ADVANCED MATERIALS

Supporting Information

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Co_9Se_8 Nanoplates as a New Theranostic Platform for Photoacoustic/Magnetic Resonance Dual-Modal-Imaging-Guided Chemo-Photothermal Combination Therapy

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Chemicals and apparatus

Cobalt (II) acetate tetrahydrate was purchased from Sinopharm Chemical Reagent Co. Ltd (China). Selenium dioxide was purchased from Sigma-Aldrich. All other chemicals were of analytical grade and were used as received from manufacturer. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ resistivity) was used in all runs.

Ultraviolet-visible-near-infrared light (UV-Vis-NIR) absorption spectra was recorded using a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan) at room temperature. Atomic force microscopy (AFM) measurement was performed with a Multimode 3D atomic force microscope (Bruker Nano Inc., USA). Photothermal irradiation was operated using a semiconductor laser unit (KS3-11312-110, BWT, Beijing Kaipulin Co. Ltd, China). The fluorescence images of cells were taken on a confocal fluorescence microscope (Nikon C2).

Preparation of PAA-Co_9Se_8 nanoplates

Co_9Se_8 nanoplates were synthesized with a modified Xie's method. Briefly, SeO_2 (0.0617 g) were added into the solution of benzyl alcohol (20 mL) containing $Co(Ac)_2 \cdot 4H_2O$ (0.1773 g). After being strongly stirred at room temperature for 1 h, the mixed solution was transferred into a 25 mL Teflon-lined autoclave and heated in a sealed autoclave at 180 ^oC for 15 h. The resulting black precipitate was centrifuged and washed with ethanol and water

several times. Then, the mixture of Co_9Se_8 and PAA was sonicated and stirred in water to obtain water-soluble PAA modified Co_9Se_8 nanoplates.

Cell culture and cytotoxicity assay

HepG2 cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) at 37 ^oC in a humidified atmosphere with 5% CO_2. Cell viability was measured with CCK-8 according to the manufacturer's protocol. In a typical experiment, HepG2 cells were seeded in 96-well plates and then incubated with 100 μL of varying concentrations of PAA-Co_9Se_8 nanoplates for 24 h at 37 ^oC in a humidified 5% CO_2 atmosphere. Cell viabilities were determined by CCK-8 according to the manufacturer's protocol.

Photothermal effect measurement

To study the photothermal effect induced by the NIR laser irradiation, 1 mL aqueous solutions containing different concentrations of PAA-Co_9Se_8 nanoplates were irradiated by a NIR laser (808 nm, 1 W/cm^2) for 10 min. The temperatures of the solutions were monitored by a digital thermometer with a thermocouple probe submerged in the solution in a 1 cm square cuvette.

Photoacoustic imaging

HepG2 cells (2×10^5) were incubated with different concentrations of PAA-Co_9Se_8 nanoplates at 37 ^oC for 12 h. Then, the cells were washed three times with PBS and harvested by trypsinization. After the cells were transferred into a 0.2 mL microtube and centrifuged, the photoacoustic images of cell pellets were obtained at 808 nm using a PA system (Endra Nexus 128, Ann Arbor, MI).

MR imaging

PAA-Co_9Se_8 nanoplates dispersions with various concentrations were prepared for the MRI phantom study using a Bruker 9.4T small animal MRI scanner (94/20 USR, Bruker, Germany). T_2-Weighted MR images were acquired using a fast spin

echo sequence with the following parameters: TR/TE = 1000/8.5 ms, 256 × 256 matrices, repetition time 1.

***In vivo* PA imaging and MRI**

BALB/c nude mice (weight ~20 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. Tumor-bearing mice were prepared by subcutaneously injecting a suspension of 2×10^6 HepG 2 cells in PBS (100 μL) into the back of the hind leg.

In vivo PA imaging was performed with an Endra Life Sciences Nexus128 instrument, which producted a 3D photoacoustic images by a hemispherical ultrasonic detector with 128 identical ultrasonic transducers spirally installed on the surface. PA images were scanned before and after the intratumor injection of 100 μL nanoplates dispersion. Images of two scan were compared to display the contrast function of the nanosheets. For *in vivo* MRI, all mice were anesthetized with 1-2% isoflurane mixed with pure oxygen via a nose cone and were placed in a stretched prone. Axial and coronal two-dimensional (2D) fast spin echo sequence images were first acquired to ensure the imaging position of the implanted tumor. The following parameters were adopted for T_2-weighted multislice spinecho images: TR/TE = 1000/8.5 ms, matrix = 256 × 256, 9 contiguous slices.

DOX loading and release experiments

To load DOX onto PAA-Co_9Se_8 nanoplates, different concentrations of DOX were incubated with PAA-Co_9Se_8 nanoplates (100 μg/mL) at pH 8.0 for 12 h. The PAA-Co_9Se_8-DOX was collected by centrifugation and washed three times with water. The loading capacity was defined as the percentage of the actual mass of doxorubicin loaded on the PAA-Co_9Se_8 nanoplates relative to the mass of the PAA-Co_9Se_8 nanoplates. The equation is provided followed. The resulting PAA-Co_9Se_8-DOX was re-suspended in PBS (pH 7.4) and stored at 4 °C.

$$\text{Loading capacity (\%)} = \frac{W_{\text{initial DOX}} - W_{\text{DOX in supernatant}}}{W_{\text{nanomaterials}}} \times 100\%$$

To study the DOX release kinetics, the PAA-Co_9Se_8-DOX complex was dispersed in PBS at pH 7.0 and 5.0, and then the supernatants were collected by centrifugation after different periods of incubation time. The release amount of DOX was determined by the absorbance spectrometer.

Photothermal therapy

For *in vitro* cancer therapy, propidium iodide staining was first carried out. HepG2 cells were incubated with 30 μg/mL of PAA-Co_9Se_8 nanoplates for 4h. Cells were then irradiated by a NIR laser. Subsequently, the cells were stained with propidium iodide and imaged by confocal laser scanning microscopy. To quantitatively analyze the photothermal cytotoxicity, HepG2 cells were incubated in 96-well cell culture plates for 24 h and then different concentrations of PAA-Co_9Se_8 nanoplates were added. After incubation for 4 h, a NIR laser (808 nm, 1 W/cm^2) was used to irradiate cells for 5 min. Cell viability was determined by CCK-8 according to the manufacturer's protocol.

For *in vivo* tumor therapy, about 100 μL of PAA-Co_9Se_8-DOX was intratumorally injected into tumors, and exposed to 808 nm at 0.75 W/cm^2 for 10 min. In parallel studies, groups with PBS, free DOX, or PAA-Co_9Se_8-DOX injection were set. The tumor sizes were measured by caliper after treatment every two days. Thermal Imaging was recorded by a FLIR Ax5 infrared camera.

Histology and immunohistochemistry

Three weeks after various treatments, animals with tumors were sacrificed and collected for analysis. The tissue sections were stained with H&E following the standard protocol.

Supporting figures

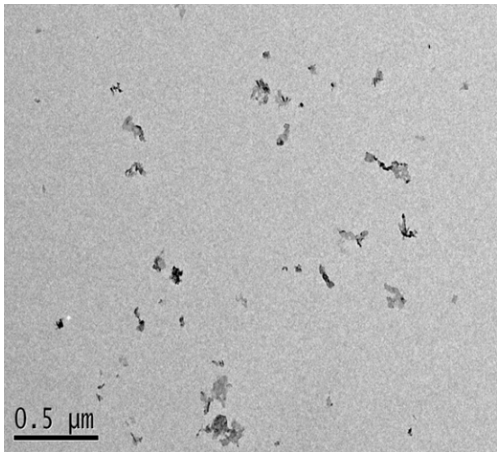

Figure S1. TEM image of the synthetic PAA-Co_9Se_8 nanoplates.

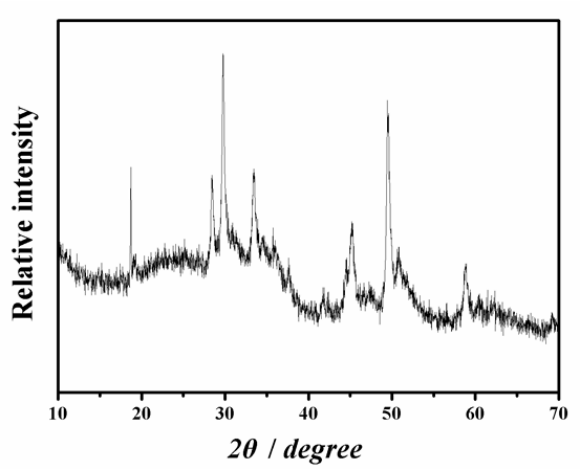

Figure S2. XRD pattern of as-synthesized PAA-Co_9Se_8 nanoplates.

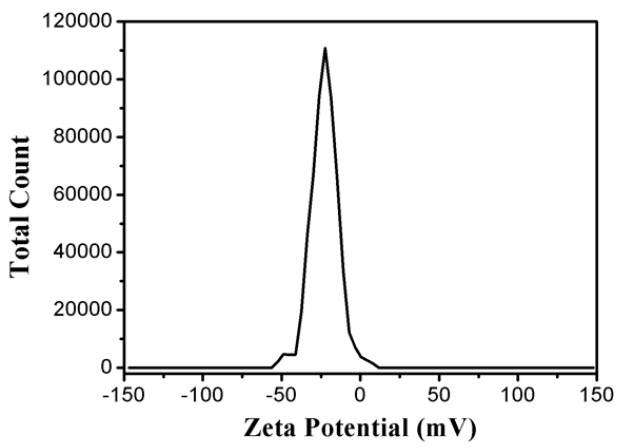

Figure S3. Zeta potential measurement of PAA-Co_9Se_8 nanoplates in water.

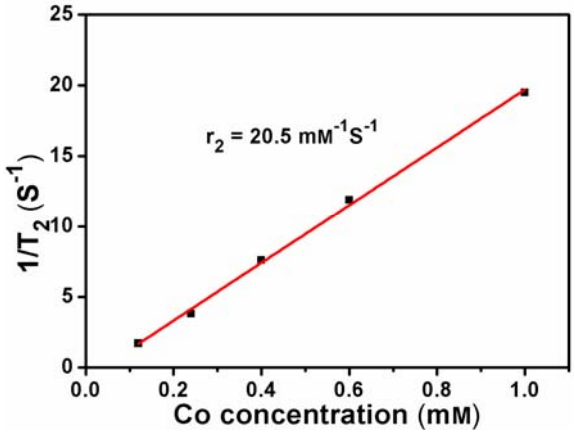

Figure S4. The analysis curve of the water proton trasverse relaxation rate ($1/T_2$) vs Co concentrations.

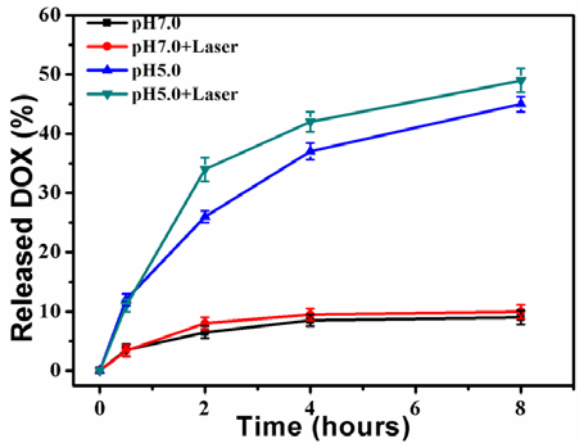

Figure S5. DOX release profile of PAA-Co_9Se_8-DOX at different pH values with and without laser irradiation.

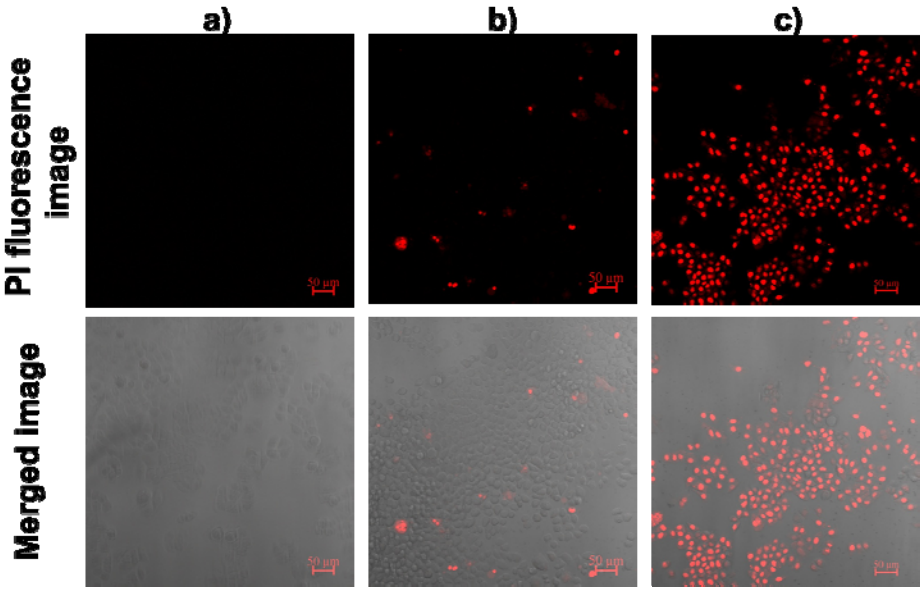

Figure S6. CLSM images of differently treated HepG2 cells stained with PI: a) 5 min of laser irradiation only; (b) PAA-Co_9Se_8 nanoplates and 2 min of laser irradiation; and (c) PAA-Co_9Se_8 nanoplates and 5 min of laser irradiation. Top panels are PI fluorescence corresponding to dead cells, and bottom panels are the overlay of PI fluorescence and the bright field image. Scale bars are 50 μm.

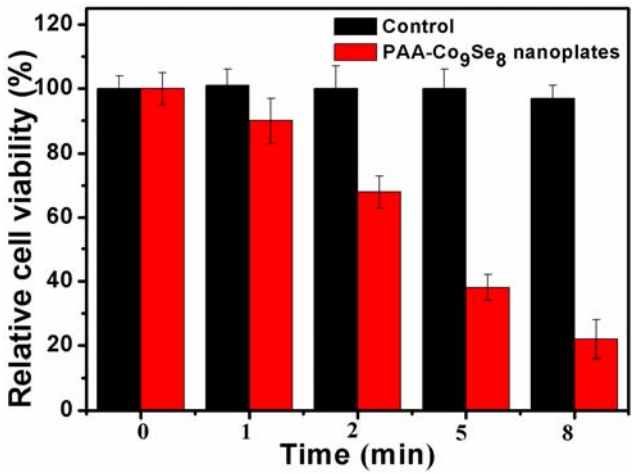

Figure S7. Cell viability of HepG2 cells exposed to different time of laser irradiation with PAA-Co_9Se_8 nanoplates.

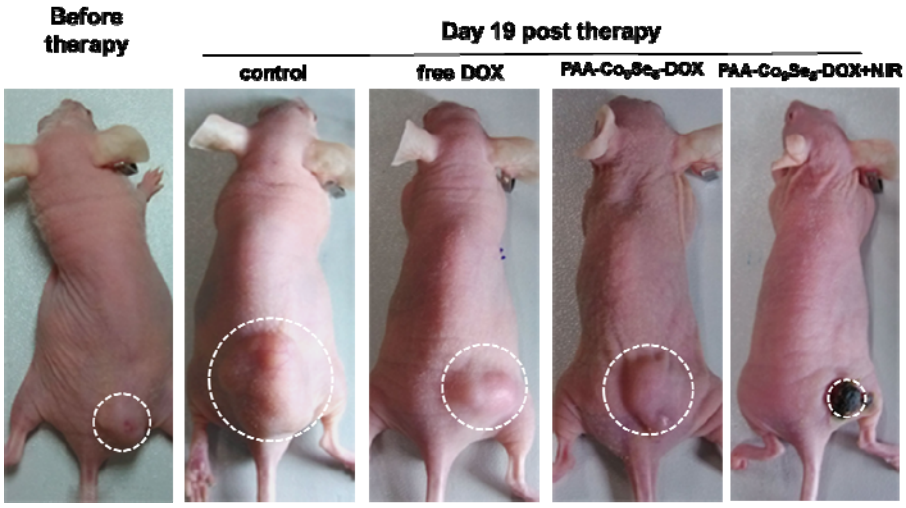

Figure S8. Photographs of the test mice before and after therapy.

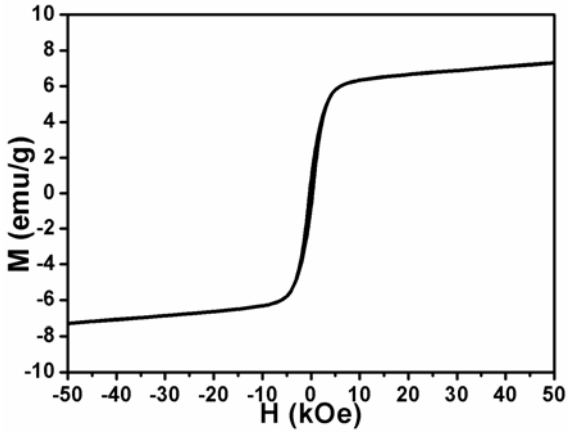

Figure S9. The room-temperature magnetization curve of PAA-Co_9Se_8 nanoplates.