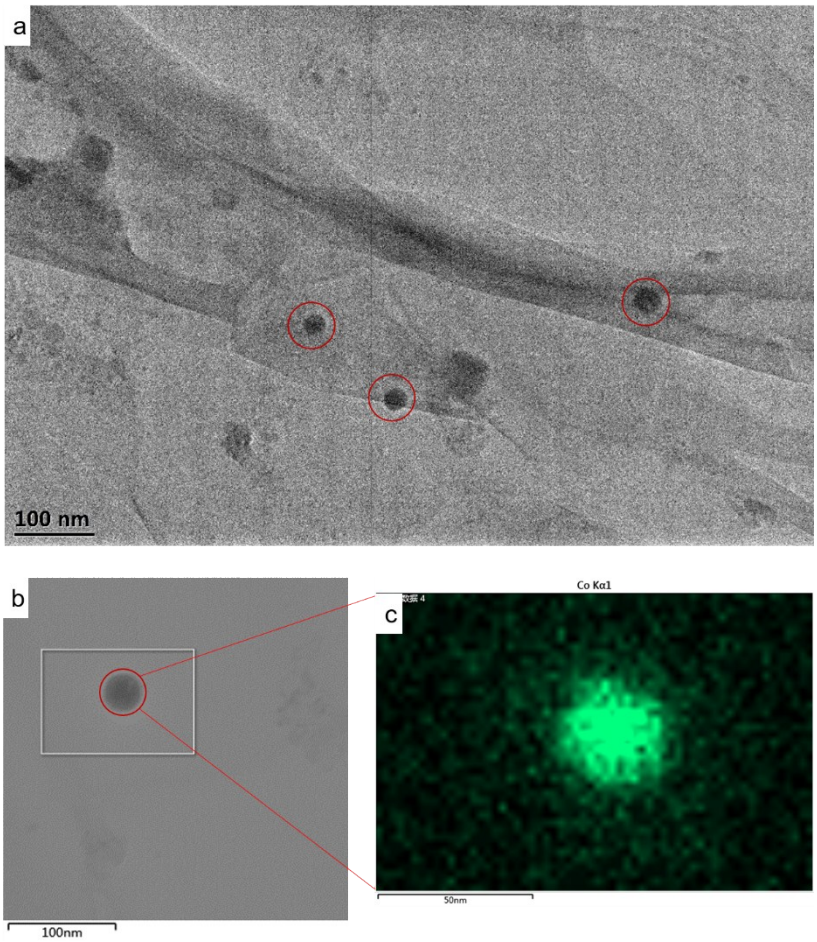

Figure S2. (a) TEM image of Co NPs indicated by red circles. (b) STEM image of Co NPs, (c) TEM elementary mapping of Co NPs.

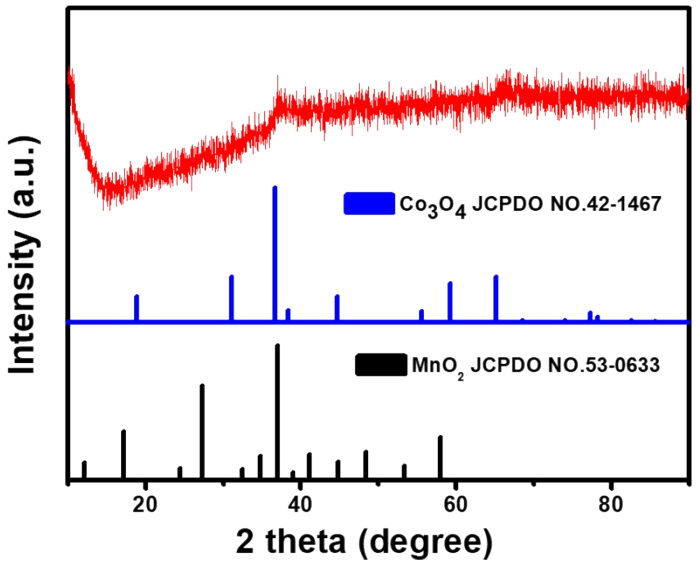

Figure S3. The XRD spectrum of MCO NPs.

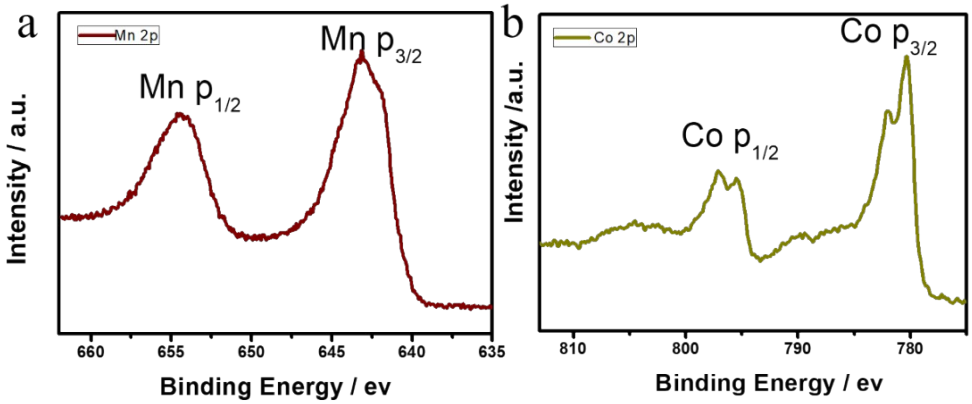

Figure S4. XPS spectra of (a) Mn 2p and (b) Co 2p, indicating that MCO NPs were composed of MnO_2 and Co_3O_4.

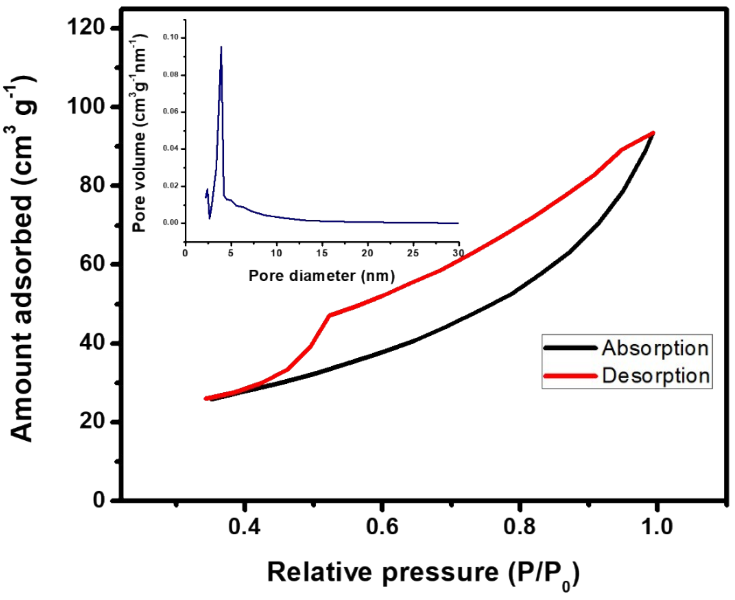

Figure S5. The N_2 adsorption-desorption isotherm and the corresponding pore size distribution of MCO NPs with pore size and surface area of 4.2 nm and 84.111 m^2/g, respectively.

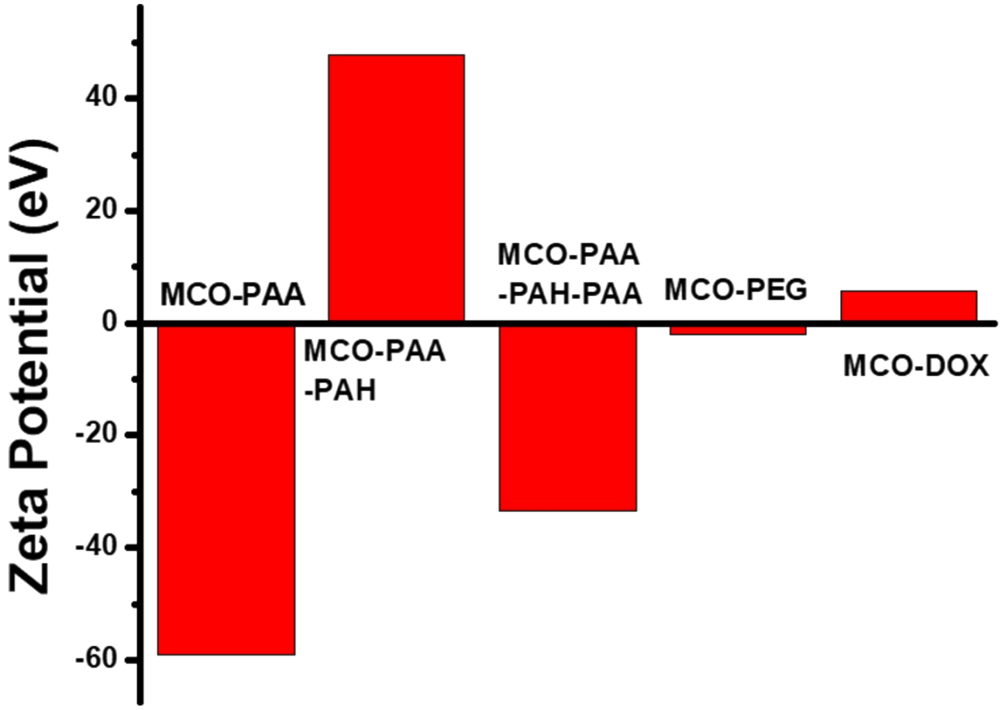

Figure S6. Zeta potential of MCO-PAA, MCO-PAA-PAH, MCO-PAA-PAH-PAA, MCO-PEG and MCO-DOX.

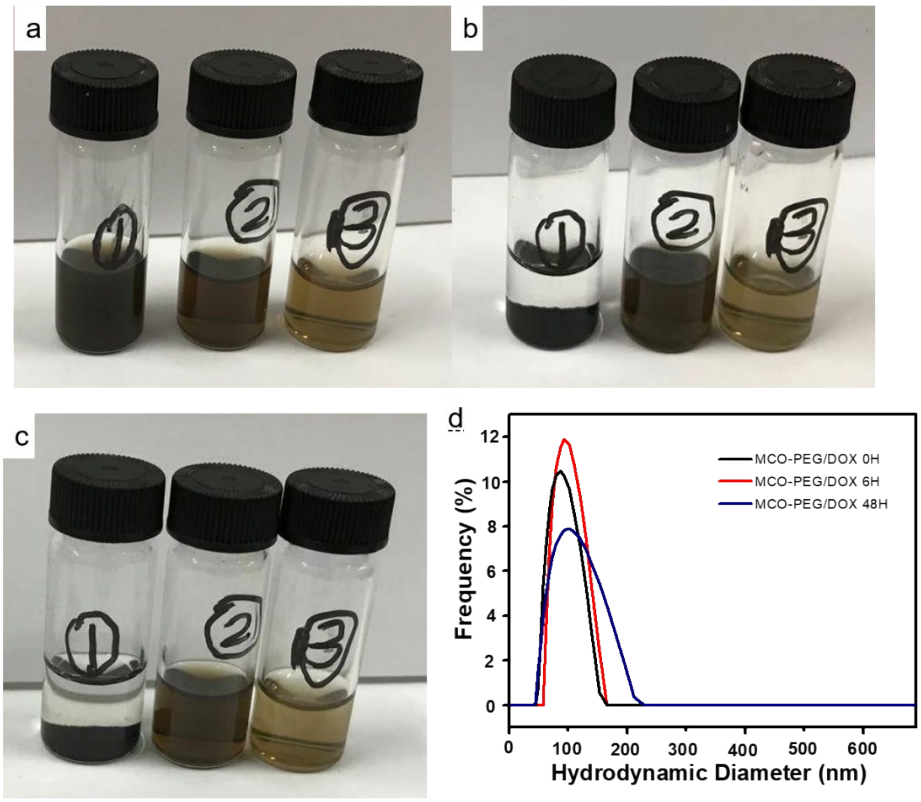

Figure S7. The pictures of MCO-PAA, MCO-PEG, and MCO-PEG/Dox NPs in PBS solution for (a) 0 h, (b) 6 h, and (c) 48 h. The vials from left to right in the pictures contain MCO-PAA NPs, MCO-PEG NPs, and MCO-PEG/Dox NPs in PBS solution，respectively; (d) DLS of MCO-PEG/Dox NPs at 0 h, 6 h, 48 h.

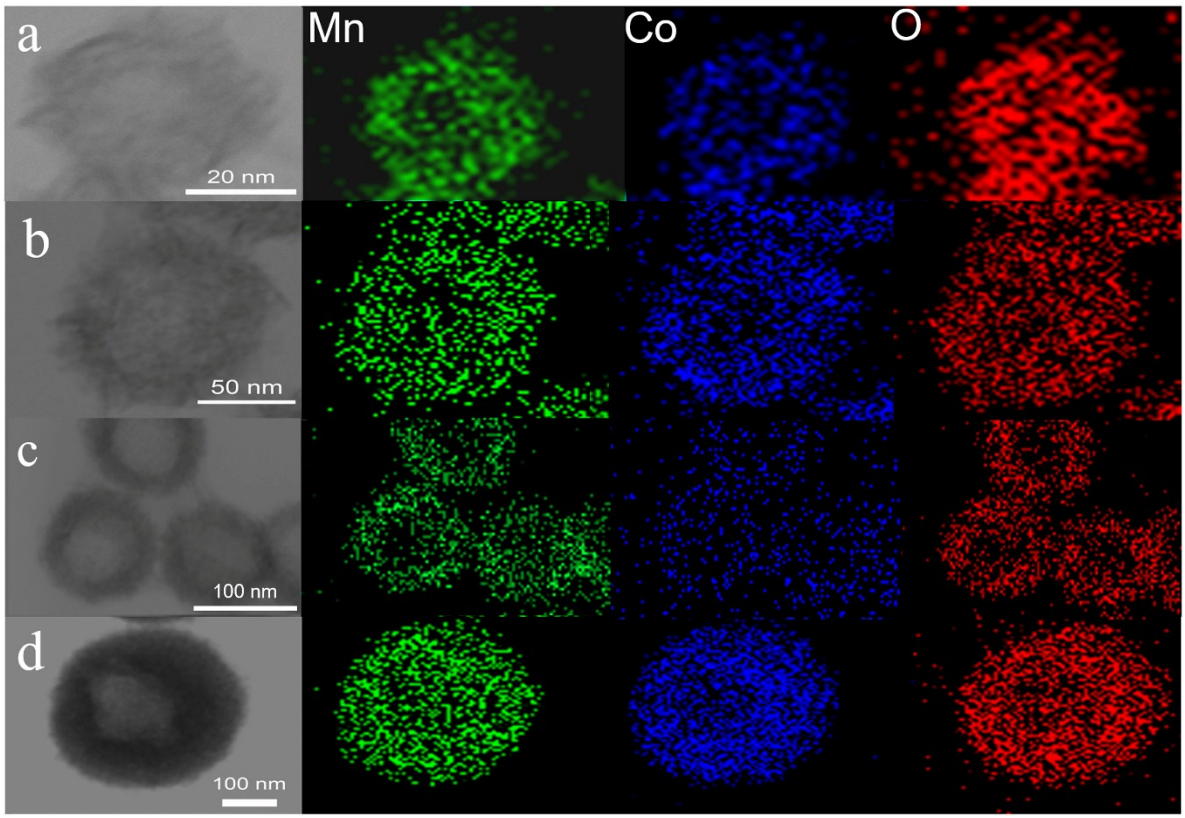

Figure S8. TEM images and elementary mapping of MCO NPs with different sizes: (a) MCO-50 NP, (b) MCO-70 NP, (c) MCO-100 NP, and (d) MCO-300 NP. The signals from Mn, Co and O are in the 2nd, 3rd and 4th columns, respectively,

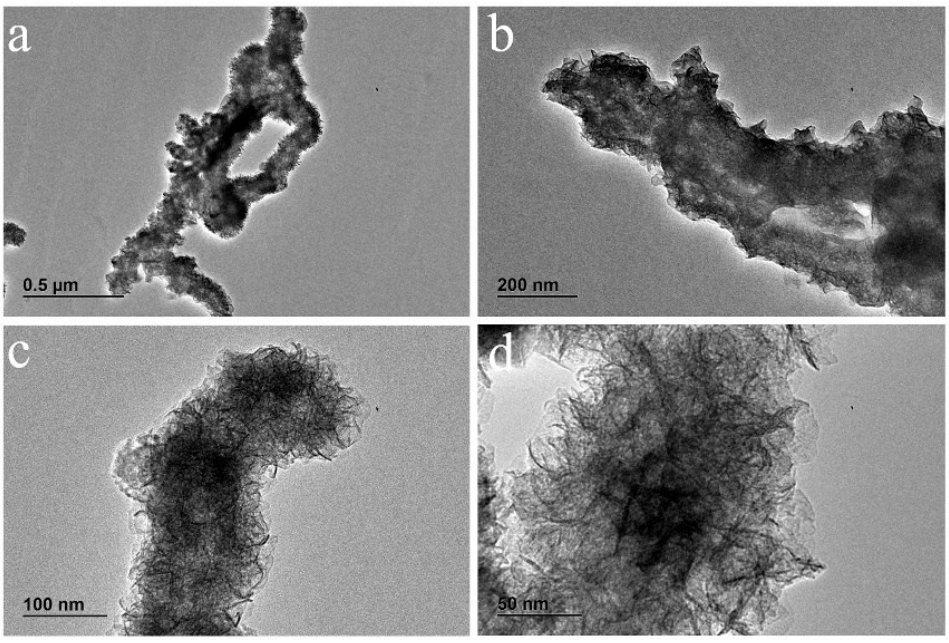

Figure S9. TEM images of MCO NPs without PAA as surfactant in different magnification. NP aggregates were formed.

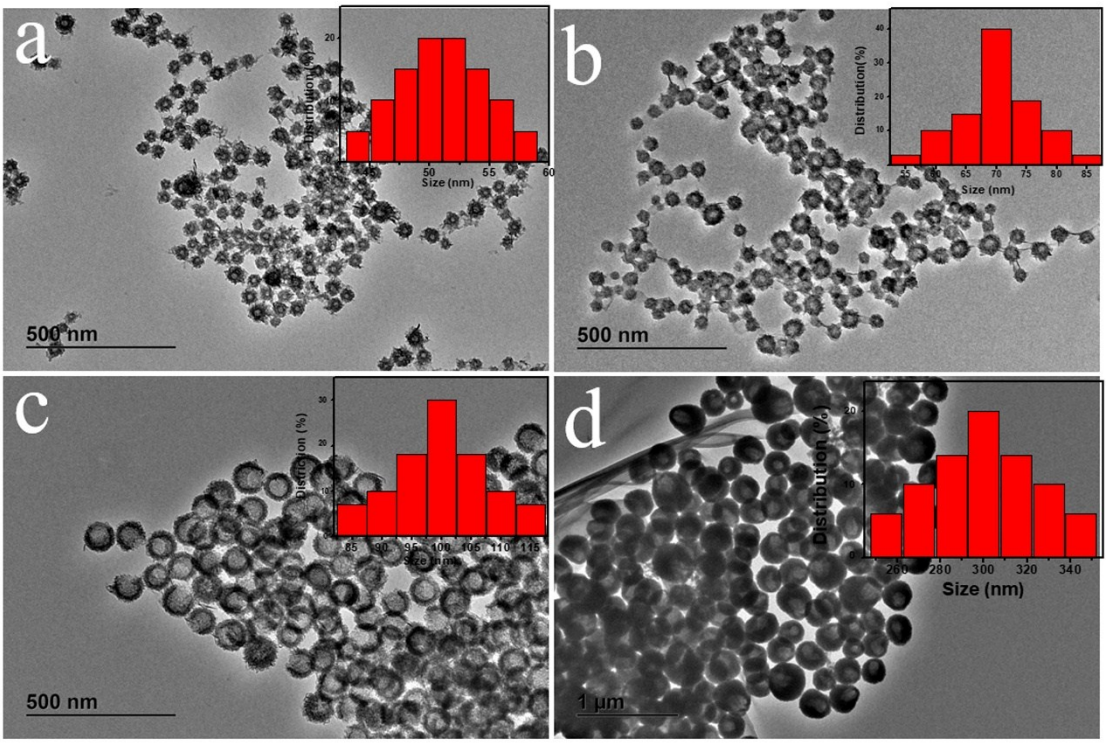

Figure S10. TEM images and histogram analysis of the size distribution of (a) MCO-50 NP, (b) MCO-70 NP, (c) MCO-100 NP, and (d) MCO-300 NP. The average sizes are 50.5 ± 6.4, 67.5 ± 9.4, 95.0 ± 7.9, and 286.0 ± 47.2 nm, respectively.

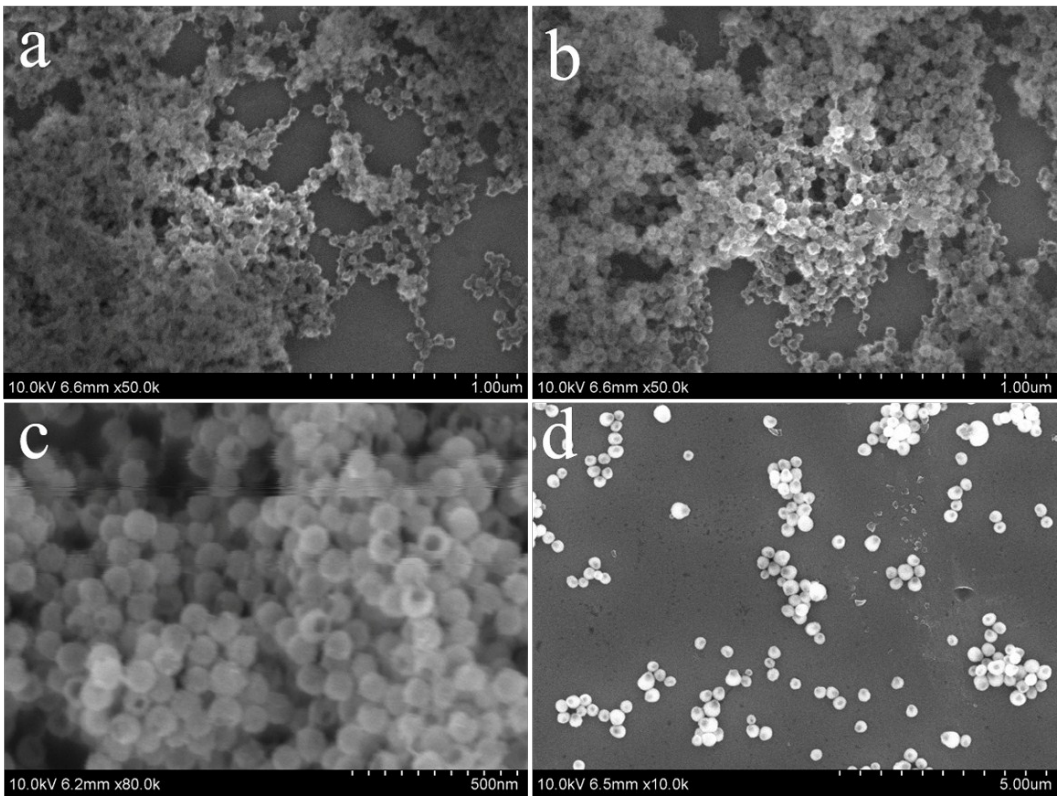

Figure S11. Large area SEM images of MCO NPs with different sizes. (a) MCO-50 NP, (b) MCO-70 NP, (c) MCO-100 NP and (d) MCO-300 NP.

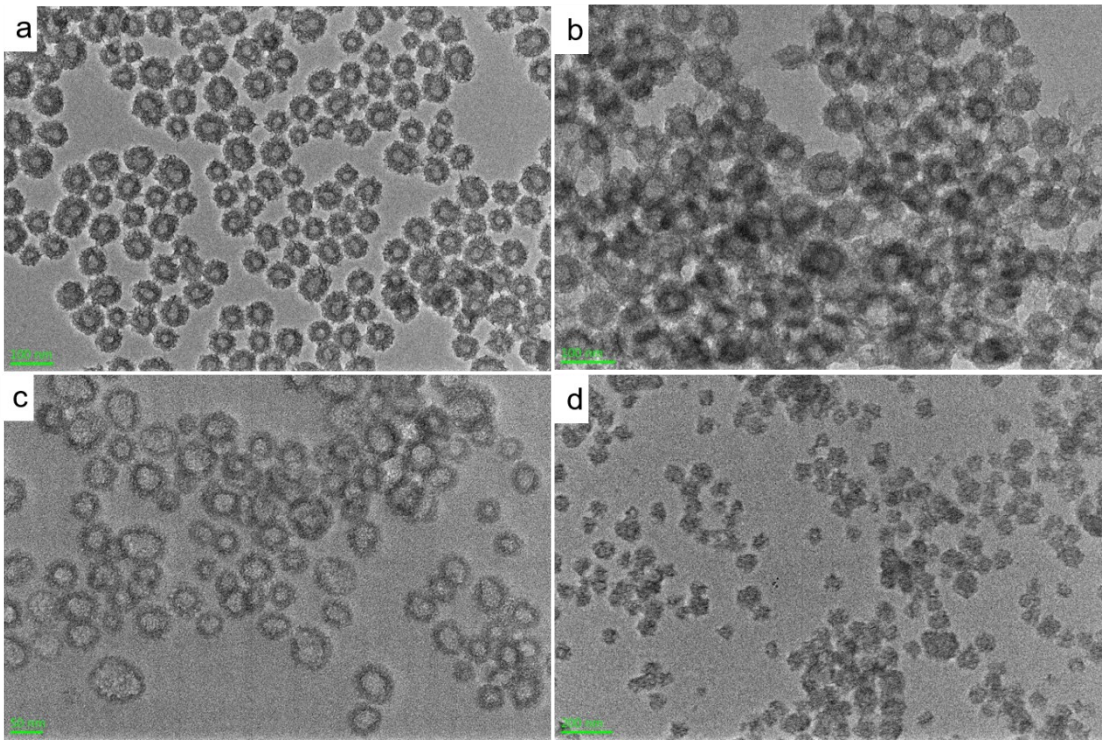

Figure S12. (a) TEM images of MCO NPs made from PAA with different molecular weights. The TEM images of MCO NPs made from PAA with molecular weight of (a) Mw: 2 K, (b) Mw: 5 K, (c) Mw: 450 K, and (d) Mw:6M. The mass of PAA was kept as 40 mg for all conditions.

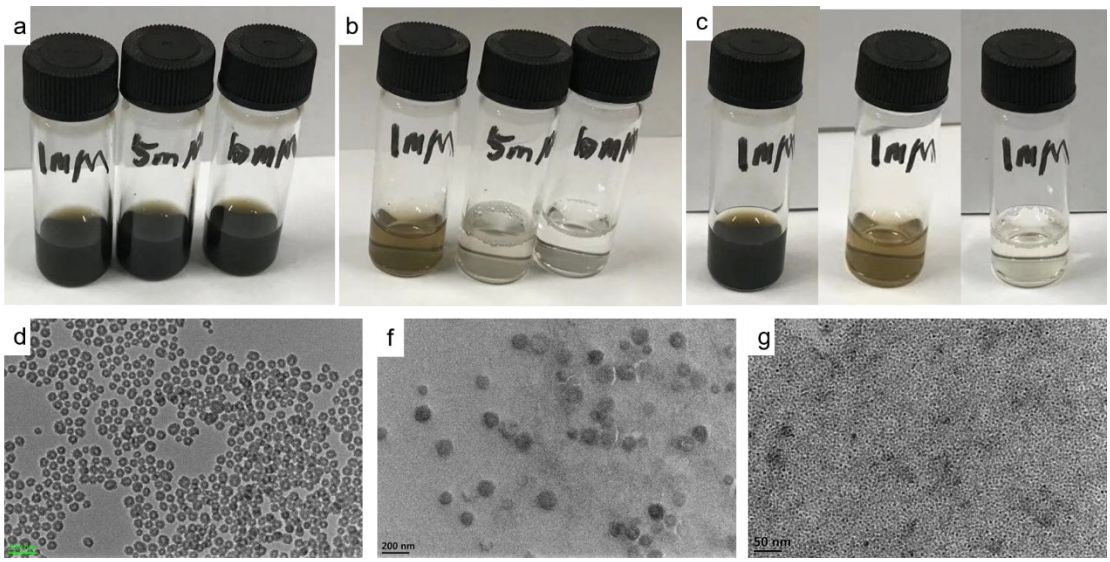

Figure S13. (a,b). The photographs of MCO NPs incubation with 1, 5, 10 mM GSH (from left to right) at (a) 0 and (b) 30 min;(c). The photographs from left to right are MCO NPs incubated with 1mM GSH at 0 and 24 h, and at 48 h after adding another 10uL 100 mM GSH at 24 h and their corresponding (d-g).TEM images.

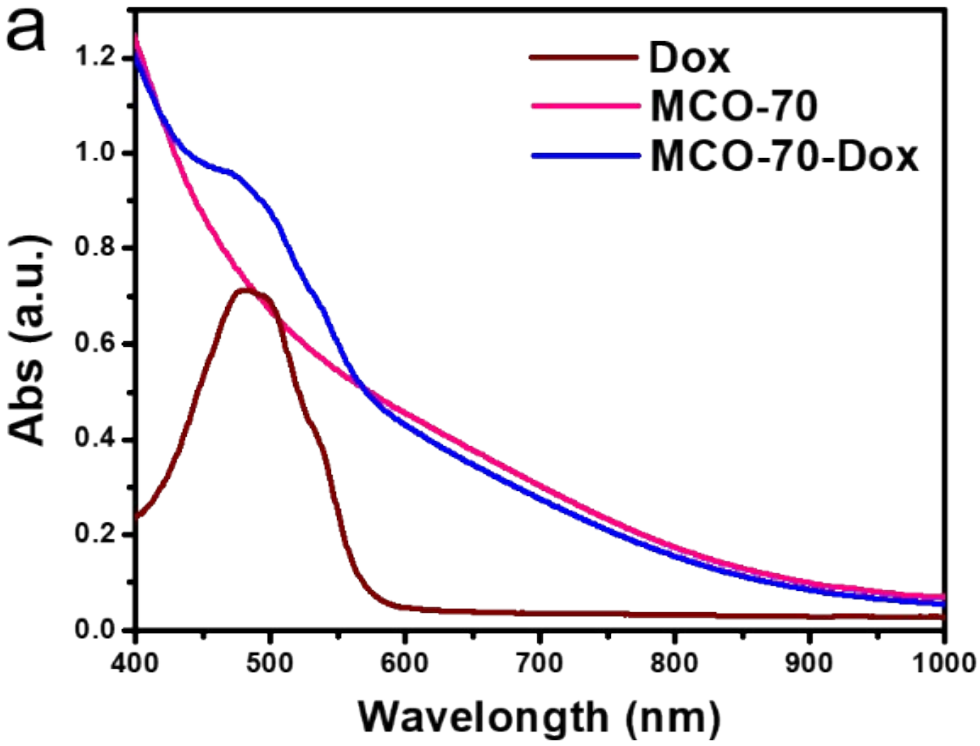

Figure S14. (a) UV-vis spectrum of the Dox, MCO-70, MCO-70-DOX. (b) Relative viabilities of U87MG cells after incubation with deferent concentration of MCO NPs.

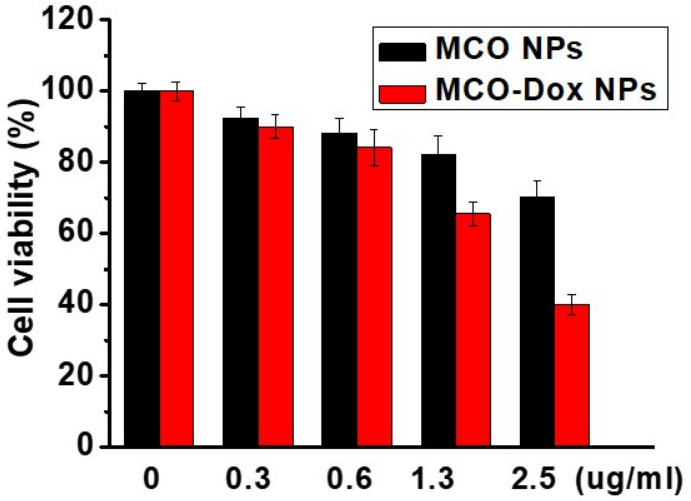

Figure S15. Relative viabilities of U87MG cells after incubation with deferent concentration of MCO NPs and MCO-Dox NPs.