Supporting information

Near Infrared Light Activatable PEI-Wrapped Bismuth Selenide Nanocomposites for Photothermal/Photodynamic Therapy Induced Bacterial Inactivation and Dye Degradation

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Materials

Analytical grade bismuth (III) nitrate pentahydrate (Bi(NO₃)₃ .5H₂O) (98%), sodium selenite pentahydrate (Na₂SeO₃・5H₂O) (99%), ethylene glycol, isopropyl alcohol, ethanol, 1,3-Diphenylisobenzofuran (DPBF), 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI), polyethyleneimine (PEI), propidium iodide (PI), methylene blue (MB), Rhodamine B (RhB), phosphate-buffered saline (PBS) solution and agar powder were purchased from Sigma-Aldrich.

Characterization

The as-prepared Bi₂Se₃ NPs and Bi₂Se₃ NPs/PEI were characterized as follows. The crystal structures of the samples were analyzed by powder X-ray diffraction (XRD) on a Rigaku D/MAX-2500 X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 20 mA. The surface modification was characterized by FTIR spectroscopy. In this, Bi₂Se₃ NPs and Bi₂Se₃ NPs/PEI were dried and grounded with KBr and FTIR spectra were recorded by a Perkin-Elmer system 2000 FTIR spectrophotometer with a resolution of 2 cm⁻¹. The UV-Vis-NIR spectra were recorded using a JASCO V-570 spectrometer in the wavelength range from 350 to 1200 nm, with a resolution of 1 nm. The morphologies and elemental composition of the Bi₂Se₃ NPs were analyzed by a JEOL JEM-2100 TEM coupled with an EDXA analyzer as well as a JEOL, JSM-7000F SEM. The zeta potential was measured with a Nicomp 380 ZLS from Particle Sizing Systems.

Photothermal Conversion Efficiency of B₂Se₃ NPs/PEI

The photothermal conversion efficiency of the Bi₂Se₃ NPs/PEI NPs was calculated according to the reported methods.¹-³ Briefly, 80 ppm Bi₂Se₃ NPs/PEI (OD₈₀₈ = 1) in a 1.7 mL centrifuge vial was irradiated with an 808 nm laser. The diameter of the laser beam was 1 cm, the power
density was 1 W/cm². A thermocouple was inserted into the solution to record the temperature. The temperature was monitored during both heating (laser on) and cooling (laser off) stages. The photothermal conversion efficiency \( \eta \) was calculated using the following equation,

\[
\eta = \frac{hS(T_{\text{max}} - T_{\text{amb}}) - Q_{\text{dis}}}{I (1 - 10^{-A_{808}})}
\]

where, \( h \) = heat transfer coefficient, \( S \) = surface area of the container, \( T_{\text{max}} \) = maximum equilibrium temperature, \( T_{\text{amb}} \) = ambient temperature of the surroundings, \( Q_{\text{dis}} \) = laser-induced heat input by the container, \( I \) = laser power, and \( A_{808} \) = absorbance of the NPs at 808 nm.

The same experiment was conducted with water as the control to determine \( Q_{\text{dis}} \) (mW) by

\[
Q_{\text{dis}} = 10^3 \cdot m \cdot C \cdot \Delta T / t
\]

where, \( m \) = mass of water (g), \( C \) = heat capacity of water (J g⁻¹ K⁻¹), \( \Delta T \) = increased temperature (K), and \( t \) = laser exposure time (s).

The term \( hS \) was calculated based on

\[
hS = \sum_{i} \frac{m_i C_{p, i}}{\tau_s}
\]

where, \( \tau_s \) = sample system time constant and \( i \) = system components (NP suspension and sample container).

\( \tau_s \) is related to a dimensionless driving force temperature \( \theta \) by

\[
t = - \tau_s \ln \theta
\]

where, \( t \) = cooling time and \( \theta \) is given by

\[
\theta = \frac{(T - T_{\text{amb}})/(T_{\text{max}} - T_{\text{amb}})}
\]

In these studies, the temperature of NPs solution under continuous laser irradiation and subsequent cooling after the laser being turned off was recorded. The cooling process was used to determine the rate of heat transfer from the NPs solution to the environment. The temperature increased by 56 °C, when the NPs solution was irradiated for 10 min and did not change significantly upon further irradiation. The temperature reached a maximum threshold value because of the equilibrium between the heat input and output. In contrast, the temperature of the control water increased by only 1.2 °C. Using eq. 2 and the heating profile of the water, the \( Q_{\text{dis}} \) was determined to be 2.51 mW. The thermal equilibrium time constant \( (\tau_s) \) was measured from the plot of cooling time \( t \) versus the term \( -\ln \theta \) which shows a linear relationship and gives a slope of 3.26 min. Using eq. 3, the term \( hS \) was calculated to be 6.43 mW/°C. On the basis of eq. 1, the efficiency of transducing the red laser to heat by the Bi²Se³ NPs/PEI NPs was calculated to be 32.07%.
Optimization of preparation conditions.

Effect of ethylene glycol: To study the effect of ethylene glycol (EG), the Bi$_2$Se$_3$ NPs were prepared in two high boiling solvents oleic acid (OA) and EG. The morphology of the obtained Bi$_2$Se$_3$ NPs was observed under a SEM. As shown in Fig. S12, only Bi$_2$Se$_3$ NPs prepared in EG exhibited definite morphology, indicating that EG serves as both solvent and structure-directing agent.

Effect of reaction time and temperature: The Bi$_2$Se$_3$ NPs were prepared under different reaction time and temperature and the morphology was observed under a SEM. The results indicate that the minimum reaction time and temperature to obtain definite structure is 5 h and 260 °C, respectively (Fig. S11).

Fig. S1. Characterization of Bi$_2$Se$_3$ NPs: (a) SEM image, (b) EDXA mapping of bismuth, (c) EDXA mapping selenium, and (d) EDXA spectrum.
Fig. S2. Zeta potential values of Bi$_2$Se$_3$ NPs and Bi$_2$Se$_3$ NPs/PEI (a); the solution appearance of Bi$_2$Se$_3$ NP before (i, iii) and after (ii, iv) after PEI conjugation (b).

Fig. S3. Temperature elevation profile: (a) Temperature elevation profile of PBS, 80 ppm Bi$_2$Se$_3$ NPs, 80 ppm Bi$_2$Se$_3$ NPs/PEI (808 nm laser, 1 W/cm$^2$, 10 min), (b) Temperature elevation profiles of Bi$_2$Se$_3$ NPs/PEI at different power densities (80 ppm, 808 nm), (c) Typical curves of the solution temperature of the Bi$_2$Se$_3$ NPs/PEI versus time in a procedure of laser-on for 10 min and then laser-off.
Fig. S4. Photographic images of *S. aureus*(a) and *E. coli*(b) viability at different conc. of Bi$_2$Se$_3$ NPs/PEI: with laser at 4 °C (1,3 rows), with laser at 28 °C (2,4 rows; 808 nm, 1W/cm$^2$, 10 min).
**Fig. S5.** Time dependent DPBF quenching experiment, (a) DPBF + Bi$_2$Se$_3$/PEI(dark) (b) DPBF + NIR light + Bi$_2$Se$_3$/PEI, and (c) Comparison of $^1$O$_2$ generation efficiency between Bi$_2$Se$_3$ NPs and Bi$_2$Se$_3$ NPs/PEI upon NIR laser irradiation.

**Fig. S6.** Fluorescence microscopy (confocal) images showing the PDT cytotoxicity of *S. aureus* (a) and *E. coli* (b) after 10 min irradiation by an 808 nm NIR laser at 1 W/cm$^2$ with Bi$_2$Se$_3$ NPs/PEI by the detection of reactive oxygen species production inside the bacteria using DCFH-DA fluorescence probe.
Fig. S7. Photocatalytic activity through photodegradation of MB under the experimental conditions (a) MB+Bi$_2$Se$_3$ NPs/PEI (in dark) and (c) MB+NIR light+Bi$_2$Se$_3$ NPs/PEI. Photocatalytic activity through photodegradation of RhB under the experimental conditions (c) RhB+ Bi$_2$Se$_3$ NPs/PEI (in dark) and (d) RhB+NIR light + Bi$_2$Se$_3$ NPs/PEI.

Fig. S8. Schematic of the charge transfer process for the photodegradation of MB and RhB dyes by Bi$_2$Se$_3$ NPs/PEI with NIR light.
Fig. S9. The recycling experiments for photodegradation of (a) MB and (b) Rh B with Bi$_2$Se$_3$ NPs and Bi$_2$Se$_3$ NPs/PEI along with NIR light.

Fig. S10. The SEM images of recycle photocatalyst (Bi$_2$Se$_3$ NPs/PEI) upon photodegradation.
Fig. S11. SEM images of Bi$_2$Se$_3$ prepared under different time and temperature parameters: (a) at 200°C for 5 h, (b) at 300°C for 5 h, (c) at 260°C for 3 h, and (d) at 260°C for 7 h.

Fig. S12. SEM images of Bi$_2$Se$_3$ prepared using oleic acid as solvent at 260°C for 5 h.
Fig. S13. Comparison of antibacterial activity between Bi$_2$Se$_3$ NPs and Bi$_2$Se$_3$ NPs/PEI at 80 ppm concentration upon NIR laser irradiation.

Table S1. Comparison of Bi$_2$Se$_3$NPs/PEI with reported materials in terms of antibacterial activity.

| Material          | Laser nm | Power density W/cm$^2$ | Concentration mg/mL | Time (min) | Killing g% (~) | Ref  |
|-------------------|----------|------------------------|---------------------|------------|---------------|------|
| MGO               | 808      | 1.5                    | 0.08                | 10         | 100           | 4    |
| PVPS: PANI        | 808      | 2                      | 1-5                 | 5          | 100           | 5    |
| Graphene sheets   | 808      | 7.5                    | 0.06-5              | 10         | 100           | 6    |
| Fe$_3$O$_4$@PEDOT | 808      | 2                      | 0.01-1              | 7          | 100           | 7    |
| PPy-SiO$_2$       | 808      | 1.5                    | 0.025-0.2           | 10         | 100           | 8    |
| Bi$_2$Se$_3$NPs/PEI | 808    | 1                      | 0.01-0.08           | 10         | 100           | Present work |
Table S2. Comparison of Bi$_2$Se$_3$NPs/PEI with reported materials in terms of photo degradation capacity

| Material          | Light | Irradiation time (h) | Concentration mg/mL | No of Cycles | Ref  |
|-------------------|-------|-----------------------|---------------------|--------------|------|
| ZnO/Au NPs       | UV    | 1.1                   | 0.4                 | NA           | 9    |
| Zn/ CdS NSs      | UV    | 4                     | 1                   | 4            | 10   |
| BiOCl-PT         | UV    | 3                     | 30                  | NA           | 11   |
| BiVO$_4$ materials | Visible | 2                   | 5                   | NA           | 12   |
| NaYF$_4$:Yb, Tm@TiO$_2$ CSNPs | NIR | 14                    | 1                   | NA           | 13   |
| Bismuth NSs      | Visible | 9.5             | 0.40                | NA           | 14   |
| GO-Ag$_3$PO$_4$   | Visible | 3                   | 20                  | 4            | 15   |
| Bi$_2$Se$_3$ NPs/PEI | NIR | 3                     | 0.25                | 4            | Present work |

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