Supporting Information

Combined chemo-photothermal anti-tumor therapy using molybdenum disulfide modified with hyperbranched polyglycidyl

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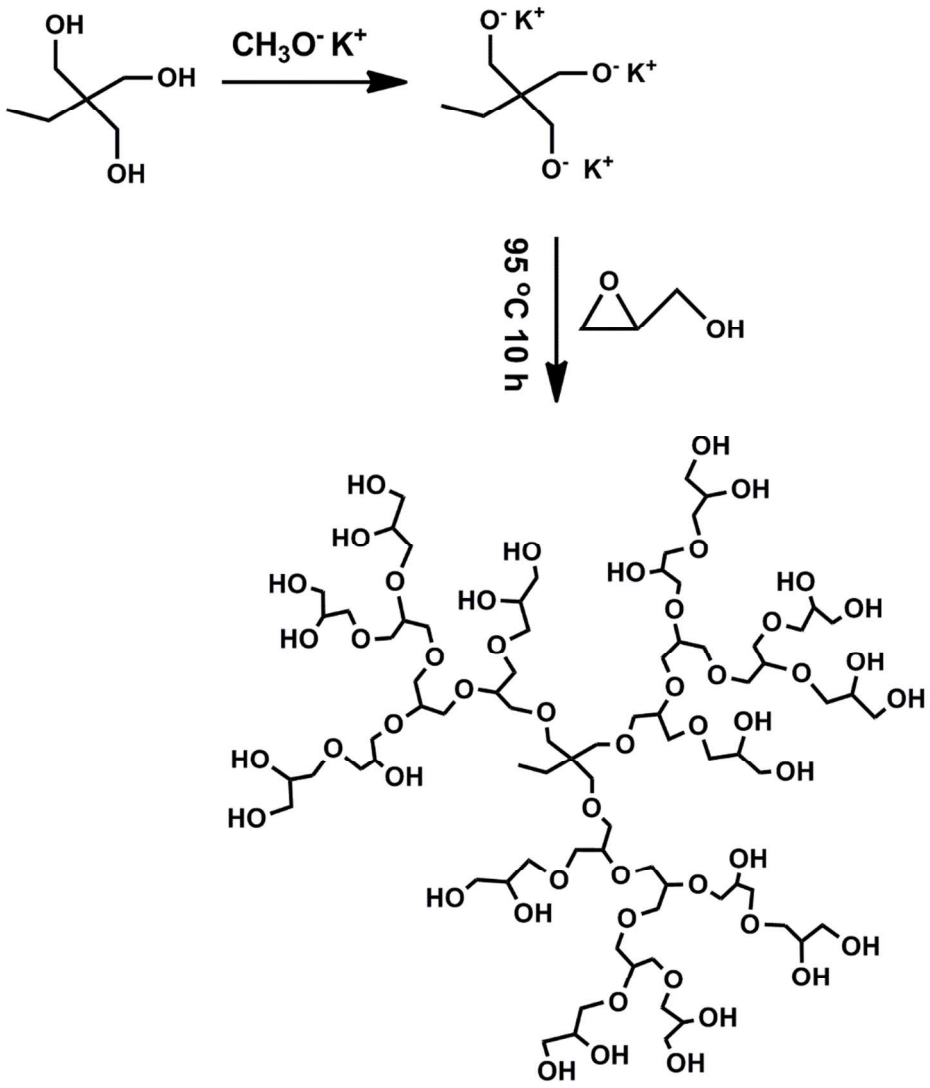
1. Preparation and characterization of hyperbranched polyglycidyl (HPG)

1.1. Materials

Glycidol and Cation exchange resin were purchased from Aladdin (Shanghai, China). Trimethylol propane was obtained from Adamas Reagent (Shanghai, China). Potassium methylate solution in methanol (20 wt%) was provided by Sigma-Aldrich (Shanghai, China). Methanol was obtained from Tianjin Chemical Reagent (Tianjin, China).

1.2. Synthesis of HPG

HPG was synthesized by anionic ring-opening multibranching polymerization, as displayed in Scheme S1.[1] Briefly, trimethyloyl propane (120 mg) was put to a three-neck round bottom flask under nitrogen atmosphere. Then, potassium methylate solution in methanol (0.1 mL, 20 wt%) was added to the flask. The mixture was stirred for 15 min. After that, excessive methanol was removed in a vacuum. Then, glycidol (5.5 mL) was dropped over 10 h into the flask at 95°C. After the reaction, the flask was cooled to room temperature. Methanol was added to the flask to dissolve the product. Then, the dissolved product and acidified polycation resin were mixed by stirring for 12 h at room temperature. After that, crude product was obtained after steaming to remove methanol. The product was then dialyzed for three days against water using cellulose acetate dialysis tubing (MWCO =1000, USA). Finally, purified HPG was obtained after freeze drying.



Scheme S1. Synthesis route of HPG.

1.3. Characterization of HPG

^{1}H NMR of HPG in D_2O was performed on a Bruker spectrometer (300 M, Bruker, Germany). The fourier transform infrared (FTIR) spectrum of HPG was obtained on a FTIR spectrometer (VERTEX70, Bruker, Germany). The weight-average molecular weight (Mw), number-average molecular weight (Mn) and polydispersity index (PDI) of the HPG were determined by using a gel permeation chromatograph (GPC) (Viscotek Model 270, Marvin, UK).

1.4 Characterization data of HPG

^{1}H NMR spectrum of HPG in D_2O was shown in Figure S1. The signal proton peak (3.1- 4.1 ppm) is the methylene and methine group of HPG.

FTIR spectrum of HPG was shown in Figure S2. The peak at 3375 cm^{-1} was owing to the O-H stretching. The peaks from 2877 to 2927 cm^{-1} were owing to the C-H stretching from CH_3 and CH_2. The peaks at 1463 and 1100 cm^{-1} were the typical peaks of CH_2 and C-O-C, respectively.

According to the GPC analysis (Figure S3), the Mw of HPG was 10.4 kDa, and the Mn of HPG was 8.17 kDa with PDI of 1.275.

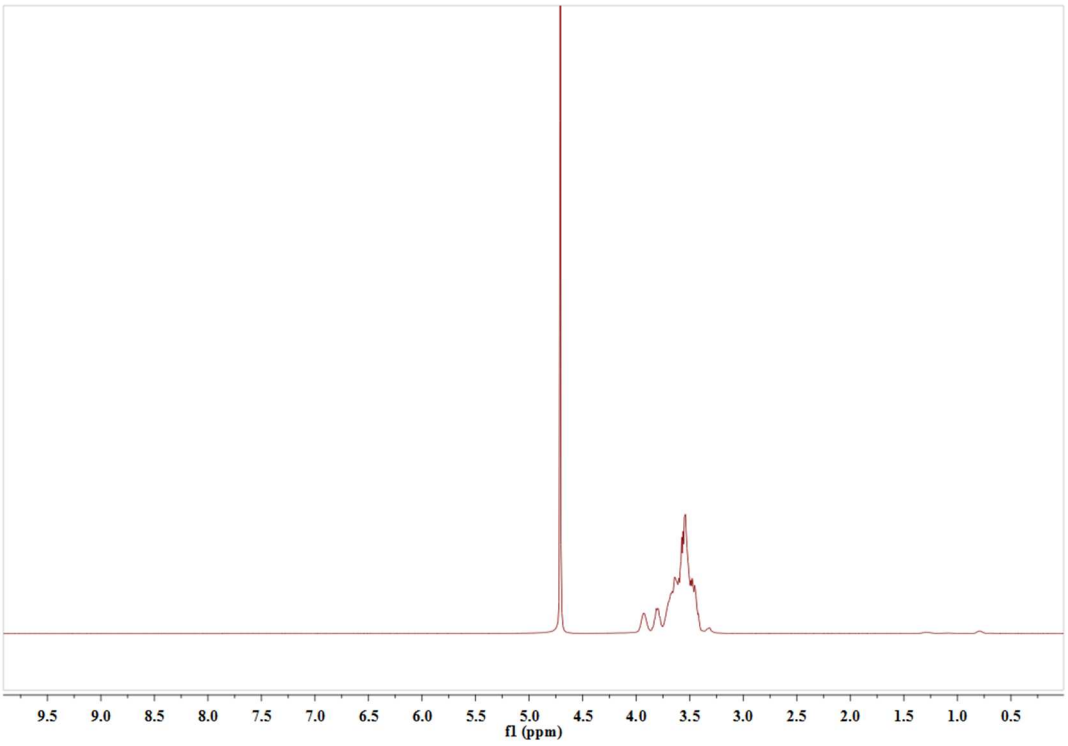


Figure S1. ^{1}H NMR spectrum of HPG in D_2O.

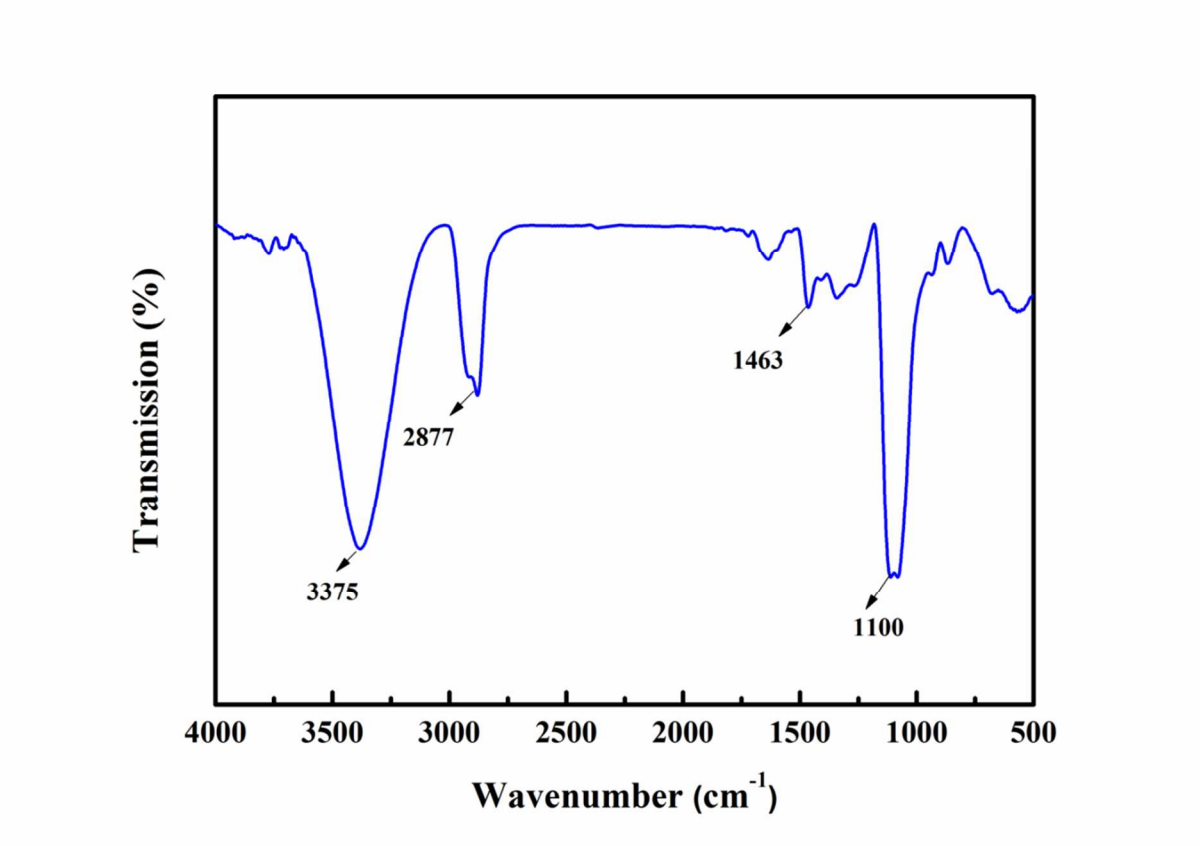


Figure S2. FTIR spectrum of HPG.

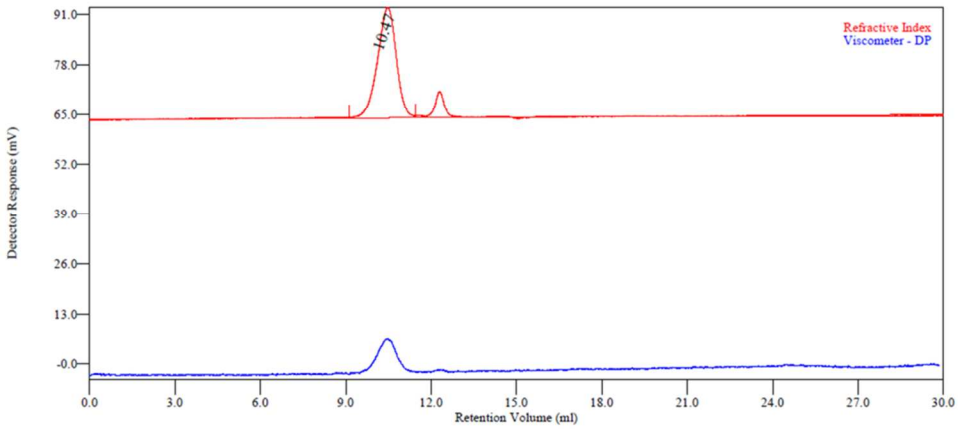


Figure S3. GPC gram of HPG.

2. Stability of MoS_2-HPG nanosheets

The stability of MoS_2-HPG was further studied by measuring their hydrodynamic sizes in different solutions or with different dilutions by using the zeta potential analyzer. Figure S4a shows the hydrodynamic sizes of MoS_2-HPG nanoparticles dispersed in different solutions (H_2O, PBS, RPMI-1640 and RPMI-1640+10%FBS). It indicates that the MoS_2-HPG nanosheets were well dispersed in the different solutions. In addition, Figure S4b shows the hydrodynamic sizes of MoS_2-HPG nanosheets dispersed in RPMI-1640 with different dilutions. It indicates that the size of MoS_2-HPG nanosheets did not varied a lot along with the dilutions, suggesting that the MoS_2-HPG nanosheets had good stability.

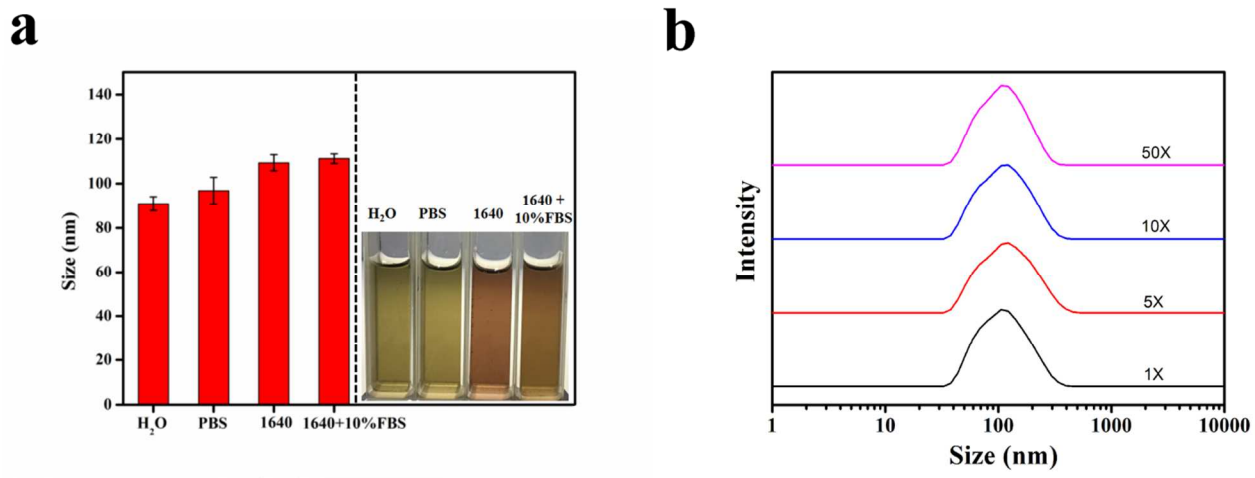


Figure S4. (a) Hydrodynamic sizes of MoS_2-HPG (100 μg/mL) dispersed in different solutions (H_2O, PBS, RPMI-1640 and RPMI-1640+10%FBS); (b) Hydrodynamic sizes of MoS_2-HPG (100 μg/mL) dispersed in RPMI-1640 with different dilutions.

3. Flow cytometry analysis of the viability of B16 cells after photothermal treatment

3.1. Method

B16 cells were cultured in 24-well plates (5×10^4 cells/well) for 24 h in DMEM complete medium. Then, the complete media in the wells were replaced with different concentrations of MoS_2-HPG suspensions in DMEM incomplete medium. After incubation for 4 h, the culture media were discarded and the cells rinsed with PBS thrice. Then, fresh complete medium was supplemented to the wells. The

cells were treated with an 808 nm laser for 10 min with different powers. After that, the cells were cultured for 12 h, stained with PI, and detected by using a flow cytometer (cytoFLEX, BECKMAN COULTER, USA).

3.2. Results

As described above, the cell viability of B16 cells after the photothermal treatment with MoS_2-HPG was measured, as shown in Figure S5. At the same irradiation power of 2 W, the viability of B16 cells decreased along with increasing the MoS_2-HPG concentration. In addition, at the same MoS_2-HPG concentration, the viability of B16 cells decreased along with increasing the irradiation power.

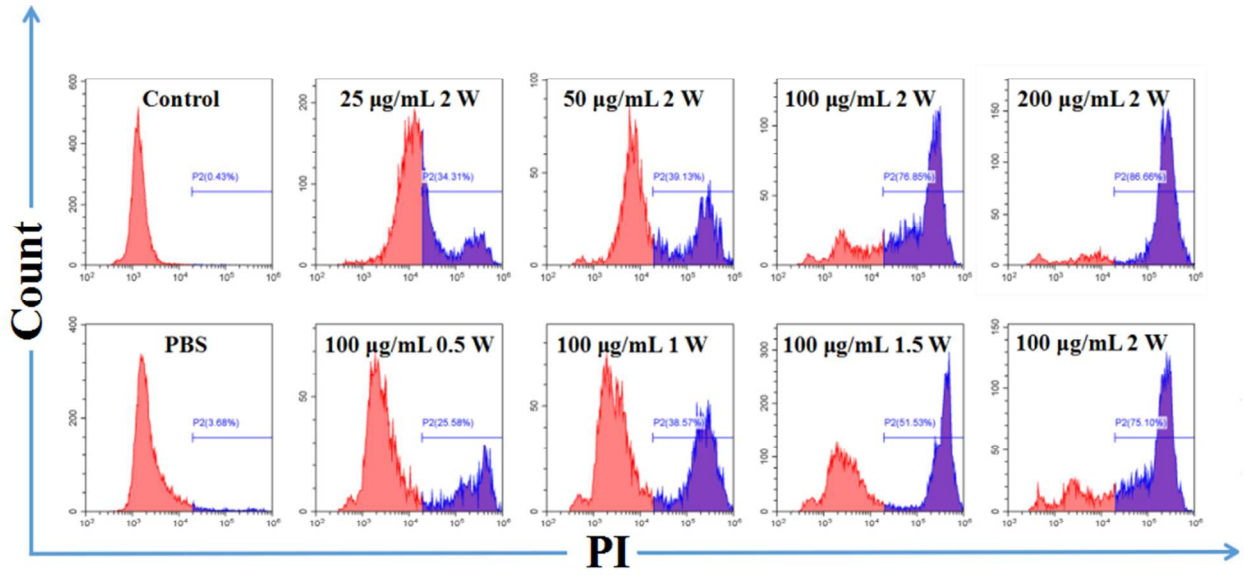


Figure S5. Flow cytometry analysis of the viability of B16 cells after photothermal treatment.

4. Fluorescent images of viable/dead B16 cells after photothermal treatment

4.1. Method

B16 cells were cultured in 24-well plates (5×10^4 cells/well) for 24 h. Then, the cells were co-cultured with different materials for 4 h. After removing the materials, the cells were rinsed three times, and cultured in fresh complete medium. Some of the cells were subject to irradiation for 10 min

under 808 nm laser (2 W/cm^2). After that, all the cells were incubated for 12 h, co-stained with Calcein-AM and PI, and observed with an inverted fluorescence microscope (Axio Observer A1, ZEISS, Germany).

4.2. Results

B16 cells with or without photothermal treatment were observed, as shown in Figure S6. Irradiation alone did not cause cell death, as shown in PBS+NIR group. MoS_2-HPG treatment alone caused a small amount of the cells to die. By contrast, the photothermal treatment with MoS_2-HPG caused a large amount of the cells to die. Further, combination of the photothermal treatment with MoS_2-HPG and the chemical therapy with DOX caused all the cells to die. From the results, the photothermal treatment could efficiently kill the cells, and the photothermal and chemical co-therapy could kill all the cells.

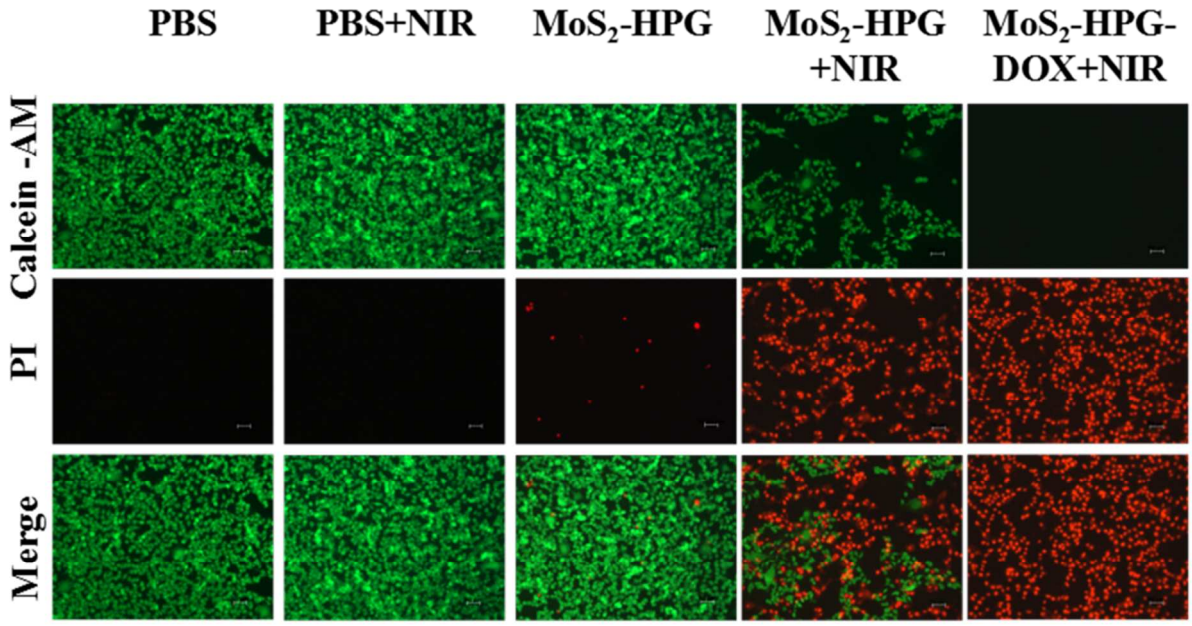


Figure S6. Fluorescent images of Calcein-AM (viable cells, green) and PI (dead cells, red) co-stained B16 cells after treated with different methods. The scale bar is 50 μm.

5. Preparation and characterization of MoS_2-PEG nanosheets

5.1. Preparation method

The preparation method of MoS_2-PEG was similar to that of MoS_2-HPG. In brief, MoS_2 nanosheets (50 mg) were dispersed in 25 mL ultrapure water. Then, 100 mg of PEG was dissolved into the MoS_2 suspension. The suspension was ultrasonic treated for half an hour, and stirred for 24 h. The product MoS_2-PEG was obtained by centrifuging at 7500 r/min for 10 min, and rinsed with deionized water, and lyophilized.

5.2. Characterization with TG

The obtained MoS_2-PEG was characterized with TG by using the same method used for the TG characterization of MoS_2 and MoS_2-HPG. The weight loss curves of MoS_2, MoS_2-PEG and MoS_2-HPG were shown in Figure S7. From 200°C to 400°C, the weight loss of MoS_2-PEG (14.56%) was close to that of MoS_2-HPG (14.6%).

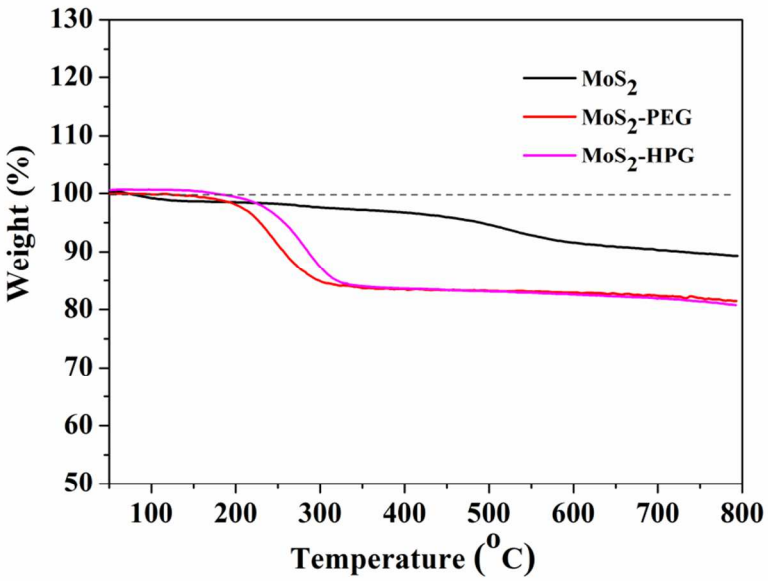


Figure S7. TG curves of MoS_2, MoS_2-PEG and MoS_2-HPG.

6. In vivo blood circulation retention time

6.1. Method

The blood circulation retention time of MoS_2-HPG, MoS_2-PEG and MoS_2 were measured.[2] Briefly, the MoS_2-HPG, MoS_2-PEG or MoS_2 suspensions (100 μL in PBS) with the same MoS_2 content were intravenously injected into female BALB/c mice through the tail vein. The blood sample (100 μL) was collected from the mice orbit at 0.5, 1, 3, 6 and 9 h after injection. All the blood samples were digested for 12 h with 3 mL of concentrated nitric acid, then mixed with 3 mL of 30% hydrogen peroxide solution and further digested for 2 h. The blood samples were placed in an oven (100^{o}C) until the residual volume decreased to 0.5 mL. Then, the residual liquid was diluted with 2% HNO_3 solution to 5 mL. The Mo element content in the samples was measured with an inductively coupled plasma mass spectrometry (ICP-MS, XSERIES 2, Thermo, USA).

6.2. Results

The Mo element contents in the digested blood samples were shown in Figure S8. From 0.5 to 9 h after injection, the Mo content was highest in MoS_2-HPG group, middle in MoS_2-PEG group, and lowest in MoS_2 group. It indicates that the blood circulation retention time of MoS_2-HPG was the longest and that of MoS_2 was the shortest among the three nanosheets. According to the data shown in Figure S8, the blood circulation half-life times of MoS_2-HPG, MoS_2-PEG and MoS_2 nanosheets in mice were calculated to be about 4.2, 3.1 and 1.4 h, respectively. The results suggest that the adsorption of PEG or HPG both increased the blood circulation retention time of MoS_2, and that the MoS_2 adsorbed with HPG had longer blood circulation retention time than that adsorbed with linear PEG.

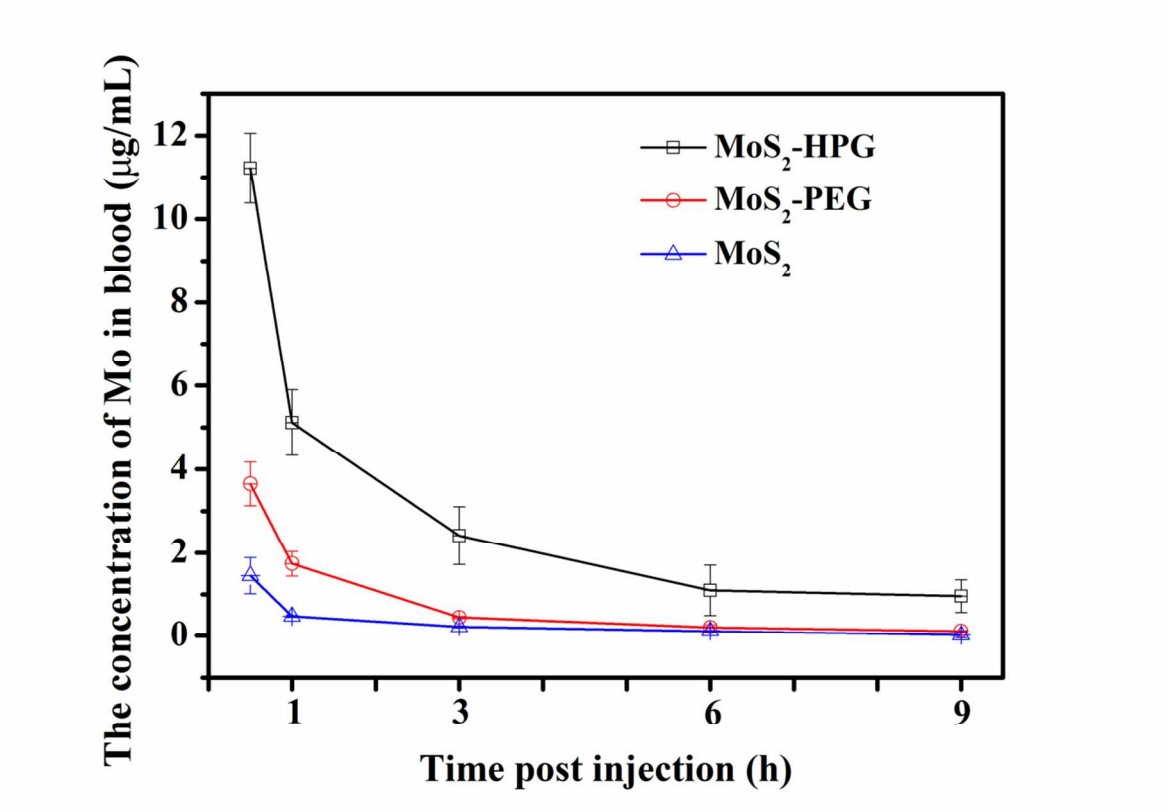


Figure S8. The concentrations of Mo element in blood at different times post injection.

7. Photographs of the tumors and H&E stained slices of the tumors

At the end of the treatment, the tumors were collected from the mice, as displayed in Figure S9a. In addition, the H&E stained slices of the tumors were shown in Figure S9b.

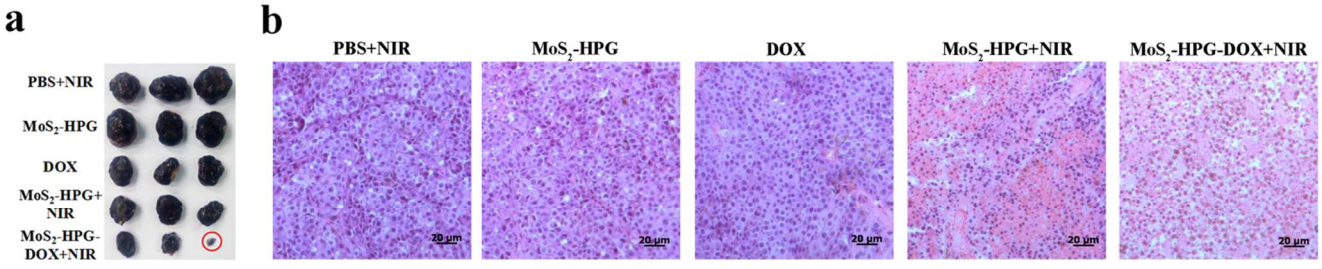


Figure S9. (a) Photographs of the tumors collected from the mice at the end of the treatment. (b) H&E stained slices of the tumors from different groups of the mice after 15 days of treatment.

REFERENCES:

(1) Kainthan, R. K.; Muliawan, E. B.; Hatzikiriakos, S. G.; Brooks, D. E. Synthesis, Characterization, and Viscoelastic Properties of High Molecular Weight Hyperbranched Polyglycerols. *Macromolecules* **2006,** *39*, 7708-17. DOI: 10.1021/ma0613483.

(2) Qin, C.; Fei, J.; Wang, A.; Yang, Y.; Li, J. Rational assembly of a biointerfaced core@shell nanocomplex towards selective and highly efficient synergistic photothermal/photodynamic therapy. *Nanoscale* **2015,** *7* (47), 20197-210. DOI: 10.1039/c5nr06501a.