Supporting Information

Fabrication of PEGylated Bi_2S_3 Nanosheets as multifunctional platform for multimodal diagnosis and combination therapy for cancer

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Experimental

Materials: Polyvinylpyrrolidone (PVP， Mw: 10000) and ethanol (C_2H_5OH, AR) were

purchased from Sinopharm Chemical Reagent Co. Ltd. Triphenyl bismuth ($(C_6H_5)_3Bi$, ≥95%) and benzyl disulfide ($C_{14}H_{14}S_2$, ≥95%) were supplied by Aladdin Co. Ltd. Alpha lipoic acid (LA, 98%) was provided by Energy Chemical Co. Ltd. Methoxypolyethylene glycols-2000 (mPEG-2000) was purchased from Tokyo Chemistry Industry. Dicyclohexylcarbodiimide (DCC, 99%) and 4-dimethylaminopyridine (DMAP, 99%) were supplied by Aladdin Co. Ltd. The distilled water used in the experiment was obtained from twice distillation process.

Preparation of Bi_2S_3 nanosheets: The bismuth sulphide nanosheets were synthesized by one pot of hydro-thermal method. Briefly, $(C_6H_5)_3Bi$ (0.132 g) and $C_{14}H_{14}S_2$ (0.0739 g) were completely dissolved into 8 ml ethanol. PVP was dispersed in another 7 ml ethanol. The above two solutions were completely dissolved by ultrasound. After that, the two solutions were mixed, shaking to make it dispersed evenly. The mixture was then transferred to a 25 ml teflon reactor, and maintain at 190℃, for 15 hours. After reaction, the obtained products was cooled down to room temperature naturally. The resulting black solution was then precipitated by centrifugation in 9000 rpm for 10 min. The black precipitate was washed three times with ethanol and water respectively, and the target product was obtained by vacuum drying.

Synthesis of Bi_2S_3-mPEG: The mPEG-SH was used a simple esterification reaction with mPEG and LA, and then ring opening reaction with sodium borohydride as reducing agent was carried on. Herein, mPEG-SH was used to functionalize Bi_2S_3. 10 mg as-prepared Bi_2S_3 was added into 2 mM PEG-SH aqueous solution under magnetic stirring in N_2 atmosphere, maintaining 6 hours, and then the precipitate was collected by centrifugation. Excess moisture was removed from the final product by vacuum drying.

Characterization

The morphologies and structures of the samples were examined by a JEOL JSM-7800F field emission scanning electron microscope (FE-SEM) and a JEOL JEM-2100 plus transmission electron microscope (TEM). Elemental analysis was measured by EDS (energy dispersive X-ray spectroscopy) equipped in SEM. The phase of the samples were detected using a Rigaku Ultima IV X-ray diffractometer (XRD, Cu-Kα radiation λ=0.15418 nm). The infrared absorption spectrum, UV-NIR absorption curve and temperature rise record were Thermo Fisher iS50, UH4150, Fotric 226 respectively. X-ray photoelectron spectroscopy (XPS) data were accumulated on a PHI Versa Probe III (ULVAC-PHI INC) X-Ray photoelectron spectrometer with a monochromatized Al K standard X-ray source and the binding energies were calibrated by referencing the C1s to 283.8 eV. Inverted Fluorescence Microscopy (Olympus-CKX53) and Molecular Devices (SpectraMax-M3) was used in cytotoxicity detection.

DOX loading and release

DOX was loaded into Bi_2S_3-PEG nanosheets by zwitterion electrostatic attraction. In brief, Bi_2S_3-PEG nanosheets (10 mg) were firstly completely dispersed into phosphate buffer saline (PBS, pH =7.4, 25 ml. The PBS solution was compound of disodium hydrogen phosphate and monometallic sodium orthophosphate in a certain proportion using double distilled water.) under ultrasonication, then DOX (4 mg) was entirely scattered to 15 ml PBS, and subsequently the two solutions were mixed together under gently stirring at room temperature for 24 h in dark. The obtained product (Bi_2S_3-PEG@DOX) was centrifuged (9000 rpm, 10 min) and then washed with DI water for several times.

To calculate the loading content of DOX, We used the concentration-absorption corresponding

standard UV curve of DOX to calculate the drug loading rate by measuring the absorption intensity of DOX before and after loading. After centrifugation, the supernatant was collected to determine the amount of loading DOX according to its Ultraviolet absorption spectrum (wavelength range: 300 nm ~ 600 nm).

To study the DOX release pattern, Bi_2S_3-PEG@DOX (10 mg) were dispersed in PBS (pH=7.4, 6.5, 5.5 10 ml), and then stirred at room temperature in dark for 2~3 h. After centrifugation, the supernatant was collected to measure UV absorption (wavelength range: 300 nm ~ 600 nm) to determine the relationship between drug release and pH.

Photothermal effect and PA imaging in vitro

In order to explore the photothermal conversion performance of Bi_2S_3 nanosheets, 1 ml solution (150 μg/ml) was used in a quartz cuvette, irradiated by a near infrared laser at 808 nm (2 W/cm^2) for 3 min, and the heating process was recorded with an infrared thermal imager every 20 seconds. To compare the heating rate of different concentration solutions, six different concentration solutions, 0 μg/ml, 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 80 μg/ml and 100 μg/ml, were prepared. The same wavelength and power were applied to the laser for 5 minutes to observe and record the temperature rise. The investigation of photothermal stability was carried out in a solution with a concentration of 100 μg/ml. The irradiation was suspended after continuous exposure in 2 W/cm^2 laser for 150 seconds. After the solution system cooled to room temperature, the laser irradiation was turned on again and the above process was repeated. The process of heating and cooling was repeated for 5 times, meanwhile, the photothermal stability of the system was recorded. For photoacoustic imaging, we prepared eight different concentrations (0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml) of bismuth

sulfide nanosheets aqueous solutions as mimics to be tested under the excitation wavelength of 680 nm ~ 900 nm.

Cell cytotoxicity and photothermal toxicity in vitro

In the cytotoxicity test, we used HeLa cell as a cancer cell model. In 24-well plates, 8.0×10^4 HeLa cells were added into each hole and cultured in the medium for 24 hours. The specific treatment methods were as follows: selected 2 lines of 6 of holes to add sterilized water and cell culture fluid as control, the remaining 4 lines were added cell culture fluid, 80 μg/ml Bi_2S_3-PEG, 80 μg/ml DOX@Bi_2S_3-PEG, 80 μg/ml DOX@Bi_2S_3-PEG respectively, then continued to train for 20 hours. Selected two lines of cell culture fluid, 80 μg/ml DOX@Bi_2S_3-PEG and then irradiated with 808 nm laser for 10 minutes. After that all cells were stained with Calcein-AM solution and PI solution. The cells were incubate in incubator for 37℃ for a period of time, and the survival rate was observed by fluorescence microscope. In order to explore the toxicity of different concentrations of Bi_2S_3 nanosheets solution, a solution with a concentration gradient (0, 6 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml) was designed to detect the toxicity of Bi_2S_3 to normal (3T3 cell) and tumor cells (Hela cell).

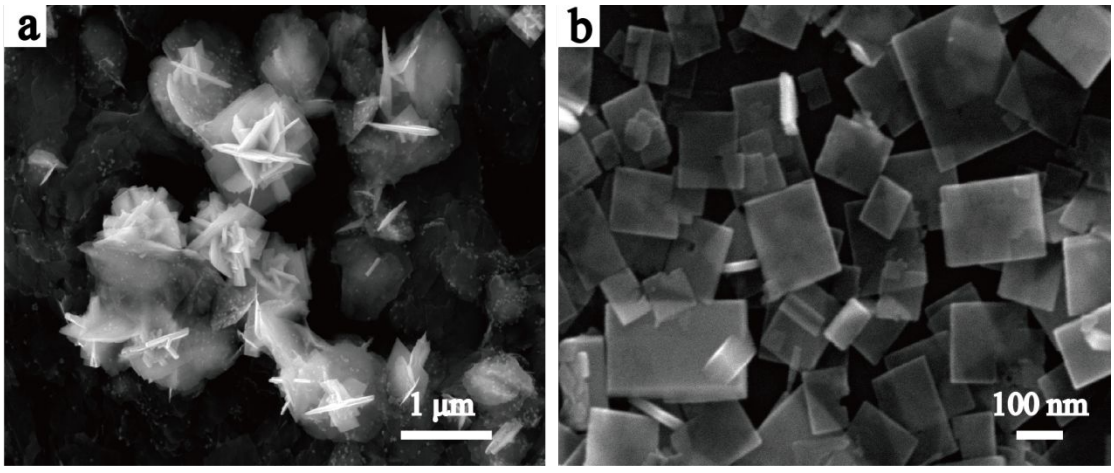

Figure S1 (a) Bi_2S_3 nano-flowers and (b) Bi_2S_3 nanosheets obtained by controlling different

hydrothermal conditions.

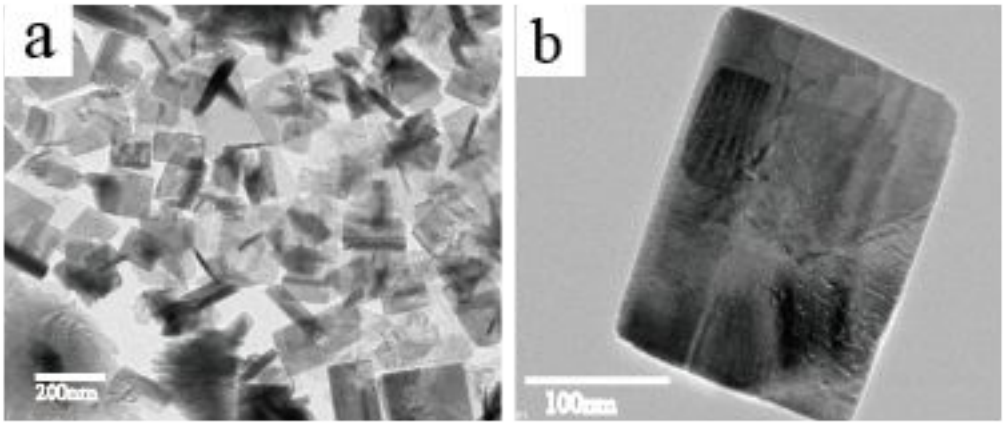

Figure S2 TEM characterization of overall (a) and local (b) graph for Bi_2S_3 nanosheets.

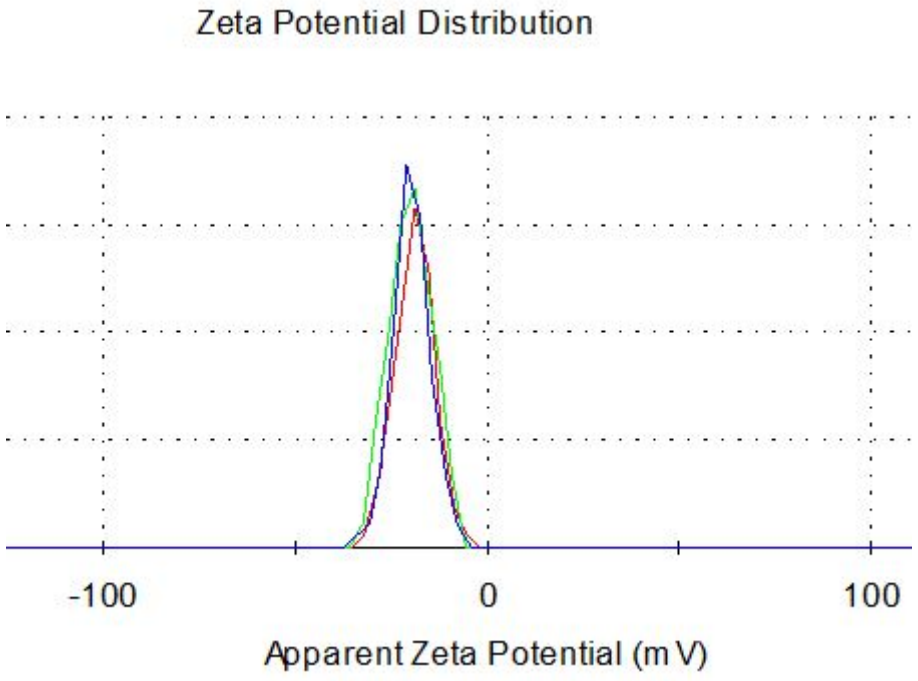

Figure S3 Test results of Zeta potentiometer of Bi_2S_3 nanosheets.

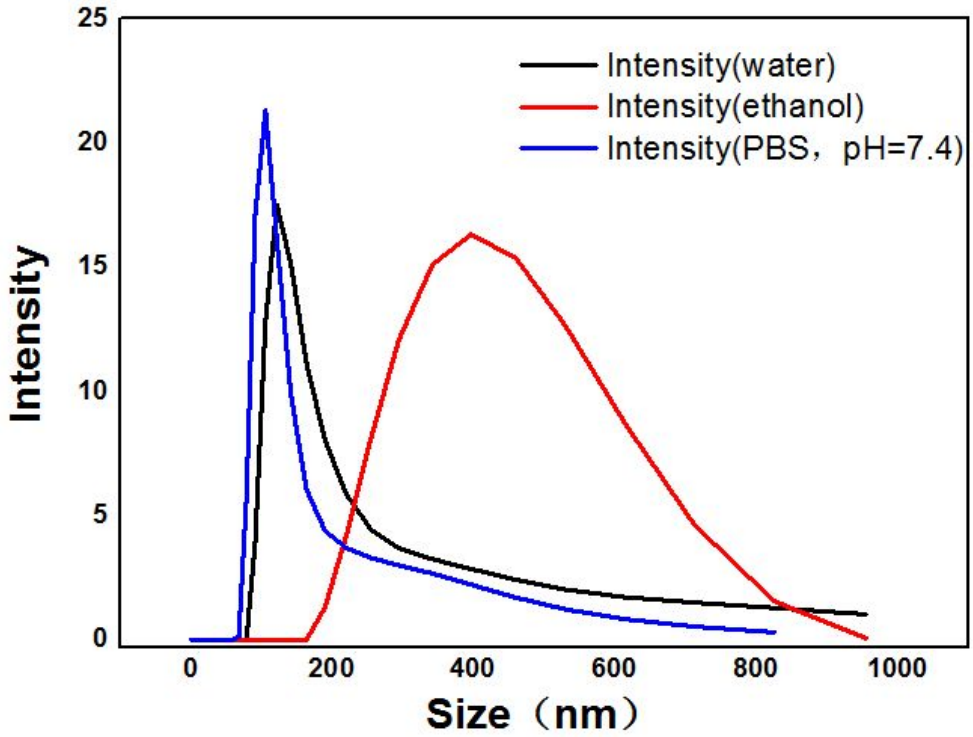

Figure S4 Dispersion of Bi_2S_3-PEG in different solvents.