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Semimetal Nanomaterials of Antimony as Highly Efficient Agent for Photoacoustic Imaging and Photothermal Therapy

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Abstract

In this study we report semimetal nanomaterials of antimony (Sb) as highly efficient agent for photoacoustic imaging (PAI) and photothermal therapy (PTT). The Sb nanorod bundles have been synthesized through a facile route by mixing 1-octadecane (ODE) and oleyl amine (OAm) as the solvent. The aqueous dispersion of PEGylated Sb NPs, due to its broad and strong photoabsorption ranging from ultraviolet (UV) to near-infrared (NIR) wavelengths, is applicable as a photothermal agent driven by 808 nm laser with photothermal conversion efficiency up to 41%, noticeably higher than most of the PTT agents reported before. Our *in vitro* experiments also showed that cancer cell ablation effect of PEGylated Sb NPs was dependent on laser power. By intratumoral administration of PEGylated Sb NPs, 100% tumor ablation can be realized by using NIR laser irradiation with a lower power of 1 W/cm² for 5 min (or 0.5 W/cm² for 10 min) and no obvious toxic side effect is identified after photothermal treatment. Moreover, intense PA signal was also observed after intratumoral injection of PEGylated Sb NPs and NIR laser irradiation due to their strong NIR photoabsorption, suggesting PEGylated Sb NPs as a potential NIR PA agent. Based on the findings of this work, further development of using other semimetal nanocrystals as highly efficient NIR agents can be achieved for *vivo* tumor imaging and PTT.

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Keywords

Semimetal; antimony; nanorod bundles; photothermal therapy (PTT); photoacoustic imaging (PAI)

1. Introduction

There is an increasing interest in using photothermal therapy (PTT) induced by near-infrared (NIR) laser as a highly effective alternative to conventional cancer treatment approaches. [1–38] To improve the therapeutic effects of PTT, it is important to develop biocompatible and effective photothermal agents with strong absorbance in the NIR wavelength region and high photothermal conversion efficiency to efficiently turn the absorbed NIR optical energy into heat. Currently, at least four types of photothermal agents, such as organic compounds of indocyanine green (ICG) dye [1] and polyaniline nanoparticles (NPs); [2] carbon-based nanomaterials of carbon nanotubes (CNTs) [3–5] and graphene; [6–11] metal nanostructures of Ge NPs, [12] Pd-based nanosheets, [13] and Au NPs; [14–30, 39–44] and copper chalcogenide semiconductors of CuS [31–35] and Cu_{2-x}Se [36] nanoparticles (NPs), have been extensively developed. Recently, tungsten oxide nanocrystal of $\text{W}_{18}\text{O}_{49}$ [37] and chemically exfoliated MoS_2 [38] were also reported to exert photothermal effect under 980 nm and 808 nm laser irradiation, respectively. However, most of the mentioned photothermal agents exhibit relatively low photothermal conversion efficiency (< 30%), thus the development of new photothermal agents with higher photothermal conversion efficiency is still necessary to further improve the therapeutic efficacy of PTT.

Photothermal conversion mechanism is very important for developing new types of photothermal agents. It is generally regarded that the heat release mechanism of crystalline NPs is related to the mobile carriers (electrons or holes) inside the NPs, which can be strongly driven by the laser electric field, then the energy obtained by carriers converts into heat. [45] In the area of plasmon resonance resulting from a considerable number of electrons' collective motion, heat generation will become especially strong in metal NPs. [45] As for semiconducting NPs of copper chalcogenides with much less carriers, strong heat generation can also be realized by self-doping the NPs, resulting in vacancy-doped semiconductor quantum dots with appreciable free carrier concentrations, [46, 47] whose photothermal conversion efficiencies are comparable to or even higher than those of metal nanostructures. [36] These intriguing features prompted us to develop a new class of materials, semimetal materials, which have fewer charge carriers than metals but more than semiconductors, potentiating the semimetal NPs as photothermal agents if they have strong NIR photoabsorption. It is well-known that antimony (Sb) is a typical group VA semimetal with an energy overlap between the valence and conduction bands. [48] Its special band structure could lead to the remarkable electronic properties. Recently, Sb has shown great technological applications in lithium ion batteries, [49] direct formic acid fuel cells, [50, 51] pH sensors, [52] and thermoelectric devices. [53] However, no study on the photothermal properties of Sb NPs and their application as photothermal agent was reported.

In this work, we successfully synthesized Sb NPs through a facile organic route by using oleyl amine (OAm) as the stabilizing and reducing agent. The aqueous dispersion of

PEGylated Sb NPs, due to its broad and strong photoabsorption ranging from ultraviolet (UV) to near-infrared (NIR) wavelengths, is applicable as a photothermal agent driven by 808 nm laser with photothermal conversion efficiency up to 41%, noticeably higher than most of the PTT agents reported before [36]. We also investigated the use of PEGylated Sb NPs for the *in vitro* and *in vivo* photothermal cancer treatment. We found that low laser power density and short irradiation time could effectively kill tumor cells incubated with Sb NPs with no noticeable side effects after treatment. Moreover, we also studied the photoacoustic (PA) properties of Sb NPs due to their strong NIR absorption, and intense PA signal was observed after intratumoral administration of Sb NPs and NIR laser irradiation. Our findings will possibly results in extensive explorations of Sb NPs and other semimetal NPs in the future for photoacoustic imaging and photothermal therapy.

2. Experimental Section

Chemicals

Oleyl amine, 1-octadecane and antimony (III) chloride (99.8%) were purchased from Fisher Scientific. 1,2-Distearoyl-sn-Glycero-3- Phosphoethanolamine conjugated Polyethylene Glycol (DSPE-PEG5000) was purchased from Nanocs Incorporation.

Synthesis of OAm-Coated Sb Nanoparticles

In a typical experiment, antimony(III) chloride (SbCl_3 , 0.5 mmol) was first added into the solution containing oleyl amine (5 mL) and octadecane (20 mL) under vigorous stirring. The mixture was heated to 140°C , then kept at the temperature for 40 min under Ar protection. After the reaction, hexane and acetone were added, and the resulting solid products were retrieved by centrifugation.

Surface Modification of OAm-Coated Sb Nanoparticles

The as-prepared Sb NPs (2 mg) were first dispersed in chloroform (1 mL), then DSPE-PEG5000 (10 mg) was added. After the mixture was sonicated for 60min, the resultant solvent was evaporated under a stream of dry nitrogen. Then the dried powder was suspended in water by sonication for 5 min. The suspension was then centrifugated to eliminate unstable aggregates. Finally, the supernatant liquid was collected and washed to remove excess DSPE-PEG5000.

Characterization

TEM (JEOL-JEM 2010) and HRTEM (JEOL JEM-2010F) were used to determine the size, morphology, and microstructure of Sb NPs. XRD measurements were carried out on a BRUKER-AXS X-ray diffractometer using $\text{Cu K}\alpha$ radiation ($\lambda = 0.15418 \text{ nm}$). EDS of the sample was performed on a Oxford INCA X-Act EDS system attached to a FEI Sirion 200 field emission scanning electron microscope. The measurement of UV-vis-NIR absorption spectra were performed on a Perkin Elmer Lambda 950 UV/vis/NIR spectrophotometer using quartz cuvettes with an optical path of 1 cm.

Cell Culture

4T1 murine breast tumor cells were cultured in standard cell media. Cells were first seeded into 96 well plates then incubated with PEGylated Sb NPs with different concentrations for 24 h. The standard MTT assay was used to determine relative cell viabilities. For *in vitro* PTT, 4T1 tumor cells (5000 cells/well, 100 μ L) were incubated with and without PEGylated Sb NPs (100 μ g/mL) for 4 h, then the cells were rinsed with PBS and immersed in 100 μ L of fresh culture medium and were irradiated under an 808 nm laser at various power densities for 10 min. Calceine AM and propidium iodide (PI) were used to stain the cells for 30 min, then they were washed with PBS, and imaged by an Olympus IX81 motorized inverted microscope.

Measurement of Photothermal Performance

For measuring the photothermal conversion performance of the Sb NPs, 1 mL PEGylated Sb NPs aqueous solution with different concentrations (0~1 mg/mL) were introduced in a quartz cuvette and irradiated under an 808 nm laser (LASERGLOW Technologies) at 2 W/cm² for 300 s, respectively. A FLIR thermal camera was used to record real-time thermal imaging of samples which could be quantified by FLIR Examiner software.

Animal Experiments

All animal operations were performed in accord with institutional regulations of animal use and care. A suspension of 2×10^6 4T1 cancer cells in 60 μ L phosphate buffered saline (PBS) were subcutaneously administered into the flank of each nude mice. When their tumor size reached about 60 mm³, the 4T1 tumor mice were treated.

Photothermal Therapy

4T1 tumor mice were intratumorally administered with 20 μ L PEGylated Sb NPs solution with concentration of 2 mg/mL. For the laser treatment groups, the tumors of mice were irradiated under the 808 nm laser at 1 W/cm² for 5 min (or 0.5 W/cm² for 10 min). The tumor sizes were measured every other day and determined by the equation: $V = L \times W^2/2$, where V is tumor volume, L is tumor length and W is tumor width. Relative tumor volumes were normalized to their initial sizes before treatment.

IR Thermal Imaging

4T1 tumor mice treated with or without PEGylated Sb NPs were irradiated under the 808 nm laser at 1 W/cm² for 5 min (or 0.5 W/cm² for 10 min), and thermal imaging was recorded by a FLIR thermal camera simultaneously.

In Vivo Photoacoustic Imaging

4T1 tumors bearing nude mice were intratumorally administered with 20 μ L PEGylated Sb NPs solution with concentration of 2 mg/mL, then PAI was carried out by a Vevo 2100 LAZR system (VisualSonics, Inc., New York, NY) equipped with a 40 MHz, 256-element linear array transducer.

Biodistribution of Sb NPs

The tissues of organs (liver, spleen, kidney, heart, lung, stomach, intestine, skin, muscle and bone) collected from mice intravenous injected with PEGylated Sb NPs (20 mg/kg) were weighed then dissolved by *aqua fortis*. Then the as-prepared samples were heated at the temperature of 150°C for 4 hours. After the samples cooled down to room temperature, deionized water was used to dilute each of them to 10 mL, then they were analyzed by ICP-MS (Iris Advantage 1000, Thermo Jarrell Ash) to determine the total amount of Sb.

Blood Analysis and Histological Examination

Five normal mice were sacrificed 60 days after intravenous injection of PEGylated Sb NPs (200 µL of 2 mg/mL, a dose of 20 mg/kg) to collect blood and organ. Five healthy mice with similar age without treatment were also sacrificed as the control group. The measurement of all the blood parameters were performed in Shanghai Research Center for Biomodel Organisms. As for histological examination, all the organs were first fixed in 4% formalin, then they were treated with paraffin embedded sections for H&E staining. A Leica Qwin digital microscope was used for the slices examination.

3. Results and discussion

In this work, Sb nanorod bundles were prepared by treating a solution of 1-octadecane (ODE) and OAm mixture containing antimony(III) chloride (SbCl_3) at 140 °C under Ar atmosphere for 40 min. Here OAm was not only used as the capping ligand, but also the reducing agent to reduce SbCl_3 into Sb NPs. The as-prepared Sb sample is composed of bundle-like nanorods of 200–300 nm long and 50–80 nm thick, and these bundles consist of many individual nanorods of about 10 nm in thickness (Figure 1a, Figure S1). Furthermore, the high resolution transmission electron microscopy (HRTEM) and selected area electron diffraction (SAED) images of Sb NPs (Figure 1a) also indicate clear lattice fringes, and determine the interplanar spacing of 0.32 nm, which is in agreement with the (012) lattice fringes of antimony rhombohedral phase, suggesting that the Sb NPs grow along the [012] direction. Moreover, Figure 1b demonstrates the X-ray diffraction (XRD) pattern of Sb NPs, and all diffraction peaks well indexed to the rhombohedral phase of antimony (JCPDS No. 85–1324) are in good accordance with the HRTEM and SAED images. Energy-dispersive X-ray spectroscopy (EDS) result (Figure 1c) indicates that these individual NPs are made of metallic antimony, and the trace amount of oxygen might be originated from the surface oxidation of the Sb NPs or adsorbed oxygen. The existence of Cu and C in the EDS spectrum may result from the copper transmission electron microscope grid with amorphous carbon coating.

Surface functional chemical groups of the Sb NPs are quite important for their further bio-applications. The OAm-coated hydrophobic Sb NPs were then treated with a solution containing 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)] (DSPE-PEG5000) (MW = 5000 g/mol) that contains a phosphate terminal group, resulting in a noncovalent micellar overcoating on the NPs. As a result, hydrophilic PEGylated Sb NPs can be easily dispersed in water with negligible morphological change (Figure S2). The PEGylated Sb NPs aqueous solution with concentration of 0.25

mg/mL exhibits a grey color (the inset of Figure 2a), and also has good stability (Figure S3, 4). UV-vis-NIR spectroscopy was used to study the optical property of the PEGylated Sb NPs aqueous solution (Figure 2a). It exhibits broad and strong absorption ranging from ultraviolet (UV) to NIR wavelengths without distinct absorption peak. The broad and strong NIR absorption makes PEGylated Sb NPs as highly promising PTT agent for cancer therapy. Thus, their photothermal conversion efficiency was first evaluated, and PEGylated Sb NPs aqueous solutions with concentrations ranging from 0 to 1 mg/mL were irradiated under an 808 nm laser at 2 W/cm² for 300 s, respectively. The results indicated that temperatures of all the PEGylated Sb NPs aqueous solutions increased with the irradiation time, and the solutions with higher concentrations showed faster temperature increase (Figure 2b). After being irradiated for 300 s, the temperature of the PEGylated Sb NPs aqueous solution with concentration of 1 mg/mL was increased by 51 °C (Figure 2b & Figure S5), while pure water showed only 3.5 °C increase. It is reported that tumor cells can be ablated when they are kept at 42 °C for 15–60 min, and the duration time can be greatly shortened to 4–6 min if the temperatures are over 50 °C.[54] Then the measurement of the photothermal conversion efficiency (η) of PEGylated Sb NPs solution was carried out to further evaluate their photothermal conversion ability. The η value was calculated according to the model reported previously,[35, 55] and the η value of PEGylated Sb NPs was determined to be 41%. In contrast, widely used PTT agents of gold nanorods and Cu_{2-x}Se NPs showed much lower η values of 21% and 22%, [36] respectively. The higher η value of PEGylated Sb NPs makes them a promising photothermal coupling agent.

To explore the biomedical applications of PEGylated Sb NPs, we first tested their potential cytotoxicity. 4T1 murine breast tumor cells and hepatic L02 cells were first incubated with PEGylated Sb NPs at different concentrations for 24 h, then their relative viabilities were determined by standard methyl thiazolyl tetrazolium (MTT) assay [56]. We did not observe significant cytotoxicity of PEGylated Sb NPs for both cells at concentrations lower than 200 µg/mL (Figure 3a). When they were incubated with PEGylated Sb NPs solution with a high concentration of 400 µg/mL, both 4T1 tumor cells and L02 cells showed a slight decrease in the viability (~60% compared to the untreated control). Then we used PEGylated Sb NPs for *in vitro* tumor cell destruction under NIR laser irradiation. We first incubated 4T1 tumor cells with PEGylated Sb NPs at the concentration of 100 µg/mL for 4 h, then an 808 nm laser at different laser power densities was used for cell ablation. After laser irradiation, Calcein AM and propidium iodide (PI) were used to stain the tumor cells to differentiate live (green) and dead (red) cells, then they were imaged by a fluorescence microscope. In our experiments, MTT assay was also performed to measure the relative viabilities of tumor cells quantitatively after they were treated under different laser power densities (Figure 3c). It is demonstrated that most of tumor cells were killed after they were incubated with 100 µg/mL of PEGylated Sb NPs and irradiated under the 808 nm laser at 1 W/cm² for 10 min (Figure 3b, c). However, the relative viabilities of tumor cells without PEGylated Sb NPs incubation showed little change even after being exposed under the laser at 2 W/cm² (Figure 3b, c). The PEGylated Sb NPs solution concentration of 100 µg/mL and the laser power density of 1 W/cm² used in our experiments are lower than many other reported photothermal agents of Au nanorods and Au nanocomposites. [26, 57–59]

Due to the effective photothermal destruction of 4T1 tumor cells *in vitro* induced by PEGylated Sb NPs, 4T1 tumor model was used to further study their *in vivo* cancer PTT. Nude mice bearing 4T1 tumors were first intratumorally administered with PEGylated Sb NPs (2 mg/kg), then they were irradiated under the 808 nm laser at 1 W/cm². We used an infrared (IR) thermal camera to record the local temperature variation (Figure 4a). It was found that the surface temperature of the tumor with PEGylated Sb NPs injection and the 808 nm laser irradiation at 1 W/cm² increased rapidly to ~53 °C within 5 min (Figure 4a,b), which was high enough for *in vivo* tumor ablation. In contrast, the surface temperature of tumor without PEGylated Sb NPs injection only increased by 4.1 °C within 5 min (Figure 4a,b). We also found that the surface temperature of the tumor with PEGylated Sb NPs injection and the 808 nm laser at 0.5 W/cm² increased to ~49 °C in 10 min (Figure S6), which was also capable of *in vivo* tumor destruction and indicated their excellent photothermal conversion performance. Moreover, we also studied the photoacoustic properties of PEGylated Sb NPs due to their strong NIR photoabsorption. It was found that when the concentration of PEGylated Sb NPs was increased, the corresponding PA signal intensity would increase linearly (Figure S7). Intense PA signal could also be observed in the tumor region with PEGylated Sb NPs injection at wavelengths of 808, 730 and 680 nm (Figure 4c). The average PA signal intensity of PEGylated Sb NPs was 2.5~3 times stronger than that before nanoparticles injection at all three wavelengths (Figure 4d), which indicates that PEGylated Sb NPs are also suitable contrast agents for PA imaging.

Next, we studied the *in vivo* PTT efficacy of PEGylated Sb NPs caused cancer treatment, and five groups of 4T1 tumor mice with 5–7 mice per group were adopted in the experiments. For the treatment groups (n = 7/group), the tumors with intratumoral injection with PEGylated Sb NPs (2 mg/kg) were irradiated under the 808 nm laser at 1 W/cm² for 5 min or 0.5 W/cm² for 10 min. Mice in other control groups (n = 5/group) included untreated mice, mice with PEGylated Sb NPs injection without 808 nm laser irradiation, and mice with 808 nm laser irradiation (1 W/cm²) without PEGylated Sb NPs injection. We measured the tumor sizes every other day after treatment (Figure 5a). The results indicated that the tumors treated by PEGylated Sb NPs injection and the 808 nm laser irradiation at 1 W/cm² or 0.5 W/cm² could be effectively ablated after treatment, resulting in black scars at their original sites (Figure 5c), and the scars finally disappeared after about 2 weeks (Figure S8). In contrast, mice in control groups demonstrated similar tumor growth speed, which suggests that either laser irradiation at 1 W/cm² or PEGylated Sb NP injection does not influence the tumor growth (Figure 5a). It is worth to note that 4T1 tumor mice with PEGylated Sb NPs injection and 808 nm laser irradiation could survive over 30 days without tumor reoccurrence (Figure 5b). However, mice in the three control groups could only survive for about 16 days after treatment (Figure 5b), further demonstrating the PEGylated Sb NPs' excellent PTT efficacy. After the 808 nm laser irradiation, we collected tumors immediately for hematoxylin and eosin (H&E) staining (Figure 5d). We noticed that cells in the tumors with both PEGylated Sb NPs administration and laser irradiation were damaged significantly, which was different from those in other three control groups. Moreover, neither death nor obvious body weight variation of mice was noticed after PEGylated Sb NPs injection at a dose of 2 mg/kg (Figure S9). Our results demonstrate that PEGylated Sb NPs could act as an effective photothermal agent for *in vivo* photothermal treatment of cancer,

and a low power density of 1 W/cm² and short irradiation time of 5 min (or power density of 0.5 W/cm² and irradiation time of 10 min) could be sufficient for them to kill tumors *in vivo*.

Moreover, in order to quantitatively study the late time point biodistribution and organ accumulation profiles of Sb NPs in mice, tissues collected from mice with intravenous injection of PEGylated Sb NPs (20 mg/kg) were dissolved by *aqua fortis* for inductively coupled plasma-atomic emission spectrometry (ICP-MS) measurement of Sb concentrations. It was found that Sb NPs mostly accumulated in the reticuloendothelial system (RES) organs of liver and spleen 1 day after intravenous injection, then in the lung and bone (Figure S10). A distinct trend of persistent decrease of Sb levels in measured major organs 30 days after intravenous injection was observed from the biodistribution data, possibly due to the clearance of Sb NPs from the mouse body. Moreover, we did not notice death nor body weight drop of mice after treatment. Meanwhile, we also sacrificed healthy mice 60 days after intravenous injection of PEGylated Sb NPs for blood analysis and histological examination to investigate the potential *in vivo* toxicity of PEGylated Sb NPs (Figure S11 and S12). The results indicate that no distinct hepatic and kidney disorder of mice was caused by PEGylated Sb NPs treatment, and all blood panel parameters in the PEGylated Sb NPs treated mice were normal compared with the control groups. Although white blood cell (WBC) counts showed slight variation, they were sensitive to external factors of housing environment, drinking water and feeding. Moreover, neither obvious organ damage nor inflammation was noticed, indicating the *in vivo* treatment based on PEGylated Sb NPs caused no distinct side effect to the treated mice. Obviously, the excellent photothermal conversion efficiency, photostability and low *in vivo* toxicity of PEGylated Sb NPs make it a promising agent for photothermal cancer treatment.

Despite the higher photothermal efficiency of the semimetal Sb particles compared to the classic photothermal agents (*i.e.* gold nanorods, nanoshells, *etc.*), one limitation of this type of nanoparticles is the relatively large size, which would be only suitable for intratumoral injection. The Sb nanoparticles reported here are too large to be accumulated in tumors *via* enhanced permeability and retention (EPR). Moreover, these nanoparticles can be cleared rapidly by the immune system and accumulated in the reticuloendothelial system (RES) organs when they are intravenously administered.

4. Conclusions

In summary, a novel photothermal agent of semimetal Sb NPs have been prepared through a facile route with by mixing ODE and OAm as the solvent. The aqueous dispersion of PEGylated Sb NPs, due to its broad and strong photoabsorption ranging from ultraviolet (UV) to near-infrared (NIR) wavelengths, is applicable as a photothermal agent driven by 808 nm laser with photothermal conversion efficiency up to 41%. Our *in vitro* experiments also showed cancer cell ablation effect of PEGylated Sb NPs was dependent on laser power. By intratumoral injection of PEGylated Sb NPs, 100% tumor ablation can be realized by using NIR laser irradiation with a lower power of 1 W/cm² for 5 min (or 0.5 W/cm² for 10 min) and no obvious toxic side effect is identified after PTT. The results of the long time biodistribution, as well as blood analysis and histological examination of healthy mice after PEGylated Sb NPs intravenous injection also demonstrated their low *in vivo* toxicity.

Moreover, intense PA signal was also observed after intratumoral injection of PEGylated Sb NPs and NIR laser irradiation due to their strong NIR photoabsorption, suggesting PEGylated Sb NPs as a potential NIR PA agent. More importantly, based on the findings of this work, further development of using other semimetal nanocrystals as highly efficient NIR agents can be achieved for vivo tumor imaging and PTT. Although our semimetal Sb NPs in their current form are relatively large in size, which may limit their use *in vivo*, our future work on Sb NPs will focus on the influence of size, shape, structure, and surface modification on their photothermal properties, toxicity, EPR effect and circulation time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
 (a) TEM (left), HRTEM (top right) and SAED (bottom right) images of the as-prepared Sb NPs, (b) XRD and (c) EDS of the as-prepared Sb NPs.

Figure 2.

(a) UV-vis-NIR absorption spectra of PEGylated Sb NPs. The inset is a image of PEGylated Sb NPs aqueous solution with concentration of 0.25 mg/mL. (b) Temperature variation of pure water and PEGylated Sb NPs solutions with various concentrations versus laser irradiation time. (c) The photothermal response of PEGylated Sb NPs solution irradiated under an 808 nm laser at 2 W/cm² for 300 s, then the laser was turned off. (d) Linear relationship between time and $-\ln\theta$ obtained from the cooling time of (c).

Figure 3.

(a) Relative viabilities of 4T1 tumor cells and hepatic L02 cells after incubation with PEGylated Sb NPs at various concentrations for 24 h. (b) Fluorescence images of Calcein AM/PI stained 4T1 cancer cells without and with PEGylated Sb NPs (100 μg/mL) incubation after irradiated under the 808 nm laser at 0.5, 1 and 2 W/cm². (c) Relative viabilities of 4T1 cancer cells with and without PEGylated Sb NPs caused photothermal destruction at various laser power densities. The standard deviations of five parallel samples were used to determine the error bars.

Figure 4.

(a) IR thermal images of 4T1 tumor mice with and without PEGylated Sb NPs injection before and after the 808 nm laser irradiation at 1 W/cm^2 for 5 min. (b) The temperature variation on tumors of mice with and without PEGylated Sb NPs injection after the 808 nm laser irradiation at 1 W/cm^2 . (c) PA imaging of mice bearing 4T1 tumors without and with $40 \mu\text{g}$ PEGylated Sb NPs injection under the irradiation of NIR laser with different wavelengths. (d) PA signal intensity ratio of 10 min after $40 \mu\text{g}$ PEGylated Sb NPs injection vs before injection at different wavelengths of 808, 730 and 680 nm.

Figure 5.

(a) The 4T1 tumor growth curves of mice after various treatments. The tumor volumes were normalized to their initial tumor sizes. Laser wavelength = 808 nm; irradiation time = 5 min (1 W/cm^2) or 10 min (0.5 W/cm^2). Standard deviations of 5–7 mice per group were used to determine error bars. (b) Survival curves of 4T1 tumor mice after various treatments. (c) Representative photographs of 4T1 tumor-bearing mice after various treatments. (d) H&E stained tumor slices collected from different groups of mice immediately after 808 nm laser irradiation.