Supporting Information

Interlayer Expansion of 2D MoS_2 Nanosheets for Highly Improved Photothermal Therapy of Tumor *in vitro* and *in vivo*

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1. Characterizations and methods

1.1 Materials

Ammonium thiomolybdate and ammonium fluoride were purchased from Aladdin Industrial Corporation (Shanghai, China). N, N-dimethylformamide (DMF), hydrochloric acid (HCl), and dimethyl sulphoxide (DMSO) were brought from Beijing Chemical Works (Beijing, China). Calcein-AM, Propidium Iodide (PI) were obtained from the sigma. MTT was obtained from beyotime. All reagents used in this work were analytical reagents (A.R.) and used as received without any further purification.

1.2. Synthesis of MoS_2 and E-MoS_2 nanosheets

The layer-expanded MoS_2 nanosheets were prepared by a solvothermal decomposition of ammonium thiomolybdate in N, N-dimethylformamide (DMF) with the presence of ammonium ion. Typically, the solution produced by completely dissolving the appropriate amount of ammonium thiomolybdate and ammonium fluoride in DMF was transferred to the Teflon reactor for solvothermal treatment at 180 °C for 24 h. The layer-expanded MoS_2 nanosheets were obtained after thoroughly washing with water and alcohol for three times followed by vacuum drying at 60 °C for 12 h. For comparison, the MoS_2 nanosheets with normal interlayer distance were prepared by a similar method without adding ammonium ions.

1.3. Characterization

TEM and SEM images were taken on JEOL-2100 transmission electron microscopy and Hitachi-4800F scanning electron microscope, respectively. UV-Vis-NIR spectra were recorded with JASCO V570 spectrophotometer. The element distribution were analyzed via energy dispersive X-ray spectroscopy (EDX).

1.4. Photothermal experiment *in vitro*

Photothermal experiment were carried out to examine the photothermal conversion effect of E-MoS_2. E-MoS_2 and MoS_2 were dispersed in the aqueous solution with the concentration of 100 μg mL^{-1}, respectively. Then the heating curves of the MoS_2 and E-MoS_2 irradiated by the NIR laser at the power of 1.0 W cm^{-2} for 5 mins were obtained with an IR thermal camera.

$$\eta = (h*S*\Delta T_{max} - Q_{diss})/[I*(1-10^{-A_{808}})] \quad (1)$$

where η is the PCE value; ΔT_{max} is indicative of the difference value of the steady-state maximum temperature and the ambient temperature; Q_{diss} refers to the thermal loss caused by the absorbance of the container measured by pure water; I is the laser power; A is the absorbance at 808 nm; h is the heat transfer coefficient; S is the heat surface area of the container.

1.5. Cell experiment *in vitro*

Human liver carcinoma cells HepG2 cell line were seeded in DMEM medium containing 10% FBS. Then MTT assay were conducted to estimate the effect of E-MoS_2 on the cell viability with or without NIR irradiation. 8×10^3 HepG2 were seeded in 96-well plate for overnight at 37 °C. Subsequently, adding 100 μL E-MoS_2 with different concentrations were co-incubated with cells for another 24 h. Then, the cells were washed with PBS for twice to remove the free E-MoS_2. For the NIR group, each well were irradiated by NIR laser at 1 W cm^{-2} for 5 min, while other well were treated without NIR irradiation. Finally, 20 μL MTT solution (3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide at 0.5 mg mL^{-1} were dropped into the 96-well plate for MTT assay. In order to observe the cell viability, live/dead cell staining were performed. After 2 $\times 10^5$ HepG2 seeded on the confocal dish for overnight were incubated with 100 μg mL^{-1} E-MoS_2 for 24 h, the medium were removed and washed the free nanomaterials with PBS. Then mixture of calcein-AM and PI were stained according to operating instructions. Cells were observed using the fluorescence confocal microscope.

1.6. Hemolysis test

Hemolysis test was employed to evaluate the toxicity effect of E-MoS_2. Red blood cells (RBCs) were obtained from the rabbit's heart and then 2% RBCs dispersed in PBS solution were prepared. E-MoS_2 with different concentrations from 20 to 400 μg mL^{-1} were mixed with the equal-volume 2% RBCs suspension. Deionized water and PBS solution were set as the positive and negative control groups, respectively. The samples were incubated for 3 h at room temperature. Then the UV-Vis absorbance of the supernatant were measured at 570 nm after the samples were centrifuged. The equation of the hemolysis rate was calculated as follows:

Hemolysis rate (%) = $(A_{sample} - A_{negative})/(A_{positive} - A_{negative})\times 100\%$ (1)

where A_{sample}, $A_{negative}$ and $A_{positive}$ represent the absorbance value of samples, positive control and negative control, respectively.

1.7. Animal experiments *in vivo*

ICR mice were obtained from Vital River Laboratory Animal Technology Co. Ltd. All animals were treated complying with the ethical standards of the practice. The ICR mice were injected in the left axillary region subcutaneously with 1×10^7 H22 cells. Tumor volume was measured with calipers. When the tumor size reached about 100 cm^3, the tumor-bearing mice were divided into 4 groups named as E-MoS_2 plus NIR, MoS_2+NIR, Control+NIR and Control group, respectively. Then E-MoS_2 and MoS_2 in PBS solution were treated at 40 mg kg^{-1} body weight via intravenous

injection and the control groups were injected with equal-volume PBS solution. After 6 h, the tumor of the mice from the NIR groups were irradiated by NIR laser at 1.0 W cm^{-2} for 5 min. During the irradiation period, an IR thermal camera was used to monitor the temperature change of the tumor regions. Then, body weight and tumor size were recorded during the experiment period. The tumor sizes were calculated as follows.

$V=ab^2/2$ (2)

Where V is the volume of the tumor. a and b are the tumor length and tumor width, respectively.

After 11 d, the mice were killed. Then the main organs including liver, spleen, lung, kidney were collected and fixed in 10% neutra formaldehyde solution. Histological sections were performed according to the standard techniques of embedding in paraffin, sectioning, and staining with hematoxylin and eosin (HE). The slides were observed with optical microscope.

1.8. Statistics

Data were presented as mean ± standard deviation (S.D). One-way analysis of variance (ANOVA) were performed to analyze the data of multigroup comparisons by test using SPSS 14.0 (SPSS Inc., Chicago, IL). The statistical significance was set at a probability of $p < 0.05$.

2. Figures and Tables

Table S1 The photothermal conversion efficiency of other 2D nanomaterials

2D Nanomaterials	PCE value	References
Ti_3C_2 nanosheets	30.60%	*Nano letter*, 2017, 17, 384-391
Bi_2Se_3 nanosheets	34.60%	*Small*, 2016,12,30,4136-4145
WS_2 nanosheets	32.83%	*Nanoscale*, 2014, 6, 10394-10403

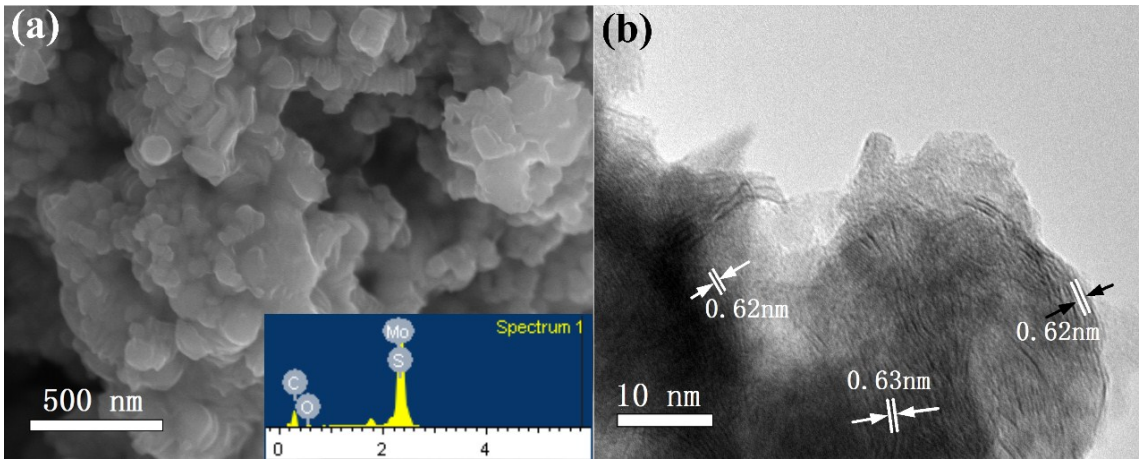

Fig. S1 (a) SEM and (b) HRTEM images of the normal MoS_2 nanosheets. The inset in (a) is the corresponding EDX. No obvious N element was detected in the EDX pattern. An average layer distance of ~0.62 nm could be obviously observed from HRTEM image, agreeing well with the XRD result.

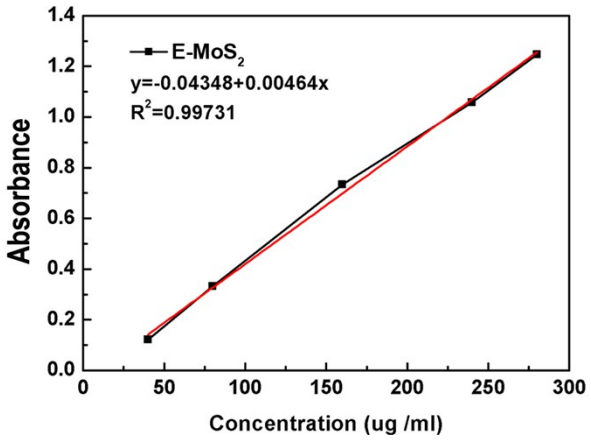

Fig. S2 The absorption intensity of E-MoS_2 with different concentrations at 808 nm.

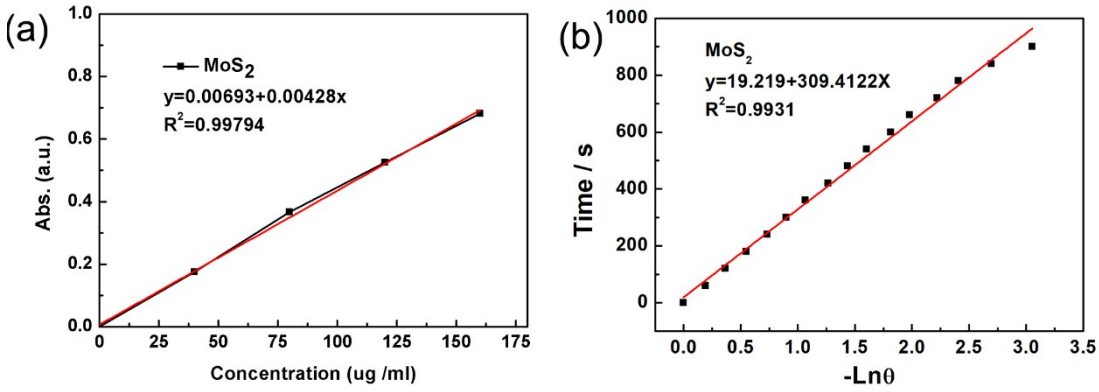

Fig. S3 (a) The absorption intensity of MoS_2 with different concentrations at 808 nm. (b) Linear time data versus -lnθ based on the cooling curve

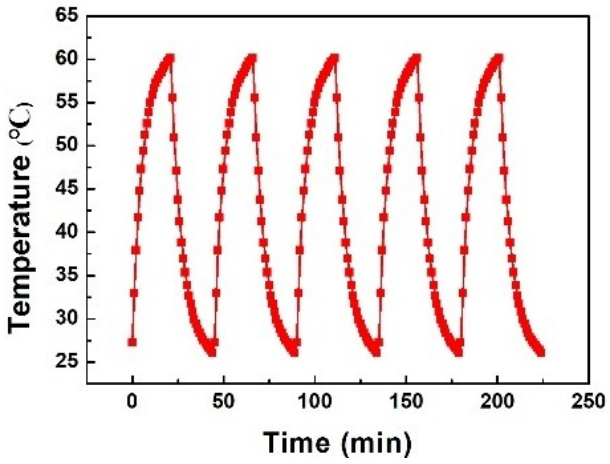

Fig. S4 The photothermal stability of the E-MoS_2 nanosheets irradiated with NIR light.

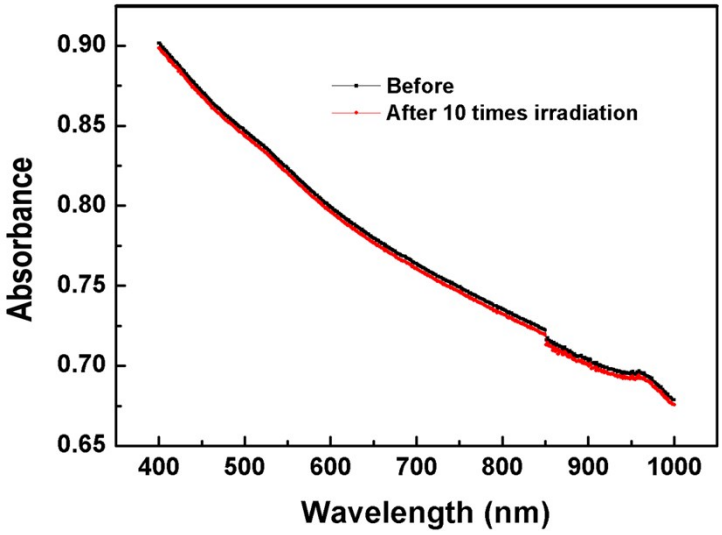

Fig. S5 UV-Vis-NIR spectra of the E-MoS_2 nanosheets before and after 10 times irradiation with NIR.

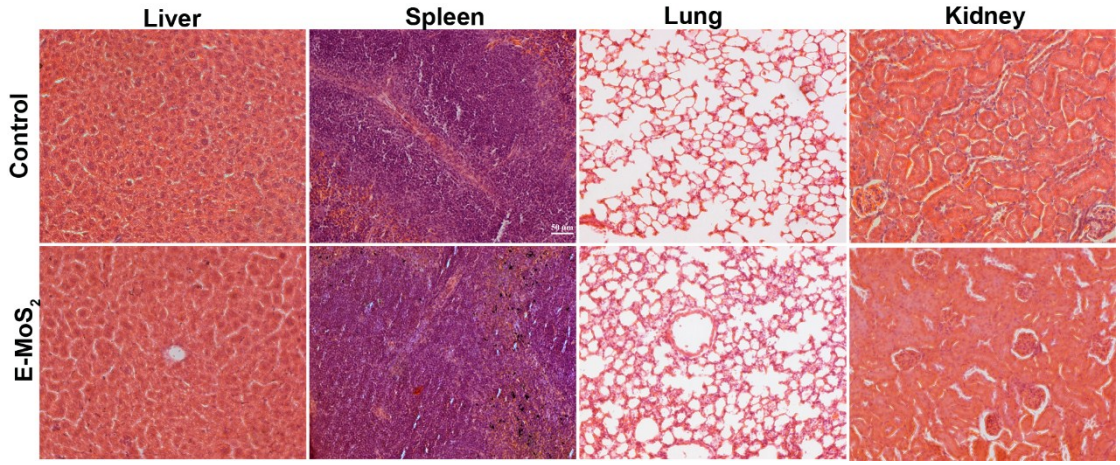

Fig. S6 Histology examination of the main organs from the mice treated with E-MoS_2 nanosheets.