2D Material-Based Nanofibrous Membrane for Photothermal Cancer Therapy

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Supporting Information

ABSTRACT: One of the clinical challenges facing photothermal cancer therapy is health risks imposed by the photothermal nanoagents in vivo. Herein, a photothermal therapy (PTT) platform composed of a 2D material-based nanofibrous membrane as the agent to deliver thermal energy to tumors under near-infrared (NIR) light irradiation is described. The photothermal membrane, which is fabricated by an electrospinning poly(ε-lactic acid) (PLLA) nanofibrous membrane loaded with bismuth selenide (Bi2Se3) nanoplates, exhibits very high photothermal conversion efficiency and long-term stability. Cell experiments and hematological analyses demonstrate that the Bi2Se3/PLLA membranes have excellent biocompatibility and low toxicity. PTT experiments performed in vivo with the Bi2Se3/PLLA membrane covering the tumor and NIR irradiation produce local hyperthermia to ablate the tumor with high efficiency. Different from the traditional systematical and local injection techniques, this membrane-based PTT platform is promising in photothermal cancer therapy, especially suitable for the treatment of multiple solid tumors or skin cancers, and long-term prevention of cancer recurrence after surgery or PTT, while eliminating the health hazards of nanoagents.

KEYWORDS: two-dimensional materials, photothermal effect, near-infrared laser, nanofibrous membrane, cancer therapy

1. INTRODUCTION

Cancer is one of biggest health threats, and nanomaterial-mediated photothermal therapy (PTT) by means of near-infrared (NIR) light illumination has aroused much attention in cancer treatment. Owing to the high tissue penetration ability of NIR light, PTT makes use of the conversion of photon energy to localized heat to destroy cancer cells with high selectivity, especially in vital organs where surgery is difficult. Compared to radiotherapy and chemotherapy with many side effects, PTT causes minimal damage to normal cells in the vicinity because tumors with a poor blood supply are more sensitive to heat-induced damage than normal tissues. Furthermore, PTT can be applied as an adjuvant therapy after surgical treatment of cancer. Research on photothermal conversion agents has spurred the development of PTT, and in particular, nanomaterials with strong NIR absorption and high photothermal conversion efficiency are excellent PTT agents for cancer ablation. Nevertheless, local and precise delivery of heat energy to the tumor remains a big challenge.

To achieve local hyperthermia, most PTT techniques rely on the administration of nanoagents via systematical or local injection. Nanoparticles introduced by systematical injection can target tumors by passive (e.g., enhanced permeability and retention effect) and/or active (e.g., antibody conjugation) targeting. However, most of the injected nanomaterials tend to accumulate in the metabolic organs such as the liver and spleen, causing long-term harmful effects. Direct local injection can circumvent the inefficiency and off-target deposition of nanoparticles during intravenous administration. However, most of them can easily diffuse to surrounding tissues because of their small size. In particular, to kill cancer cells efficiently with a sufficient heat energy, a high-power laser or a high-concentration agent introduces safety risks and so, more effective PTT strategies with less side effects are crucial to clinical adoption.

PTT exploits local hyperthermia of tumor tissues, and the main purpose of the nanoagents is to produce thermal energy locally in the presence of NIR light. Owing to potential side effects of nanoagents, the more attractive PTT approach is to deliver sufficient thermal energy to tumors without them. In this paper, a membrane-based PTT platform is described. In this technique, the photothermal nanoagents are embedded in the membrane as a porous structure to provide a controlled delivery of heat energy to the target tissue.
polymer instead of being injected into the human body. The membrane is fabricated by electrospinning two-dimensional (2D) Bi$_2$Se$_3$ into the poly(1-lactic acid) (PLLA) nanofibrous structure. In vitro and in vivo experiments confirm that the photothermal membrane produces local hyperthermia to tumor tissues while avoiding the potential hazards of injected nanogagents.

2. EXPERIMENTAL SECTION

2.1. Synthesis of Bi$_2$Se$_3$ Nanoplates. Poly(vinylpyrrolidone) (PVP, 1.0 g, $M_w = 55$ 000, Sigma-Aldrich, USA) was dissolved in 50 mL of ethylene glycol (99%, Sigma-Aldrich, USA) and was poured into a 250 mL round-bottom flask. A solution containing Na$_2$SeO$_3$ (99%, Sigma-Aldrich, USA, 0.242 g dissolved in 40 mL of ethylene glycol) and Bi(NO$_3$_)$_3$.5H$_2$O (99.99%, Sigma-Aldrich, USA, 0.452 g dissolved in 15 mL of ethylene glycol) was added under magnetic stirring at room temperature. The flask was sealed and heated to 160 °C under nitrogen. The reaction commenced after rapid injection of a hydroxylamine solution (NH$_2$OH, Sigma-Aldrich, USA, 2.4 mL dissolved in 20 mL of ethylene glycol), and the mixture turned dark, which is immediately indicative of the formation of Bi$_2$Se$_3$ nanoparticles. After reacting for 10 min, the products were cooled to room temperature, centrifuged at 12 000 rpm for 10 min, washed thrice with a mixture of acetone and deionized water (300 mL:60 mL).

2.2. Synthesis of Bi$_2$Se$_3$/PLLA Nanofibrous Membranes. The PLLA-based nanofibrous membranes were fabricated by electrospinning. In particular, the PLLA with a viscosity of 1.46 dL/g (Daigang Biomaterial Co. Ltd., China) was dissolved in a 4:1 solvent of deionized water and N,N-dimethylformamide (Beijing Chem. Co., China) to obtain a 10% (w/v) homogeneous solution, followed by the addition of Bi$_2$Se$_3$ nanoparticles (10 mg/mL) and gentle agitation at room temperature for 12 h. During electrospinning, the mixture was placed in a syringe with a stainless steel needle (inner diameter of 0.6 mm) and pumped continuously at a rate of 2 mL/h, when the distance between the needle tip and the collecting drum covered with an aluminum foil was 15 cm. A high voltage power supply was employed to apply a 20 kV potential to the tip. After electrospinning for 2.5 h, a 10 × 20 cm$^2$ Bi$_2$Se$_3$/PLLA nanofibrous membrane (Bi$_2$Se$_3$ content: 50 μg/cm$^2$) was obtained and removed for the residual solvent by vacuum drying overnight. The pristine PLLA nanofibrous membrane was synthesized using the same procedures but without adding Bi$_2$Se$_3$ nanoparticles.

2.3. Characterization. The obtained Bi$_2$Se$_3$ nanoparticles were characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM), energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD) as described in our previous work.12,13 The amount of Bi$_2$Se$_3$ solution and in the Bi$_2$Se$_3$/PLLA membrane was determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES) using the IRIS Intrepid II XSP (Thermo Electron Corporation, USA). The UV–vis–NIR absorption spectra were obtained on the TU-1810 spectrophotometer (Purkinje General Instrument Co. Ltd. China). The surface morphology of the Bi$_2$Se$_3$/PLLA membrane was subjected to 120 s of gold coating and observed by scanning electron microscopy (SEM) with a Nova NanoSEM 430 instrument (FEI, USA) at an accelerating voltage of 5 kV (Theta Lite, Finland).

2.4. NIR Laser-Induced Heat Conversion. Because the 808 nm semiconductor laser is cheap and commonly used in PTT studies, an 808 nm semiconductor laser (KS-810F-8000, Kai Site Electronic Technology Co., Ltd., China) was used in this study. To evaluate the photothermal performance of the Bi$_2$Se$_3$ nanoparticles, aqueous solutions with different concentrations of Bi$_2$Se$_3$ nanoparticles in quartz cuvettes were subjected to the 1.0 W/cm$^2$ laser irradiation for 10 min, and the temperatures of the solutions were monitored by an infrared thermal imaging camera (Fluke Ti27, USA). To compare the photothermal effects between the Bi$_2$Se$_3$/PLLA membrane and the pristine PLLA membrane, the Bi$_2$Se$_3$/PLLA membrane (containing 50 μg Bi$_2$Se$_3$) was immersed in water and exposed to the 808 nm laser (1.0 W/cm$^2$) for 5 min. A Bi$_2$Se$_3$ solution containing the same amount of Bi$_2$Se$_3$ nanoparticles (50 μg) was excited under the same conditions. To study the photothermal tissue penetration, a piece of fresh pigskin purchased from the local food market was used as the barrier layer. The quartz cuvette was coated with a 2 mm thick pigskin, and similar procedures as described above were implemented to measure the temperature.

2.5. Cell Cultures. The HeLa cancer cells and human skin fibroblast (HSF) cells were obtained from American Type Culture Collection (ATCC, USA) and cultivated in humidified atmosphere with 5% CO$_2$ at 37 °C by using the high-glucose Dulbecco’s modified Eagle’s medium (H-DMEM) containing 10% (v/v) fetal bovine serum as the culture medium. The PLLA and Bi$_2$Se$_3$/PLLA membranes were sterilized by immersing the samples in 70% (v/v) ethanol for 30 min, and then rinsed twice with phosphate-buffered saline (PBS) and twice with H-DMEM for 30 min each. Subsequently, the cells were seeded onto the samples at a density of 1 × 10$^4$ per sample (1 cm in diameter) by using 24-well plates as the holders. During the cell culture, the medium was refreshed every 2 days.

2.6. Cell Viability and Proliferation. To determine cell viability, the cells on samples were examined by using a live/dead viability/cytotoxicity assay kit according to the manufacturer’s test specification. The cells were co-stained with calcein AM and PI for 30 min, rinsed with PBS, and imaged immediately under an upright fluorescence microscope (BX53, Olympus, Germany). Cell proliferation was determined by the cell counting kit-8 (CCK-8) assay by measuring the absorbance at 450 nm on a microplate reader (Varioskan Flash 4.0.53, Finland).

2.7. In Vivo Toxicity. Fifteen healthy female nude mice (6 weeks old, Balb/c) were employed in our experiments, and we followed the protocols approved by the Animal Care and Use Committee of the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences. The mice were randomly divided into 3 groups: (1) control group without treatment, (2) subcutaneously implanted PLLA membrane, and (3) subcutaneously implanted Bi$_2$Se$_3$/PLLA membrane. A skin incision was made at the right rear flank of each mouse before the membrane was implanted. After 30 days implantation, all mice were sacrificed, and organs such as liver, spleen, kidney, heart, and lung were collected and fixed with 10% buffered formalin. Afterward, the tissue was embedded in paraffin and sectioned on a microtome into 8 μm thick slices. All the slices obtained were stained with hematoxylin and eosin (H&E) for further examination.

2.8. In Vivo Photothermal Experiments. The HeLa cancer cells and the HSF cells were cultured on the Bi$_2$Se$_3$/PLLA or pristine PLLA membranes for 24 h, and the cell membrane samples were irradiated with the 808 nm laser at a power density of 0.5 W/cm$^2$ for different periods of time. The laser spot was adjusted to fully cover the area of each well. After further incubation for 4 h, the cell membrane samples were rinsed twice with PBS, co-stained with calcine AM and PI for 30 min each, and then observed by fluorescence microscopy. The cell viability was determined by CCK-8 assay as mentioned above.

2.9. In Vivo Photothermal Experiments. The tumor-bearing mice model was established by the subcutaneous injection of HeLa cancer cells (1 × 10$^7$ cells in 100 μL PBS) into the right rear flank of nude mice (Balb/c, 6 weeks old). When the volume of tumors in mice reached about 100 mm$^3$, the mice were separated into 3 groups as mentioned above. After anesthesia, a skin incision was made at the edge of the tumor, and the surface of the tumor tissue was covered by the membrane (about 1 cm$^2$). After laser illumination (808 nm, 0.5 W/ cm$^2$) for 5 min, the wound was routinely sutured and coated with an antibiotic ointment. The corresponding histological changes of the tumors after NIR irradiation were examined by H&E staining as mentioned above. The tumor size was measured by a caliper every other day, and T2-weighted images were calculated. During the course of therapy, no mice died, and the relative tumor volumes were determined by V/V$_0$ (V$_0$ was the initial tumor volume before initiating the treatments).

2.10. Statistical Analysis. All results are presented as mean ± SD, and the analysis of variance was used for statistical analysis. In all the
statistical evaluations, \( P < 0.05 \) was considered significant, \( P < 0.01 \) was considered highly significant, and \( P < 0.001 \) was considered very highly significant.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Bi\(_2\)Se\(_3\) Nano-plates. As one of the biodegradable biopolymers, PLLA is an eco-friendly nontoxic product derived from renewable feedstock.\(^{30}\) By means of electrospinning, biomedical PLLA can be processed into a nanofibrous structure with high porosity and large surface area, which is quite suitable for tissue regeneration.\(^{31,32}\) In our process, to fabricate a photothermally active PLLA membrane, 2D Bi\(_2\)Se\(_3\) nanoplates are incorporated into the PLLA nanofibrous structure. It has been reported that Bi\(_2\)Se\(_3\) nanoplates are an efficient NIR photothermal agent\(^{33−37}\) having low cytotoxicity\(^{38−40}\) and being easily metabolized.\(^{41}\) The Bi\(_2\)Se\(_3\) nanoplates are prepared by a simple method using PVP as reported previously.\(^{35}\) The TEM image in Figure 1a reveals that the products are mainly bilayered nanoplates with a relatively uniform size distribution without aggregation. As shown in Figure 1b, the average size of the Bi\(_2\)Se\(_3\) nanoplates (statistical TEM analysis of 200 particles) is 81.3 ± 16.5 nm. The high-resolution TEM image in Figure 1c shows lattice fringes of 0.21 nm corresponding to the (110) plane of the Bi\(_2\)Se\(_3\) crystal.\(^{42}\) The three-dimensional AFM image in Figure 1d depicts the topography of Bi\(_2\)Se\(_3\) nanoplates, and the corresponding thickness is determined by sectional analysis. Because Bi\(_2\)Se\(_3\) nanoplates have a layered structure with each layer about 4 nm thick,\(^{43}\) the measured heights of ~8 and ~12 nm along lines 1 and 2 can be ascribed to Bi\(_2\)Se\(_3\) nanoplates with 2 and 3 layers. The products are analyzed by EDS to confirm the expected atomic ratio of bismuth (Bi)/selenium (Se) = 2:3 (Figure S1).

The XRD pattern in Figure 1e shows that the Bi\(_2\)Se\(_3\) nanoplates have a rhombohedral crystal structure, which is in good agreement with bulk Bi\(_2\)Se\(_3\) (JCPDS Card no. 33-0214).\(^{44}\) The Bi\(_2\)Se\(_3\) nanoplates dispersed in aqueous solutions with different concentrations are characterized for the absorbance spectra, as shown in Figure 1f. Similar to the other 2D layered materials such as black phosphorus\(^{45,46}\) and graphene oxide,\(^{37}\) the Bi\(_2\)Se\(_3\) nanoplates reveal a broad absorption across the UV and NIR regions. The absorption intensity over the characteristic length of the cuvette (\(A/L\)) at 808 nm was normalized to the concentration (C) measured by ICP-AES (inset in Figure 1f). According to the Lambert–Beer law: 
\[
\frac{A}{L} = \alpha C,
\]
where \(\alpha\) is the extinction coefficient, there is a linear trend observed from \(A/L\) versus C, and \(\alpha\) of the Bi\(_2\)Se\(_3\) nanoplates is determined to be 10.9 \(L \, g^{-1} \, cm^{-1}\), which is much higher than the common photothermal agent Au nanorods (3.9 \(L \, g^{-1} \, cm^{-1}\), Figure S2).

To evaluate the photothermal properties of the Bi\(_2\)Se\(_3\) nanoplates, the aqueous solutions with different concentrations of Bi\(_2\)Se\(_3\) nanoplates (0, 5, 10, 20, and 50 \(\mu g/mL\)) are irradiated with the 808 nm laser (1.0 \(W/cm^2\)), and the solution temperature is monitored as a function of irradiation time. As shown in Figure 1g, when the concentration of the Bi\(_2\)Se\(_3\) solution is 50 \(\mu g/mL\), the solution temperature increases by 27.5 \(^\circ C\) after irradiation for 10 min. In contrast, the temperature of pure water only increases by 3.9 \(^\circ C\), indicating that the Bi\(_2\)Se\(_3\) nanoplates can efficiently convert NIR light into thermal energy. On the basis of a previously reported method,\(^{47}\) the photothermal conversion efficiency of Bi\(_2\)Se\(_3\) nanoplates is determined.

Figure 1. Characterization of the Bi\(_2\)Se\(_3\) nanoplates: (a) TEM image; (b) statistical size analysis of 200 particles measured by TEM; (c) HR-TEM image; (d) three-dimensional AFM images and section analysis; (e) XRD spectra; (f) absorption spectra; (g) photothermal heating curves acquired from the Bi\(_2\)Se\(_3\) nanoplates dispersed in water with different concentrations (0, 5, 10, 20, 50 \(\mu g/mL\)) irradiated with the 808 nm laser (1.0 \(W/cm^2\)). Inset: Plot of temperature change (\(\Delta T\)) for a period of 10 min vs Bi\(_2\)Se\(_3\) concentration.

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to be about 26.4%, which is higher than that of Au nanorods (21.0%).

3.2. Preparation and Characterization of Bi$_2$Se$_3$/PLLA Nanofibrous Membranes. The Bi$_2$Se$_3$/PLLA nanofibrous membranes are fabricated by dispersing Bi$_2$Se$_3$ nanoplates in the PLLA solution and electrospinning (Figure 2a). For comparison, the pristine PLLA membrane by electrospinning; (b) membranes are fabricated by dispersing Bi$_2$Se$_3$ nanoplates in the PLLA solution and electrospinning (Figure 2a). For comparison, the dispersed Bi$_2$Se$_3$ nanoplates (50 μg/mL), pristine PLLA membrane, and pure water serve as control samples. As shown in Figure 3a, the temperature of the solution immersed with the Bi$_2$Se$_3$/PLLA membrane increases by 26.4 °C after irradiation for 5 min. In contrast, the temperature of the solution containing dispersed Bi$_2$Se$_3$ nanoplates and the PLLA membrane increases by 22.3 and 8.8 °C, respectively. These results indicate that the photothermal property of the Bi$_2$Se$_3$/PLLA membrane is better than that of the Bi$_2$Se$_3$ dispersion for the same Bi$_2$Se$_3$ amount. This is probably due to the fact that the Bi$_2$Se$_3$/PLLA membrane is more efficient than the Bi$_2$Se$_3$ solution for light absorption, which is ascribed to the opaqueness of the membrane. During the first 1 min of irradiation, the temperature of the solution containing the Bi$_2$Se$_3$/PLLA membrane increases by 21.8 °C, whereas that of the Bi$_2$Se$_3$ dispersion increases by only 8.9 °C. Evidently, the Bi$_2$Se$_3$/PLLA membrane can convert NIR light into heat more efficiently than the dispersed Bi$_2$Se$_3$ nanoplates. The surface morphology (Figure S3) and the contact angle (Figure S4) of the Bi$_2$Se$_3$/PLLA nanofibrous membrane are compared before and after laser irradiation for 5 min. Laser irradiation does not damage the structure or degrade the surface properties of the Bi$_2$Se$_3$/PLLA nanofibrous membrane.

Because NIR light can penetrate more deeply into biological tissues than visible light, a piece of fresh pigskin with a thickness of 2 mm is used as a model barrier layer to study the photothermal effects on biological tissues. As shown in the insets of Figure 3b, the aqueous solution with the Bi$_2$Se$_3$/PLLA membrane containing 50 μg Bi$_2$Se$_3$ is covered by the pigskin and illuminated by the 808 nm laser (1.0 W/cm$^2$). The temperature of the solution increases from 25.1 to 38.3 °C after laser illumination for 5 min, and the temperature rise is reduced from 26.4 °C in the absence of the pigskin (Figure 3a) to 13.2 °C in the presence of the pigskin (Figure 3b). The 50% reduction can be attributed to the decrease in the usable power of the 808 nm laser which is partially absorbed and scattered by the pigskin. It is well-known that a temperature of over 43 °C can sterilize cancer cells because of the relatively lower heat tolerance of cancer tissues caused by a poor blood supply. Assuming that the temperature in the human body is 37 °C, the Bi$_2$Se$_3$/PLLA membrane covered by the 2 mm thick pigskin can be easily heated to over 43 °C under NIR light irradiation. Furthermore, no obvious damage can be found from the pigskin after the photothermal measurements, thus confirming the safety of laser irradiation. These results reveal the effectiveness and large potential of the Bi$_2$Se$_3$/PLLA membranes in PTT.

To assess the photothermal stability, the aqueous solution containing the Bi$_2$Se$_3$/PLLA membrane is irradiated with 808 nm laser radiation for 5 cycles, and the time-dependent temperature is monitored. In each cycle, the laser is turned on for 1 min (laser on), followed by natural cooling to room temperature (laser off for about 5 min). As shown in Figure 3c, the photothermal effects do not degrade, suggesting good photothermal stability.
The desirable photothermal response agent should also be stable during long-term storage. Hence, the stability of Bi$_2$Se$_3$/PLLA membranes is evaluated by comparing the photothermal performance of a Bi$_2$Se$_3$/PLLA membrane newly prepared and one stored under ambient conditions for 2 months. Figure 3d reveals that storage for 2 months leads to only 1.9% reduction in the temperature elevation. In contrast, the temperature elevation observed from the Bi$_2$Se$_3$ nanoplates in aqueous solution is reduced by 27.4%, probably because of oxidation of Bi$_2$Se$_3$ in the ambient environment. Hence, the PLLA membrane can even

**Figure 3.** (a) Photothermal heating curves of pure water and aqueous solutions immersed with the PLLA membranes, Bi$_2$Se$_3$/PLLA membranes (containing 50 μg Bi$_2$Se$_3$), and dispersed with Bi$_2$Se$_3$ nanoplates (50 μg Bi$_2$Se$_3$). (b) Photothermal heating curve of the aqueous solution immersed with the Bi$_2$Se$_3$/PLLA membrane and covered with the pigskin after 808 nm laser irradiation (1.0 W/cm$^2$). The insets show the photothermal experiments performed with a piece of 2 mm thick pigskin as a model barrier layer: left: preirradiation; middle: during irradiation; right: postirradiation. (c) Temperature variation in the aqueous solution immersed with the Bi$_2$Se$_3$/PLLA membrane during the five laser on/off cycles. (d) Photothermal heating curves of the aqueous solution containing the freshly prepared Bi$_2$Se$_3$/PLLA membrane (containing 50 μg Bi$_2$Se$_3$) and dispersed Bi$_2$Se$_3$ nanoplates (50 μg Bi$_2$Se$_3$) as well as samples after storage for 2 months.

**Figure 4.** (a) Relative viability of HSF cells and HeLa cancer cells on the PLLA and Bi$_2$Se$_3$/PLLA membrane after culturing for 1, 4, and 7 days; (b) proliferation of HSF cells on PLLA and Bi$_2$Se$_3$/PLLA membranes. Inset: Corresponding fluorescence images (scale bar = 50 μm) of HSF cells stained with calcein AM (live cells, green fluorescence) and PI (dead cells, red fluorescence); (c) histological data (H&E stained images) obtained from liver, spleen, kidney, heart, and lung of the mice after membrane implantation for 30 days.

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Figure S5 shows six samples studied in parallel. The results involving NIR irradiation are compared to the result without NIR irradiation to determine the level of significance.***p < 0.001.

To further assess the possible toxicity of the Bi$_2$Se$_3$/PLLA membrane in vivo, both membranes are systematically investigated after subcutaneous implantation into female Balb/c mice. Because the release of Bi and Se elements from the fibers is probable because of the degradation of PLLA, long-term toxicity studies of the Bi$_2$Se$_3$/PLLA membrane are necessary. The mice implanted with the PLLA and Bi$_2$Se$_3$/PLLA membranes are monitored carefully, and neither death nor obvious toxicity is observed within 30 days. After 30 days of implantation, all mice were sacrificed, and organs such as liver, spleen, kidney, heart, and lung are sliced and stained with H&E for histology analysis. As shown in Figure 4c, no organ damage or inflammatory lesion is observed from the organs, and the negligible histopathological abnormalities or lesions observed from both treated groups bode well for the application of the PLLA and Bi$_2$Se$_3$/PLLA membranes in vivo.

3.4. In Vitro and in Vivo Biocompatibility. The potential cytotoxicity of the Bi$_2$Se$_3$/PLLA membranes toward normal and cancer cells is examined. Each membrane is seeded with the same amount of HSF normal cells or HeLa cancer cells and incubated for 1, 4, and 7 days. The relative viability of HSF and HeLa cells is determined by the CCK-8 assay. As shown in Figure 4a, no significant cytotoxicity can be detected from both types of cells, demonstrating that incorporation of Bi$_2$Se$_3$ nanoparticles does not compromise the biocompatibility of the PLLA nanofibrous membranes. In addition to the CCK-8 assay, the HSF cells are co-stained with calcein AM (live cells, green fluorescence) and PI (dead cells, red fluorescence) to evaluate cell proliferation on the membranes at different time intervals. Figure 4b shows that most of the HSF cells exhibit green fluorescence, further corroborating the good cytocompatibility of both membranes. After incubation for 4 and 7 days, the cells proliferate on both the PLLA and Bi$_2$Se$_3$/PLLA membranes, and Figure S5 confirms that the HSF cells adhere well onto the Bi$_2$Se$_3$/PLLA membrane after 7 days.

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3.5. In Vitro Photothermal Performance. The photothermal therapeutic ability of the Bi$_2$Se$_3$/PLLA membranes to cancer cells is investigated with HeLa cells. Prior to 808 nm laser irradiation (0.5 W/cm$^2$) for up to 5 min, the cells are preincubated with the PLLA and Bi$_2$Se$_3$/PLLA membranes for 24 h. Afterward, the live/dead cells are differentiated by calcein AM and PI co-staining when a time-dependent photothermal effect on the HeLa cancer cells is observed. It is evident from Figure 5a that almost all the HeLa cells on the Bi$_2$Se$_3$/PLLA membrane are killed after laser irradiation for 5 min, but, conversely, the cell viability on the PLLA membrane does not change. Similar results are obtained by the CCK-8 assay (Figure S6b,c), and the percentage of dead cells on the Bi$_2$Se$_3$/PLLA membrane increases with irradiation time. This poses a unique advantage in clinical applications because this photothermal system can modulate the therapeutic effects externally by simply altering the irradiation time. On the basis of our results, irradiation with the 808 nm laser at 0.5 W/cm$^2$ for 5 min is sufficient to kill cancer cells.

The difference in the photothermal effects on cancer and normal cells is examined. After incubation on the Bi$_2$Se$_3$/PLLA membrane for 24 h, the HeLa cancer cells and HSF normal cells are irradiated with the 808 nm laser (0.5 W/cm$^2$) for 5 and 10 min. The cells are differentiated for life/death by calcein AM and PI co-staining and quantitatively assessed by the CCK-8 assay. As shown in Figure S6, both the cancer and normal cells are sterilized after NIR irradiation for 10 min. However, when the irradiation time is decreased to 5 min, obvious cytotoxicity can only be found from the cancer cells because of their lower thermal tolerance than normal cells. Hence, NIR radiation can...
selectively induce cancer cell death without casing significant harm to normal cells by controlling the irradiation time. This selectivity, which is more difficult to achieve by radiotherapy and chemotherapy, minimizes the side effects in photothermal cancer therapy.

3.6. In Vivo Photothermal Cancer Therapy. The in vivo therapeutic efficacy of the Bi$_2$Se$_3$/PLLA membranes is evaluated in an in situ established tumor model by subcutaneous injection of HeLa cells into the right rear flank of the Balb/c mice. When the volume of tumors reaches approximately 100 mm$^3$, the mice are separated into 3 groups as mentioned above. As shown in Figure S7, a skin incision is made at the edge of the tumor after the mice are anesthetized, and the tumor locations are covered with the Bi$_2$Se$_3$/PLLA membranes (about 1 cm$^2$). After laser irradiation (808 nm, 0.5 W/cm$^2$) for 5 min, the wound is routinely sutured and coated with an antibiotic ointment. For comparison, the mice without any treatment or treated with the PLLA membranes serve as the control.

For in vivo monitoring of the photothermal effects, the infrared thermographic maps (Figure 6a) and the maximum temperature (Figure 6b) are measured simultaneously by using an infrared thermal imaging camera. After NIR irradiation of the tumor sites for 5 min, the temperature increase of the PLLA group is only 9.1 °C, which is slightly larger than that of the control group without membranes (5.2 °C). In the Bi$_2$Se$_3$/PLLA group, the temperature of the tumor site rises rapidly by 22.2 °C during the first 1 min of irradiation and reaches 54.5 °C within 5 min, which is high enough for tumor ablation. The corresponding histological changes of tumors after NIR irradiation are examined by H&E staining. As shown in Figure S8, the tumor tissue of the mice in the Bi$_2$Se$_3$/PLLA group degenerates partly with the sign of coagulative necrosis of tumor cells.

After NIR irradiation, the mice in the different experimental groups are monitored for signs of distress daily, and the tumor volume is measured every 2 days. Neither death nor obvious toxicity such as abnormal body weight, drinking, or eating is observed during the posttherapy period. The variation in the tumor volume is consistent with that of the tumor temperature. As shown in Figures 6c,d and S9, the tumors in the mice in the Bi$_2$Se$_3$/PLLA group shrink gradually after NIR irradiation. They are completely cured without recurrence within 14 days. The tumor-bearing mice after the Bi$_2$Se$_3$/PLLA + NIR treatment can survive for over 40 days without a single death. In contrast, tumor inhibition cannot be observed from the control and PLLA groups, and all the mice show an average life span of 24–30 days.

These results corroborate the excellent efficacy of the Bi$_2$Se$_3$/PLLA membranes in photothermal cancer therapy in vivo.

In conventional photothermal cancer therapy, the photothermal agents are usually administrated by systematical or local injection, and the injected nanoparticles may produce long-term detrimental effects. Herein, the Bi$_2$Se$_3$/PLLA membrane acts as a photothermal agent and avoids the potential health detriments of the nanoagents in the body and offers the following advantages: (1) the membrane covers the whole tumor locally, and so it is especially suitable for the treatment of multiple solid tumors (Figure S7) and skin cancers; (2) it can be used to selectively target tumors which are difficult to access by intravenous injection; (3) the membrane-based PTT platform possesses more controllable heat generation because of the homogeneous distribution of the photothermal nanomaterials in the polymer matrix; (4) it can supplement surgical treatment of cancer, and the stable photothermal activity enables long-term prevention of cancer recurrence after surgery; (5) because PLLA is a widely used biocompatible material, the use of the Bi$_2$Se$_3$/PLLA membrane may be safer than direct injection of the Bi$_2$Se$_3$ nanosheets; (6) as biodegradable membranes are widely used in surgery, the PLLA-based membranes with excellent biocompatibility are reliable and safe and especially useful for skin regeneration and wound healing after PTT treatment.

Figure 6. (a) Infrared thermographic maps and (b) time-dependent temperature increase of the tumor-bearing nude mice irradiated by an 808 nm laser (0.5 W/cm$^2$); (c) typical photographs of the tumor-bearing mice before (day 0) and after (day 14) PTT treatment; and (d) corresponding growth curves of tumor in different groups of mice after NIR laser irradiation.
**4. CONCLUSION**

A 2D material/polymer composite membrane fabricated by electrospinning PLLA nanofibrous membranes with Bi$_2$Se$_3$ nanotubes is demonstrated for NIR photothermal cancer therapy. Owing to incorporation of Bi$_2$Se$_3$ nanotubes into the PLLA nanofiber, the Bi$_2$Se$_3$/PLLA composite membranes exhibit improved photothermal conversion efficiency and long-term stability compared to the Bi$_2$Se$_3$ dispersion. Cell experiments and hematomal analyses reveal that the membranes have excellent biocompatibility and low toxicity. In vitro and in vivo photothermal experiments corroborate that the Bi$_2$Se$_3$/PLLA membranes possess excellent PTT efficiency under NIR laser irradiation, thus boding well for minimally invasive PTT treatment of cancer. The membrane-based PTT platform is promising in photothermal cancer therapy, especially for long-time prevention of cancer recurrence while avoiding the potential safety hazards of nanoagents in vivo.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b17117.

   Energy-dispersive X-ray spectroscopy analysis, absorbance spectra of aqueous solutions, SEM images of the Bi$_2$Se$_3$/PLLA nanofibrous membranes, contact angles of the Bi$_2$Se$_3$/PLLA membranes, SEM images of HSF cells, calcein AM and PI, schematic presentation of the in vivo photothermal cancer therapy, histological analysis of tumors, and typical photographs of the tumor-bearing nude mice (PDF).

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**Notes**

The authors declare no competing financial interest.

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Supporting Information

**2D material-based nanofibrous membrane for photothermal cancer therapy**

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**KEYWORDS:** two-dimensional materials, photothermal effect, near-infrared laser, nanofibrous membrane, cancer therapy
Characterization of Bi$_2$Se$_3$ nanoplates

Figure S1. Energy dispersive X-ray spectroscopy analysis of Bi$_2$Se$_3$ nanoplates.
Absorbance spectra of Au nanorods

Figure S2. Absorbance spectra of aqueous solutions containing Au nanorods of different concentrations. Inset: normalized absorbance intensity over characteristic length of the cell ($A/L$) at different concentrations for $\lambda = 808$ nm.
SEM images of Bi$_2$Se$_3$/PLLA membranes

Figure S3. SEM images of the Bi$_2$Se$_3$/PLLA nanofibrous membranes (a) before and (b) after 808 nm laser irradiation (1.0 W/cm$^2$) for 10 min.
Contact angle measurements

Figure S4. Contact angles of the Bi$_2$Se$_3$/PLLA membranes before and after 808 nm laser irradiation (1.0 W/cm$^2$) for 10 min.
SEM images of HSF cells on Bi$_2$Se$_3$/PLLA membranes

Figure S5. SEM images of HSF cells on the Bi$_2$Se$_3$/PLLA membrane after incubation for 7 days.
Photothermal ablation effects of Bi$_2$Se$_3$/PLLA membranes to cancer cells and normal cells

**Figure S6.** Calcein AM (live cells, green fluorescence) and PI (dead cells, red fluorescence) co-staining of HeLa cancer cells (a, b, c) and HSF normal cells (d, e, f) on the Bi$_2$Se$_3$/PLLA membrane after 808 nm laser irradiation (0.5 W/cm$^2$) for 0 (a, d), 5 (b, e), and 10 min (c, f), scale bar = 100 µm. The inset spectra show the relative cell viabilities and the error bars are based on the standard deviations (SD) of six parallel samples.
Figure S7. Schematic presentation of the *in vivo* photothermal cancer therapy.
Histological analysis

Figure S8. Histological analysis (haematoxylin and eosin stained images) of the tumors treated with the Bi$_2$Se$_3$/PLLA membrane after 5 min NIR laser irradiation (0.5 W/cm$^2$).
Photographs of the tumor-bearing nude mice after treatments

![Typical photographs of the tumor-bearing nude mice treated with the Bi$_2$Se$_3$/PLLA membrane after NIR laser irradiation (0.5 W/cm$^2$) for 5 minutes.]

**Figure S9.** Typical photographs of the tumor-bearing nude mice treated with the Bi$_2$Se$_3$/PLLA membrane after NIR laser irradiation (0.5 W/cm$^2$) for 5 minutes.