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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/cm^2 for 5 min (or 0.5 W/cm^2 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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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 $W_{18}O_{49}$ [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.

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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 appliable 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

2.1. Chemicals

Oleyl amine, 1-octadecance 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.

2.2. 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 octadecence (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.

2.3. 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 60 min, 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 centrifuged to eliminate unstable aggregates. Finally, the supernatant liquid was collected and washed to remove excess DSPE-PEG5000.

2.4. 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 Cu Kα radiation ($\lambda = 0.15418$ 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.

2.5. 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 μL) were incubated with and without PEGylated Sb NPs (100 μg/mL) for 4 h, then the cells were rinsed with PBS and immersed in 100 μL 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.

2.6. 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/cm^2 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.

2.7. 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 μL phosphate buffered saline (PBS) were subcutaneously administered into the flank of each nude mice. When their tumor size reached about 60 mm^3, the 4T1 tumor mice were treated.

2.8. Photothermal therapy

4T1 tumor mice were intratumorally administered with 20 μL 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/cm^2 for 5 min (or 0.5 W/cm^2 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