

Rapid Biofilm Elimination on Bone Implants Using Near-Infrared-Activated Inorganic Semiconductor Heterostructures

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Bacterial infections often cause orthopedic surgery failures. It is hard for the immune system and antibiotics to clear bacteria adhered to implants after they form a mature biofilm, and a secondary surgery is required to remove the infected implants. To avoid this, a hybrid coating of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ is prepared to eliminate biofilm using near-infrared (NIR) light. Bi_2S_3 nanorod (NR) arrays are prepared on titanium (Ti) implants through hydrothermal methods, and Ag_3PO_4 nanoparticles (NPs) are loaded on Bi_2S_3 NR arrays using a stepwise electrostatic adsorption strategy. The introduction of Ag_3PO_4 NPs enhances the photocatalysis performances of Bi_2S_3 , and the hybrid coating also exhibits good photothermal effects. After 808 nm light irradiation for 15 min, it shows superior bactericidal efficiency of 99.45% against *Staphylococcus aureus*, 99.74% against *Escherichia coli* in vitro, and 94.54% against *S. aureus* biofilm in vivo. $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ also shows good cell viability compared to pure Ti. This NIR-activated-inorganic hybrid semiconductor heterojunction coating is biocompatible and could be employed to eliminate biofilm effectively, which makes it a very promising strategy for the surface modification of bone implant materials.

unavoidable, and it is possible for them to adhere to implants. Traditional treatment requires repeated and long-term intravenous or oral antibiotics, which can damage the immunologic system and may also lead to bacterial resistance.^[2] Furthermore, if the bacteria attached to the material surface are not removed in time, they can multiply in large quantity to form a biofilm and secrete polysaccharides, which protect them from antibiotics and the immune system.^[3] This outcome necessitates a second surgery to remove the infected implants and means that patients suffer additional physical pain and financial loss.^[4]

Photodynamic therapy (PDT) introduced by reactive oxygen species (ROS) has been widely studied in sterilization.^[5] ROS produced by a photodynamic system can kill cells in a short time.^[6] Compared to ultraviolet and visible (vis) light, near-

infrared (NIR) light possesses deeper biological tissue penetration ability, causes minimal photodamage to living bodies, and has been widely used in phototherapy.^[7] As demonstrated by Faris et al.,^[8] the effective penetration depth of NIR into a neonatal head could be several millimeters, so biofilm infections

1. Introduction

The failure of orthopedic implantation surgery because of bacterial infection is very common.^[1] Although the surgical environment is strictly sterilized, pathogenic bacteria are

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of artificial knee joints, skulls, elbow joints, and knuckles implanted under the skin could be treated by NIR phototherapy. Recent research by Wu and co-workers,^[9] led to the development of a photoresponsive antibiofilm coating composed of organic photosensitizer IR780 iodide and inorganic semiconductor photothermal agent molybdenum disulfide (MoS_2). The *Staphylococcus aureus* biofilm on the implants was eradicated within 20 min under NIR light due to the combined action of PDT, photothermal therapy (PTT), and glutathione oxidation. However, using IR780 as the photosensitizer often suffers from poor stability and lack of bacteriostatic effect under dark conditions.^[10] In comparison, inorganic semiconductors with high stability can be used as a ROS generator for PDT. Using NIR-activated semiconductors as a coating strategy combines rapid biofilm elimination capacity with long-term antimicrobial effect is a promising surface modification for implant materials.

Bismuth sulfide (Bi_2S_3) is a promising photocatalyst with a narrow bandgap (1.3–1.7 eV). Owing to its large absorption coefficient (10^4 – 10^5 cm^{-1}) and high photoconductivity,^[11] this abundant material has been widely used in the field of photodegradation of photovoltaic cells, photodetectors, and organic dyes.^[12] The good biocompatibility and high photothermal conversion efficiency of Bi_2S_3 lends itself to wide application in the biomedical field.^[13] Theoretically, Bi_2S_3 can generate electrons and holes under NIR light, and they can react with surrounding water or oxygen to produce ROS. Bi_2S_3 also provides an additional advantage of photothermal properties that help kill bacteria, which is different from some other bismuth-based semiconductors, such as bismuth oxychloride (BiOCl).^[14] Nevertheless, Bi_2S_3 is rarely employed singly in photocatalytic reactions because of the severe recombination of its photogenerated carriers.

Recent studies have focused on the combination of narrow bandgap semiconductors with the wider one to promote light absorption and increase the speed of charge separation, and in turn, improve the photocatalytic effect.^[15] Among the photocatalysts under investigation, trisilver phosphate (Ag_3PO_4), has a bandgap of 2.36 eV, possesses good bacteriostatic properties, and expresses low toxicity to mammalian cells, making it a potential antimicrobial material in vivo.^[16] Therefore, Bi_2S_3 NR arrays grown on Ti implants were combined with Ag_3PO_4 NPs to achieve higher photocatalytic effects and rapid biofilm elimination, and relevant mechanisms are discussed in the paper. Bi_2S_3 NR arrays were grown on Ti implants through hydrothermal methods, and Ag_3PO_4 nanoparticles (NPs) were loaded onto Bi_2S_3 NR arrays using a stepwise electrostatic adsorption strategy. Based on the synergistic therapy of PDT and PTT under an 808 nm NIR light, this coating on the Ti plates was expected to accomplish biofilm elimination within 15 min. This was expected to work better than general phototherapy strategies like bismuth ferrite ($\text{Bi}_{2.88}\text{Fe}_5\text{O}_{12}$),^[17] because the trace amount of silver (Ag) ions released from the coating enabled it an effective bacteriostatic action in the absence of light. To the best of our knowledge, this type of coating on bone implants, composed of NIR-triggered inorganic semiconductor heterojunction photocatalysts, has not been reported yet. The hybrid semiconductor heterojunction coating, with efficient biofilm elimination and good cell activity, is encouraging for the future of antimicrobial coatings building on bone implant surfaces.

2. Results and Discussion

2.1. Synthesis and Characterizations of $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$

The schematic of the synthesis procedure for the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ NR arrays on Ti plates is illustrated in **Figure 1**. The alkali-treated Ti plates with a network-like surface showed homogeneous distribution of “S” element of 4-mercaptobenzoic acid (4-MBA) (Figure S1a,b, Supporting Information). Bi_2S_3 NR arrays were derived from an aqueous solution at 50 °C on the 4-MBA-treated Ti plate. **Figure 2a,b** shows sharp-tip Bi_2S_3 NRs grown densely and homogeneously on the surface of Ti plates. After being compounded with Ag_3PO_4 , the coating remained intact (Figure 2c), but the surface of Bi_2S_3 NR arrays was coated by Ag_3PO_4 NPs with an average size of $21.1 \pm 0.2 \text{ nm}$ (Figure 2d). Meanwhile, Ag_3PO_4 grown in solution had a larger and more heterogeneous particle size of $420 \pm 2 \text{ nm}$ (Figure S2, Supporting Information). The reduced Ag_3PO_4 particle size was ascribed to the space-confined effect of Bi_2S_3 hierarchical nanorods.^[18] Transmission electron microscope (TEM) images showed that Bi_2S_3 NRs were subuliform (Figure 2e), which was identical to the scanning electron microscope (SEM) observation in Figure 2b. The lattice fringe of 0.40, 0.36, and 0.31 nm located on the high-resolution image (Figure 2f) was indexed to the planes (220), (130), and (211) of Bi_2S_3 (JCPDS Card File No. 17-0320, orthorhombic lattice).^[19] The inset image in Figure 2f disclosed that the fast Fourier transform (FFT) pattern (corresponding to the red rectangle) affirmed the existence of the Bi_2S_3 crystal, which showed planes (130) of 0.36 nm *d*-spacing and (211) of 0.35 nm. After treatment with AgNO_3 and Na_2HPO_4 solutions successively, the formed NPs were wrapped around Bi_2S_3 NR arrays (Figure 2g) and the high-resolution image (Figure 2h) and FFT pattern (corresponding to the red rectangle in the Figure 2h inset) showed the coexistence of Ag_3PO_4 and Bi_2S_3 . The former showed planes (200) and (211) with corresponding spacing of 0.30 and 0.25 nm (JCPDS Card File No. 06-0505, body-centered cubic),^[15a] while the latter exhibited planes of (211) and (310) with spacing of 0.31 and 0.35 nm (JCPDS Card File No. 17-0320, orthorhombic lattice). The results revealed the compact contact interface between Bi_2S_3 and Ag_3PO_4 . Energy-dispersive X-ray spectroscopy (EDS) mapping disclosed the uniform distribution of Bi, S, Ag, P, and O in $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ NRs (Figure S3, Supporting Information). Cross-section images in Figure S4a (Supporting Information)

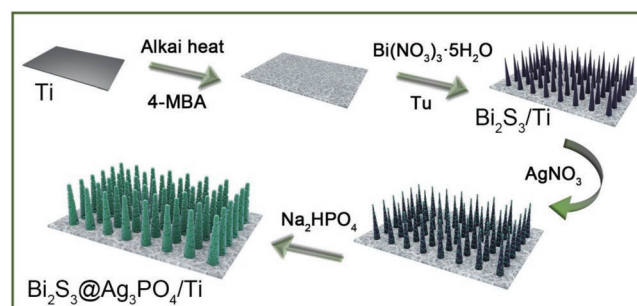


Figure 1. Illustration for the synthesis procedures for $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ nanorod arrays on Ti plates.

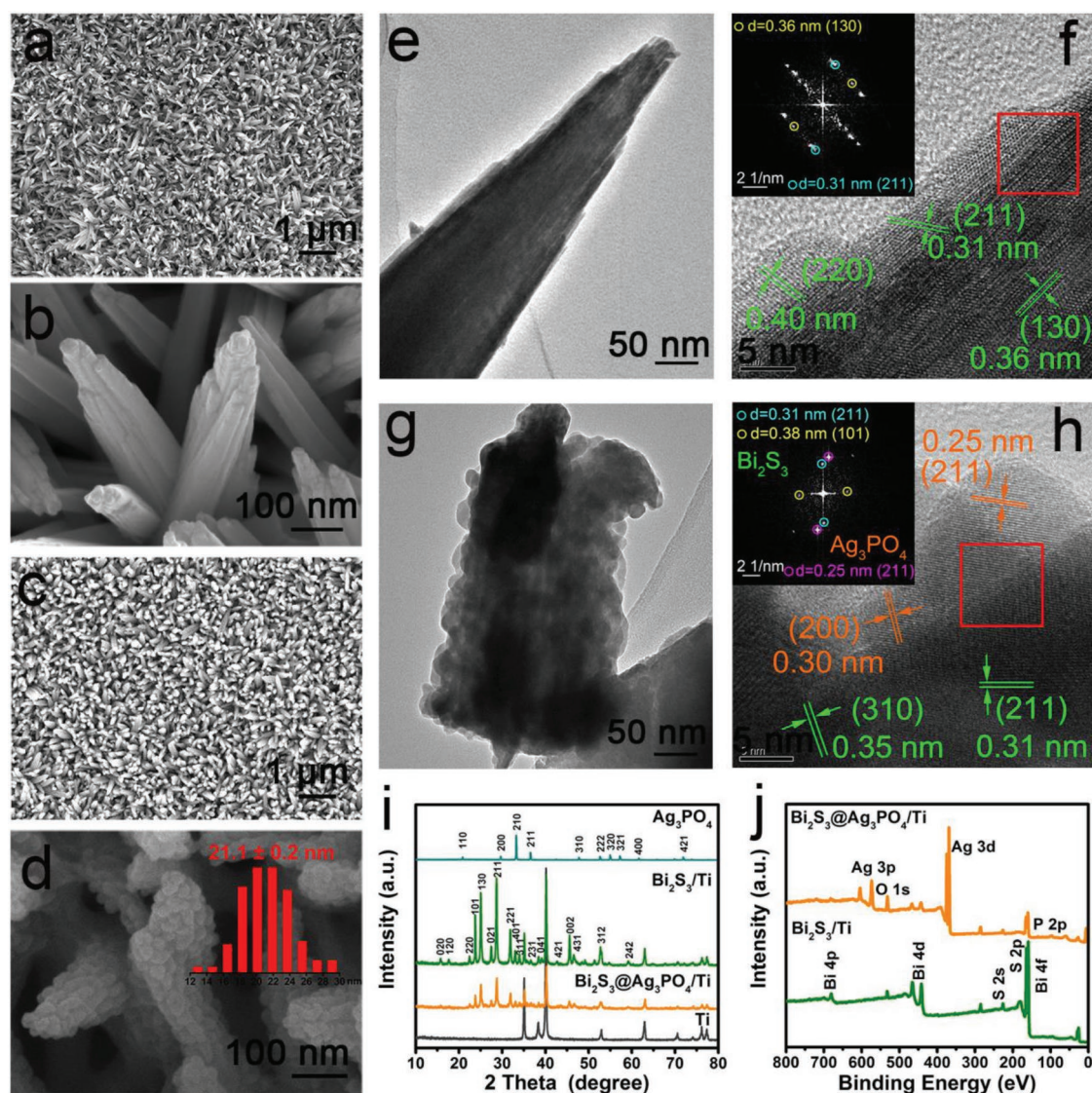


Figure 2. FESEM images of a,b) $\text{Bi}_2\text{S}_3/\text{Ti}$ and c,d) $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$. TEM and HRTEM images of e,f) Bi_2S_3 and g,h) $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ separated from $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$, respectively (inset FFT patterns are corresponding to the red rectangle). i) XRD patterns. j) XPS spectra.

show that the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ coating was about 2 μm thick. Elemental line scanning (Figure S4b, Supporting Information) and mapping images (Figure S4c, Supporting Information) indicated the homogeneous distribution of Bi, S, Ag, P, and O in the coating.

The phase compositions and chemical compositions of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ were further determined by X-ray powder diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). As shown in Figure 2i, the lattice planes (110), (210), and (211) of Ag_3PO_4 powder, prepared using the same methods as the coating, were fully consistent with body-centered cubic structure of Ag_3PO_4 (JCPDS Card File No. 06-0505).^[16a] The $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ composites showed almost the same diffraction peaks with different peak intensities as the $\text{Bi}_2\text{S}_3/\text{Ti}$, which was readily indexed to the orthorhombic lattice of Bi_2S_3 (JCPDS Card File No. 17-0320),^[19] except for the diffraction peaks from pure Ti. The result illustrated that the structure of Bi_2S_3

phase was unchanged after modification with Ag_3PO_4 NPs.^[20] The Ag_3PO_4 signal was not detected in the XRD pattern of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ due to its low content in the composite.^[21] The XPS survey scan (Figure 2j) indicated that the surface of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ included Bi, S, Ag, P, and O elements, while pure $\text{Bi}_2\text{S}_3/\text{Ti}$ was composed of Bi and S. Figure S5a (Supporting Information) shows the high-resolution Bi 4f and S 2p spectra, with peaks at 163.9 and 158.6 eV in $\text{Bi}_2\text{S}_3/\text{Ti}$, and at 164.8 and 159.5 eV in $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$. These corresponded to Bi 4f_{5/2} and Bi 4f_{7/2}. The peaks at 162.5 and 161.3 eV in $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ were attributed to S 2p_{1/2} and S 2p_{3/2}, respectively.^[22] The Bi 4f peaks in $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ shifted toward higher binding energy compared to those in $\text{Bi}_2\text{S}_3/\text{Ti}$, which was attributed to the strong bond between Bi_2S_3 and Ag_3PO_4 because of the electronic shielding effect.^[23] A slight drop of the Bi peak in $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ compared to $\text{Bi}_2\text{S}_3/\text{Ti}$ was due to the partial, rather than full,

coverage of Ag_3PO_4 NPs, because the Ag_3PO_4 NP diameter was greater than the XPS penetration depth. The binding energy located at 374.2 and 368.2 eV in Figure S5b (Supporting Information) was ascribed to Ag 3d3/2 and Ag 3d5/2, respectively, as was characteristic of Ag^+ .^[24] There was no evidence showing the existence of other valence states of the Ag element, which was consistent with Figure 2h. The binding energy value of P 2p (Figure S5c, Supporting Information) and O 1s (Figure S5d, Supporting Information) was 133.1 and 531.0 eV, respectively, indicating the existence of phosphorus (P^{5+}) and lattice oxygen in Ag_3PO_4 .^[25]

2.2. Photodynamic and Photothermal Properties

The UV-vis diffuse reflectance spectroscopy (DRS) spectra of different samples were tested to evaluate optical properties.

According to Figure 3a, pure Ti showed poor capability to absorb light from 300 to 1200 nm wavelengths and $\text{Bi}_2\text{S}_3/\text{Ti}$ displayed a strong absorption peak centered at 800 nm, whereas Ag_3PO_4 showed stronger absorption capacity for vis light than NIR light (inset image in Figure 3a). However, the integration of Ag_3PO_4 with Bi_2S_3 not only made a redshift of the characteristic absorption peak of silver phosphate but also enhanced the absorption capacity of $\text{Bi}_2\text{S}_3/\text{Ti}$ over the entire spectrum (300–1200 nm), indicating that $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ possessed superior light absorption ability than $\text{Bi}_2\text{S}_3/\text{Ti}$ or Ag_3PO_4 separately. Figure 3b was the bandgap diagram of different samples, and the bandgap can be calculated using the following equation^[26]

$$(\alpha h\nu)^n = C(h\nu - E_g) \quad (1)$$

From the Tauc plot, the bandgap of $\text{Bi}_2\text{S}_3/\text{Ti}$ decreased from 1.37 to 1.32 eV with the addition of Ag_3PO_4 NPs. For Ag_3PO_4 ,

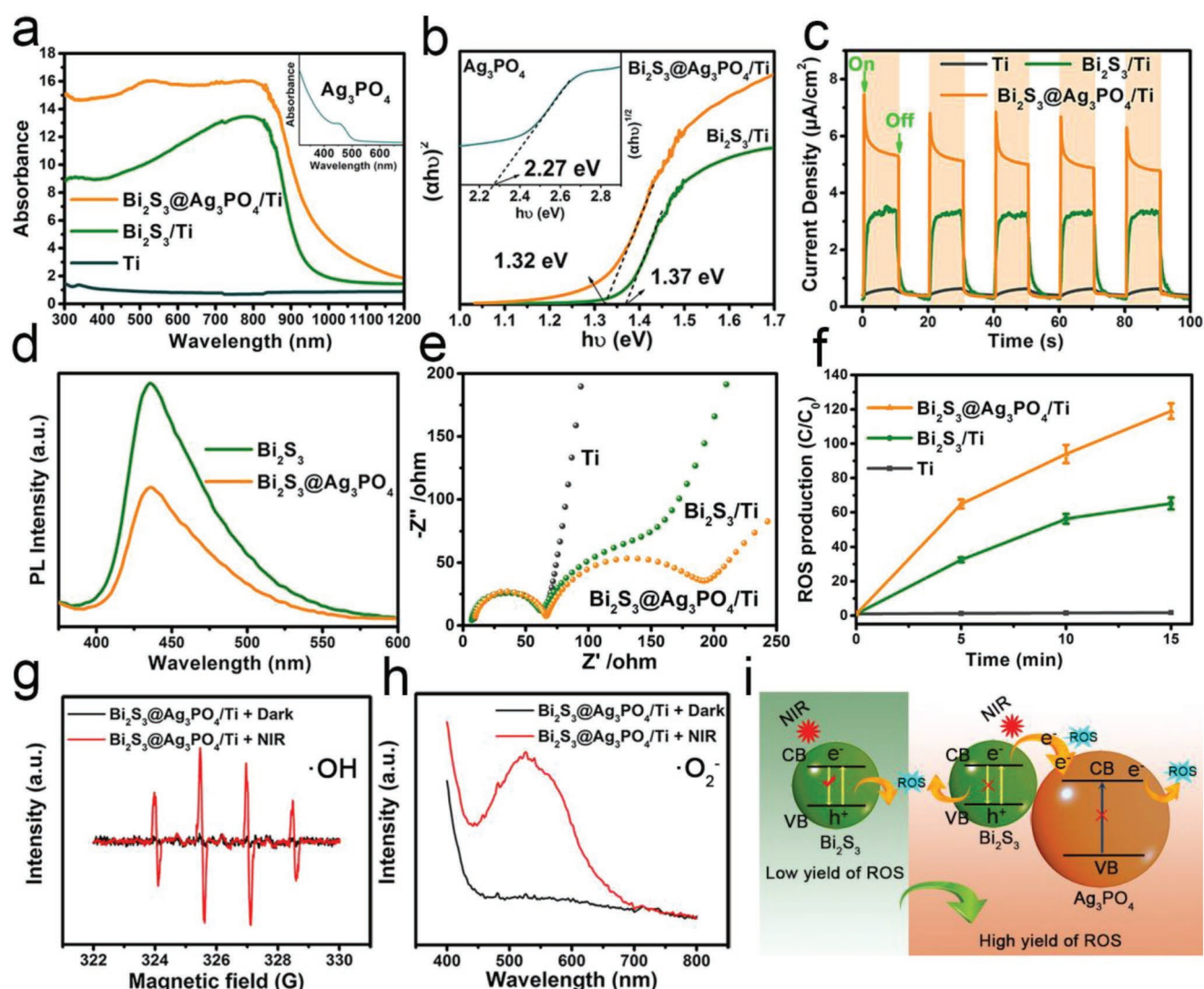


Figure 3. a) UV-vis DRS spectra and b) corresponding Tauc plot. c) Photocurrent response curves and d) PL spectra of Bi_2S_3 and $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4$ solution prepared with powder scraped from $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$, respectively. e) EIS spectra of the photocatalysts under 808 nm NIR light irradiation. f) ROS production (the error bars represent means $\pm$ SD, $n = 3$). g) ESR spectra of hydroxyl radicals. h) Superoxide radicals detected by NBT methods. i) The diagram of band alignment of Bi_2S_3 and Ag_3PO_4 and mechanism of ROS production before and after combination.

the indirect bandgap was calculated as 2.27 eV (inset curve in Figure 3b), indicating that pure Ag_3PO_4 could not be excited by the 808 nm laser. However, the modification of Ag_3PO_4 on $\text{Bi}_2\text{S}_3/\text{Ti}$ broadened the band edge absorption of $\text{Bi}_2\text{S}_3/\text{Ti}$ from 940 to 1000 nm.

The ability to absorb light is important for enhancing photocatalytic reactions, whereas the separation and transfer efficiency of photoinduced electrons and holes is critical in determining the photocatalytic properties of materials. The promoted charge separation capacity of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ compared to both $\text{Bi}_2\text{S}_3/\text{Ti}$ and pure Ti was determined by the enhanced photocurrent density under 808 nm NIR light illumination. As displayed in Figure 3c, $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited the highest photocurrent response among three kinds of samples, indicating the rapid charge separation between Bi_2S_3 and Ag_3PO_4 and the lower recombination rate of photogenerated carriers, which was proven by the reduced photoluminescence (PL) intensity of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4$ compared to Bi_2S_3 (Figure 3d). Generally, higher PL intensity means a stronger probability for photogenerated electron–hole pair recombination.^[27] The PL emission intensity of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4$ weakened dramatically in comparison with that of Bi_2S_3 , indicating suppression of photogenerated electron–hole recombination by the introduction of Ag_3PO_4 . In addition, Ag_3PO_4 NPs accelerated the transfer of photoinspired electrons of $\text{Bi}_2\text{S}_3/\text{Ti}$, which was confirmed by the lowest impedance shown in Figure 3e. The first arc radius can be categorized as the resistance of platinum (Pt) counter electrode/electrolyte interface while the second arc radius can be assigned to the charge-transfer resistance of different samples.^[28] Obviously, $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ had a smaller arc radius than $\text{Bi}_2\text{S}_3/\text{Ti}$ with 808 nm NIR light illumination, indicating a faster photoinspired electron–hole pairs separation of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$.

ROS is a strong oxidant with excellent bacteria-killing ability, with the reaction generated between the photogenerated charges and water or oxygen in the surrounding environment. A higher concentration of total ROS can lead to a lower bacterial survival rate,^[29] and $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ showed a maximum yield of total ROS under NIR light (Figure 3f); therefore, it was expected to have the best antimicrobial sterilization effect. Different reactive oxygen species were verified by electron spin resonance (ESR) spectroscopy and nitro tetrazolium blue chloride (NBT) methods.^[30] The production of $\cdot\text{OH}$ was characterized by ESR in water with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as the spin-trapping agent,^[31] and the generation of $\cdot\text{O}_2^-$ could be detected by its characteristic reaction with NBT.^[32] As shown in Figure 3g,h, both $\cdot\text{OH}$ and $\cdot\text{O}_2^-$ could be detected from $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ samples under 808 nm NIR light illumination. Beyond that, both $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited better photothermal effects in comparison with pure Ti, as observed in Figure S6a (Supporting Information). After immersion in 200 μL phosphate-buffered saline (PBS) solution, the surface temperature of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ increased from 17 to 52.4 °C within 3 min under the illumination of 0.5 W cm^{-2} 808 nm NIR light, followed by $\text{Bi}_2\text{S}_3/\text{Ti}$ (17 to 50.4 °C) and pure Ti (17 to 37 °C). The evolution of surface temperature compared to irradiation time was directly observed by thermal images shown in Figure S6b (Supporting Information).

2.3. Photocatalytic Mechanism

The above results revealed that $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4$ was a typical type II heterostructure as illustrated in Figure 3i. The construction of the heterojunction enhanced photocatalytic activity, as well as the total ROS yield of Bi_2S_3 . Bi_2S_3 with a narrow bandgap of 1.37 eV could generate ROS under 808 nm NIR light irradiation, but it suffered from severe recombination of photogenerated electron–holes, leading to a low ROS yield. After having been covered with Ag_3PO_4 NPs, the hybrid semiconductor heterojunctions bandgap narrowed to 1.32 eV, which was beneficial to producing photogenerated charges. The photocatalytic performance was also improved dramatically. Since the conduction band (CB) potentials of Ag_3PO_4 were lower than Bi_2S_3 ,^[16a,19] it was inferred that photogenerated Bi_2S_3 electrons could be transferred to Ag_3PO_4 smoothly, and in turn, move to the surface, promoting the separation of photocarriers. The well-designed core–shell structure of $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4$ made a full close-knit interfacial contact between Bi_2S_3 and Ag_3PO_4 , thus providing sufficient transport channels for photogenerated carriers and facilitating the separation of photogenerated electron–hole pairs from Bi_2S_3 . In addition, the enlarged specific surface area introduced by Ag_3PO_4 NPs provided more active sites, which was constructive for the photocatalytic redox reaction. In this way, $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ produced more total ROS compared to $\text{Bi}_2\text{S}_3/\text{Ti}$.

2.4. In Vitro Antibacterial Ability and Relative Mechanisms for Biofilm Elimination

The spread plate method was used to transfer and distribute bacteria to an agar plate evenly to count bacterial survival in different groups. The spread plate images (Figure S7a, Supporting Information) of the dark group (all samples cocultured with bacteria without light irradiation) showed no reduction of bacterial colonies compared to Ti, indicating that all samples, including $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$, had no antibacterial efficacy in darkness within 15 min. Even under NIR light irradiation, pure Ti displayed no antibacterial performance. In contrast, after exposure to 808 nm NIR, the two groups of $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited considerably fewer bacteria survival, especially for the latter group, indicating that $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ had the best antibacterial efficacy. As shown in Figure 4a, with 15 min NIR light illumination, the calculated antibacterial efficacy of $\text{Bi}_2\text{S}_3/\text{Ti}$ against *S. aureus* and *Escherichia coli* was 82.75 and 80.05%, respectively. However, $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited a higher antimicrobial efficiency of 99.32 and 99.52% under the same conditions. The higher sterilization effect was ascribed to the enhanced photocatalytic performance, stronger NIR light absorption intensity, and quicker separation of photogenerated carriers, through the addition of Ag_3PO_4 NPs. In addition, the combination of Bi_2S_3 and Ag_3PO_4 suppressed Ag^+ leaching from Ag_3PO_4 , thus providing the coating a slow, long-term Ag^+ release (Figure S7b, Supporting Information), which ensured that the implant materials had lasting protection from bacterial infection. As shown in Figure S7c (Supporting Information), after coculturing with $\text{Bi}_2\text{S}_3@ \text{Ag}_3\text{PO}_4/\text{Ti}$ for 24 h in darkness, 99.45% of *S. aureus* and 99.74% of *E. coli* were killed because

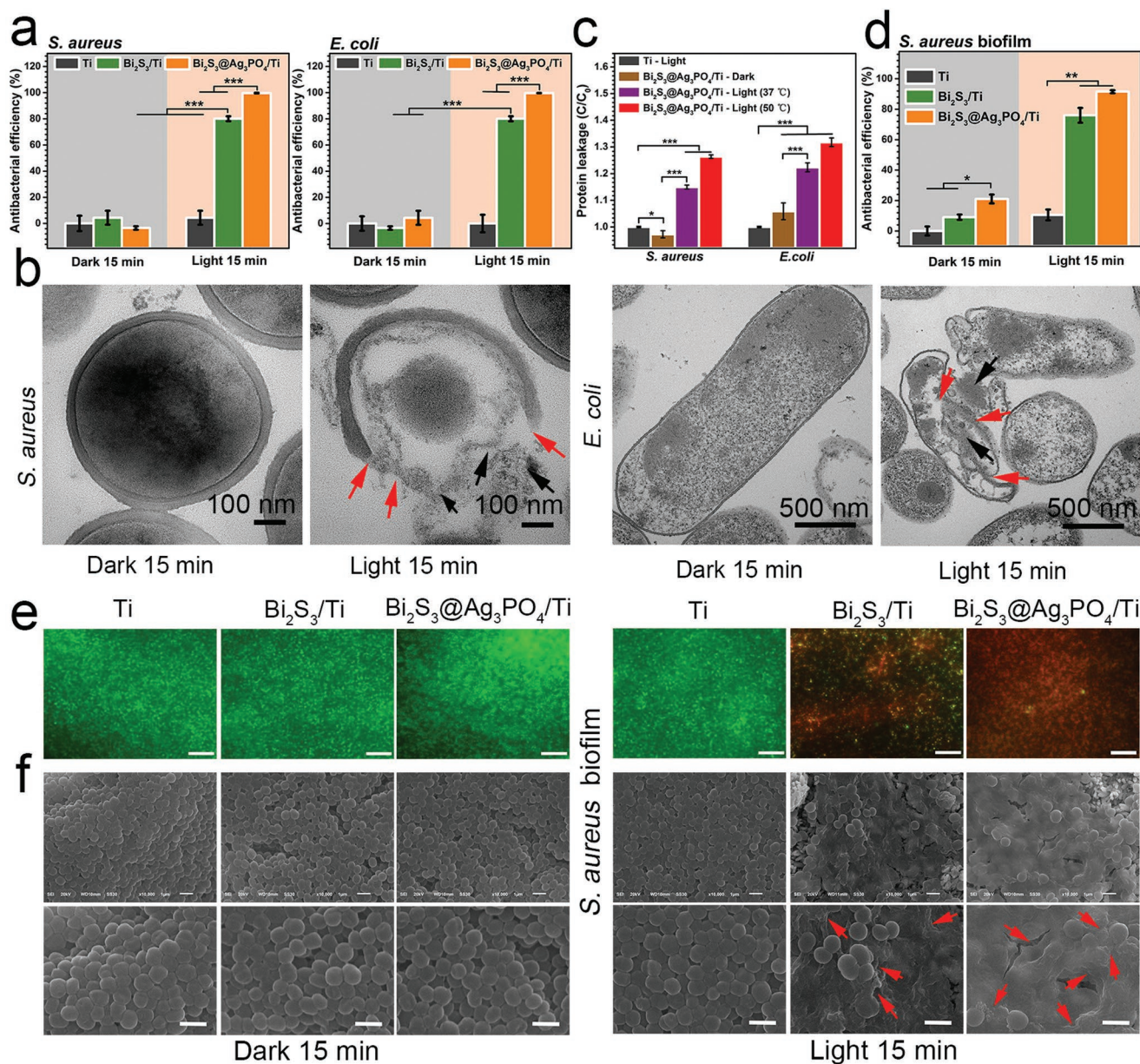


Figure 4. Antibacterial properties analysis in vitro. a) Antibacterial efficiency against *S. aureus* and *E. coli*. b) Protein leakage (OD₅₆₂). c) TEM images of the bacterial sections. d) Antibiofilm efficiency of the samples. e) Live/dead (green/red) staining fluorescence images (scale bar, 20 μm) and f) corresponding SEM images (scale bar, 1 μm) of *S. aureus* biofilm. The error bars represent means ± SD, *n* = 3: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

of the broad-spectrum bactericidal properties of released Ag⁺. In addition, as shown in Figure S7d (Supporting Information), both Ti and Bi₂S₃@Ag₃PO₄/Ti at 50 °C for 15 min in the absence of light (the temperature was on account of an extra water bath) showed antibacterial efficiency below 20%, indicating that heat-accelerated release of Ag ions did not kill bacteria alone effectively within 15 min.

ROS destroyed bacteria cell membrane structures by interacting with organisms in the bacteria and then penetrating the cell membrane, leading to gradual oxidative damage and leakage of intracellular substances, especially proteins and DNA, thus killing bacteria completely.^[29a] TEM was employed to observe the membrane damage and intracellular bacteria structural change. As illustrated in Figure 4b, both *S. aureus*

and *E. coli* in the dark group (Bi₂S₃@Ag₃PO₄/Ti, dark 15 min) remained in normal morphology with integrated intracellular substrate and smooth membranes, while the light group (Bi₂S₃@Ag₃PO₄/Ti, light 15 min) were distorted or obviously broken (red arrows), and even suffered loss of cytoplasmic or intracellular bacteria proteins.

Figure 4c shows the protein leakage compared to the light group of pure Ti (808 nm NIR, 37 °C, 15 min) from *S. aureus* and *E. coli* processed under different conditions. The dark group of Bi₂S₃@Ag₃PO₄/Ti displayed almost no difference from the control group, which meant the structure and composition of Bi₂S₃@Ag₃PO₄/Ti could not cause structural damage to bacteria in a short time. The light group of Bi₂S₃@Ag₃PO₄/Ti (37 °C, 15 min, the temperature was because of the power density

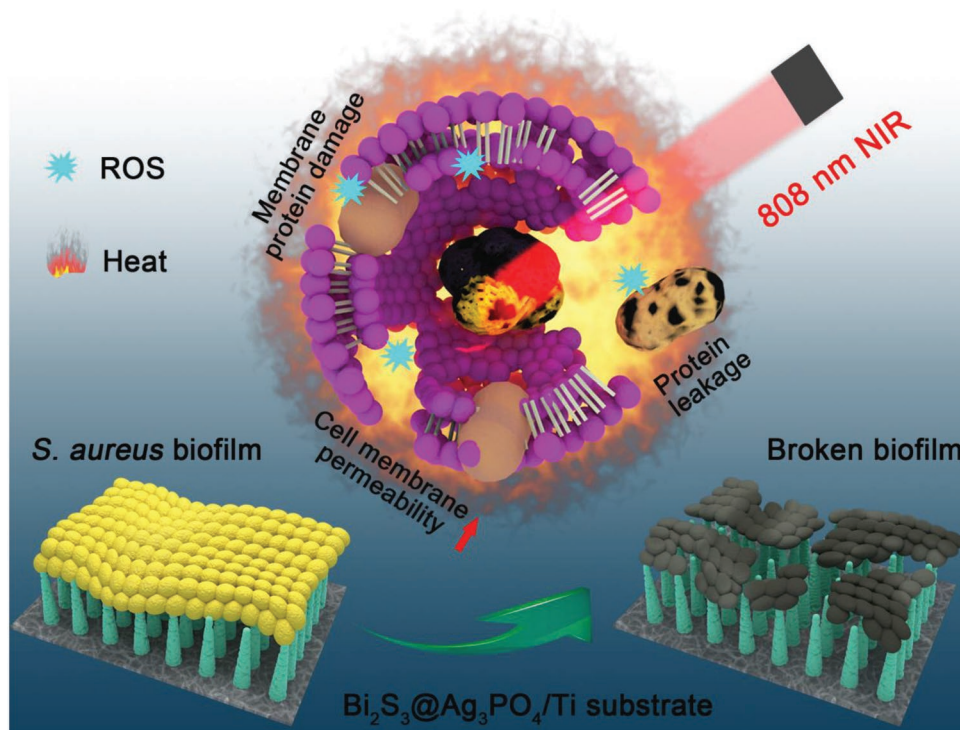
of the 808 nm NIR laser and an extra water bath) exhibited 1.15-fold protein leakage of *S. aureus* compared to the control group while $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ (50 °C, 15 min, the temperature was depended on the power density of the 808 nm NIR laser) showed that of 1.28-fold. Similar phenomena had been observed in *E. coli*. The results of protein leakage illustrated that the photothermal effect achieved sterilization by affecting the permeability of cell membranes, thus accelerating the ROS sterilization rate.

The results of antimicrobial tests against *S. aureus* and *E. coli* showed that $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ had a good germicidal effect on normal bacteria in orthopedic infections. We then further studied the effect on elimination of biofilm, a more lethal infection in bone-implant-related surgery. Although Ag^+ released from $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ eliminated most normal bacteria within 24 h, *S. aureus* biofilm still formed on the surface of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ in a high concentration bacterial solution, which is difficult to eliminate in traditional ways.^[33] The viability of *S. aureus* biofilm was evaluated by spread plate and the qualitative live/dead staining assay. As shown in Figure S7e (Supporting Information) and Figure 4d–f, all samples including $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited no obvious antibiofilm effects in darkness. In contrast, after 15 min NIR light irradiation, both $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ displayed obvious antibiofilm efficacy, especially for the latter, which owned 91.54% eradication of *S. aureus* biofilm. The fluorescence images of the dark group showed the green *S. aureus* (Figure 4e), indicating no obvious toxicity of samples including $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$. *S. aureus* biofilm easily formed on the samples in the absence of light; however, after 15 min 808 nm NIR light irradiation, the $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ samples displayed both green

and red in different ratios, whereas pure Ti remained green. In addition, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ showed more red fluorescence than $\text{Bi}_2\text{S}_3/\text{Ti}$. These results suggested that $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ had the best antibiofilm performance due to the synergistic action of photothermal effect and enhanced photocatalytic properties.

SEM was conducted to observe the morphology of biofilm and investigate the biofilm elimination mechanism. As shown in Figure 4f, the *S. aureus* biofilm, on the surfaces of the unilluminated Ti, $\text{Bi}_2\text{S}_3/\text{Ti}$, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ and illuminated Ti, showed dense and intact cell walls with smooth surfaces. These meant that none of these samples including $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ could inhibit the formation of *S. aureus* biofilm by themselves in the dark. In contrast, after exposure to NIR for 15 min, some of the cell walls were wrinkled and damaged (red arrow) on the surface of $\text{Bi}_2\text{S}_3/\text{Ti}$ while the *S. aureus* biofilm on $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ was almost wholly destroyed with several layers of collapsed, broken bacteria.

Based on the results above, the elimination mechanism of biofilm is proposed in Scheme 1. After 48 h coculture of samples and a high concentration bacterial solution, a mature *S. aureus* biofilm was formed on the surfaces of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$. Under 808 nm NIR irradiation, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ generated ROS and local heat (50 °C) simultaneously. The hyperthermia produced by the coating under NIR increased the bacteria cell membrane permeability and caused membrane protein damage, accelerating ROS penetration into the cell membranes, which led to bacterial metabolic disorders and protein leakage. Suffering from the synergy of PDT and PTT, the inactivated bacteria increased as the time of illumination increases. After 15 min of phototherapy, the entire biofilm surface was broken.



Scheme 1. The antibiofilm mechanism diagram of PDT and PTT induced by $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ under 808 nm NIR light illumination toward bacteria.

2.5. In Vitro Cell Activity

Fluorescein isothiocyanate (FITC) (green) and 4',6-diamidino-2-phenylindole (DAPI) (blue) stains were used to visualize initial cell adhesion and spreading activity of cells qualitatively cocultured with different samples after 24 h. As shown in Figure 5a, $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited higher adhesion than pure Ti. The number and spreading area of cells on the surface of pure Ti was less than that of $\text{Bi}_2\text{S}_3/\text{Ti}$. Cells on $\text{Bi}_2\text{S}_3/\text{Ti}$ extended a large number of fine filamentous pseudopodia, and the actin stress fibers had extended the complete cell morphology, while those on pure Ti were slender and spindle-shaped. Cells on $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ showed a lower viability and spreading area than $\text{Bi}_2\text{S}_3/\text{Ti}$, owing to the cytotoxicity of a small amount of Ag^+ released from Ag_3PO_4 to the cells, but it still exhibited good biocompatibility compared to pure Ti.

The methyl thiazolyl tetrazolium (MTT) method was used for quantitative analysis of sample biocompatibility. As seen in Figure 5b, $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited better biocompatibility than pure Ti after coculturing for 1, 3, and 7 d. Specifically, after coculturing for 1 d, the optical density (OD) of $\text{Bi}_2\text{S}_3/\text{Ti}$ reached 1.2, and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ also had an OD value of 0.99 which was 0.29 higher than pure Ti. However, the cell density of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ decreased to 0.93 after 3 d culturing, while pure Ti was 0.89, which was ascribed to the toxicity of the trace amount of Ag^+ released from $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$. However, the biological activity of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ was still superior to pure Ti after 7 d. Although Ag_3PO_4 NPs alone exhibited some toxicity due to the release of Ag^+ ,^[35] the strong electrostatic adsorption between Bi_2S_3 and Ag_3PO_4 suppressed the leaching of Ag^+ from $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$, which reduced the cytotoxicity of composite coatings. Meanwhile, Bi_2S_3 exhibited good biocompatibility, no residues in the organism were

reported,^[36] and it has been widely applied in the biomedical field.^[13] In this work, the in vitro tests also demonstrated that $\text{Bi}_2\text{S}_3/\text{Ti}$ showed good cell viability (see Figure 5a,b). Therefore, the excellent biocompatibility of Bi_2S_3 could offset the adverse effects of possible release of trace Ag^+ amounts. The impact of NIR light on cell activities is shown in Figure S8 (Supporting Information). After 15 min of 808 nm NIR light illumination, followed by 1 d culturing, the cells on $\text{Bi}_2\text{S}_3/\text{Ti}$ exhibited similar viability to those on Ti plates, indicating that the excellent biocompatibility of $\text{Bi}_2\text{S}_3/\text{Ti}$ can offset the adverse effects caused by lower photothermal effect and less ROS production. However, under the same conditions, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ cell density decreased significantly, which was ascribed to the photothermal effect and a higher ROS yield compared to $\text{Bi}_2\text{S}_3/\text{Ti}$, as well as the released Ag ions. However, after 3 and 7 d of coculturing, both cells cocultured with $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ became normal, indicating that the effects of NIR light on cell viability could be ignored after recovery with a long period of incubation.

As shown in Figure 5c, both $\text{Bi}_2\text{S}_3/\text{Ti}$ and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited promoted alkaline phosphatase (ALP) activity compared to pure Ti. In addition, the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ showed the highest ALP expression because of the stimulus of the structure of nanorod arrays and moderate number of Ag ions. It has been reported that nanorod array structure can influence osteoblast behaviors.^[37] Furthermore, a trace amount of Ag^+ can also help to influence osteoblast behaviors to promote osteoblast differentiation.^[38] The extent of extracellular matrix (ECM) mineralization was evaluated to further analyze osteogenic differentiation after 10 d culturing in osteogenic medium extracts. Quantitative measurement of mineralization (Figure 5d) showed the highest mineralization levels in cells on $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$, which was in accordance with Figure 5c.

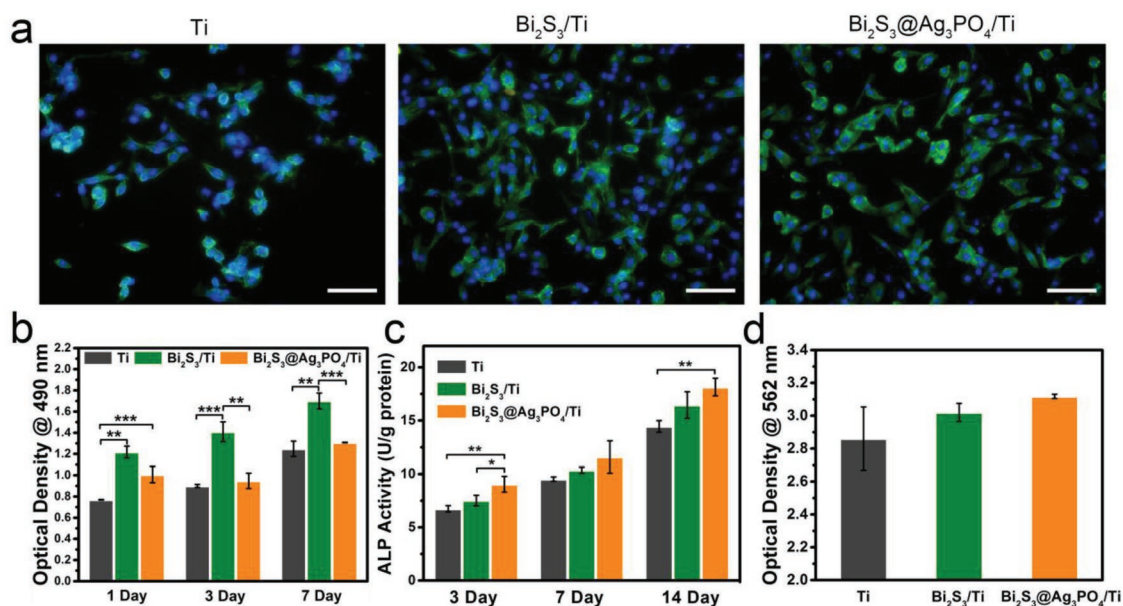


Figure 5. a) Fluorescence microscopy images of MC3T3-E1 cells cocultured with different samples after 24 h. The cytoskeletons were stained with FITC (green) and the nuclei were stained with DAPI (blue) (scale bar, 100 μm). b) MTT assay and c) ALP activity. d) Quantitative measurement of the Alizarin Red staining after 10 d coculture. The error bars represent means $\pm$ SD, $n = 3$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

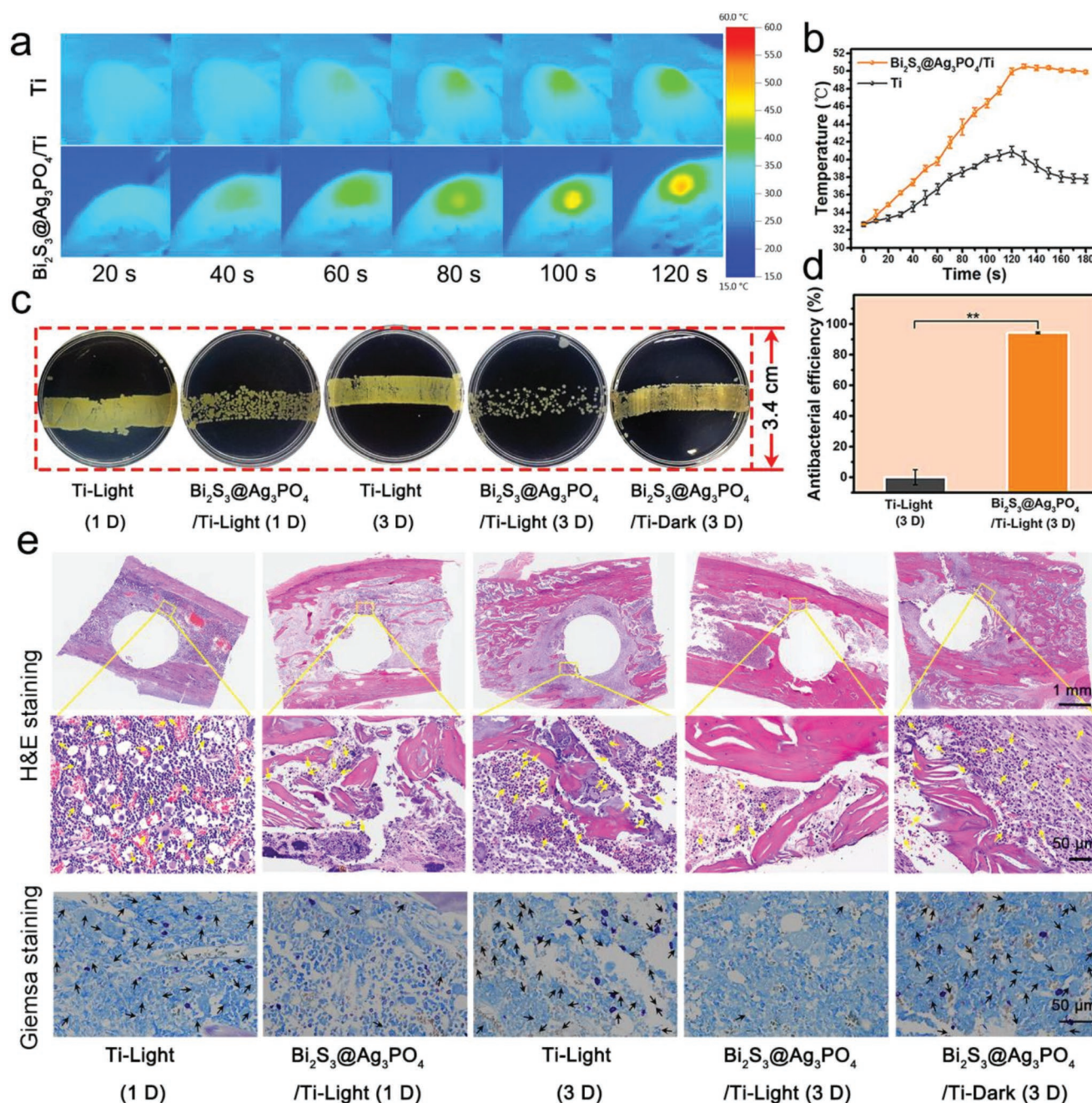


Figure 6. a) In situ thermal images and b) temperature changes of Ti and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ in vivo irradiated with 808 nm NIR light (the error bars represent means $\pm$ SD, $n = 3$). c) Photograph of bacteria colonies. d) Antibiofilm efficiency of Ti-light (3D) and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light (3D) in vivo in rats after 15 min 808 nm NIR light illumination followed by 3 d feeding, the error bars represent means $\pm$ SD, $n = 4$; ** $p < 0.01$). e) H&E and Giemsa staining images showing bone tissue infection (neutrophil infiltration and necrotic cells were indicated by yellow arrows, bacteria were indicated by black arrows).

2.6. In Vivo Biofilm Elimination

Considering the good biocompatibility and excellent sterilization ability of the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ in vitro, we performed an experiment with *S. aureus* biofilm-infected rats as the model to evaluate antibiofilm efficacy in vivo. **Figure 6a** shows the evolution of in situ photothermal images with irradiation time. Obviously, the temperature of the implant site with $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ rods increased rapidly with the irradiation time while the

pure Ti group showed little change, which was similar to the results in vitro (Figure S4, Supporting Information). As shown in Figure 6b, after 2 min NIR light (808 nm, 1.5 W cm^{-2}) illumination, the temperature increased from 32 to 50 °C, and then maintained at about 50 °C after turning down the light intensity to 1 W cm^{-2} . In contrast, the temperature of the pure Ti rod changed little (increased from 32 to 40 °C), and remained at 37 °C even with the same light conditions. These results illustrated that the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited good photothermal

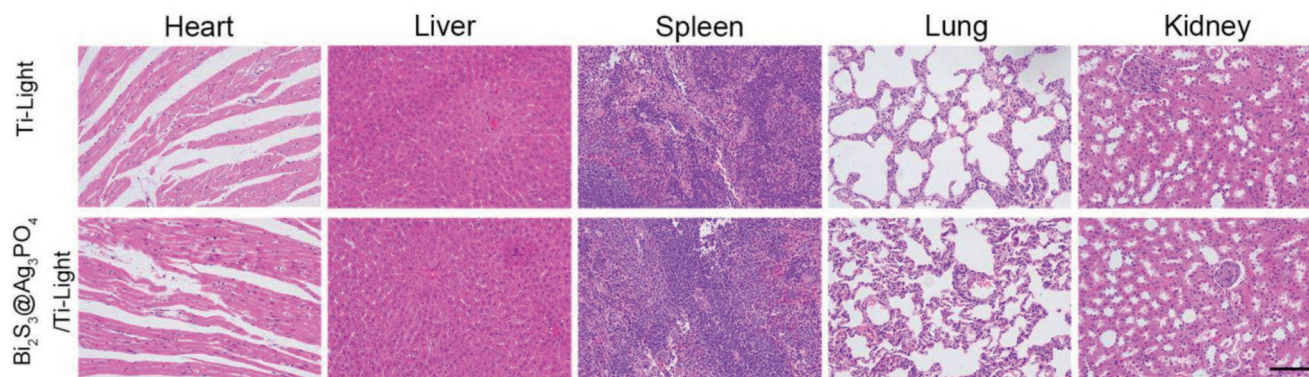


Figure 7. In vivo major organs (heart, liver, spleen, lung, and kidney) H&E staining of rats after 3 d after implant treatment by pure Ti and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ (scale bar, 100 μm).

effects in vivo thanks to the tissue penetration ability of an 808 nm laser NIR light. Figure 6c,d shows the antimicrobial results in vivo. The Ti-light group showed densely stacked colonies, while $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light exhibited sporadic sparse colonies on both the first and third days. It was noted that the bacterial survival rate in the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light group after 3 d decreased significantly compared to the first day. However, the 3 d plate for the $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -dark group showed no antibiofilm effect in vivo. It was deduced that the trace amount of Ag^+ released from $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ did not eliminate bacterial biofilm in vivo in the absence of light, which was consistent with those obtained in vitro. However, after 15 min 808 nm NIR light irradiation, *S. aureus* biofilm was broken, and then the released Ag^+ could collaborate with the immune system to kill the remaining bacteria. The 3 d antimicrobial efficiency calculated from the bacterial plate was 94.30%, which was higher than the one in vitro, because of the released Ag^+ from the coating and the immune system of the rats.

Hematoxylin and eosin (H&E) staining was conducted to observe histomorphological changes and evaluate skeletal tissue inflammation and in after phototherapy.^[39] As shown in Figure 6e, both the Ti-light (1D/3D) and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -dark (3D) groups exhibited a severe infection induced by biofilm, that is, a high neutrophil infiltration and necrotic cell exudation in H&E stained sections (indicated by yellow arrows). Quantification results of H&E staining in Figure S9 (Supporting Information) indicated inflammatory cell ratios of Ti-light (1D), Ti-light (3D), and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -dark (3D) were 29.00, 25.32, and 24.68%, respectively. However, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light (1D) showed lower inflammatory cell ratios of 8.13% because the biofilm was eliminated. Therefore, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ caused less damage to normal tissue and cells compared to pure Ti. In addition, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light (3D) showed the lowest inflammatory cell ratios of 5.53% in skeletal tissue, which indicated $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ alleviated tissue inflammation and normal tissues and cells were recovering from the damage of biofilm infections, which was similar to previous studies.^[1b,9] Giemsa-stained sections also indicated $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ -light (3D) host numerous bacteria (indicated by black arrows) on the bone tissues. The results showed that $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ also exhibited a high bactericidal effect in vivo with NIR light control. As shown in Figure 7, no abnormal effects or damage were observed during histological analysis of

the heart, liver, spleen, lung, and kidney of rats after 3 d implant treatment by pure Ti and $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$.

3. Conclusions

In summary, we developed an inorganic semiconductor heterojunction photocatalyst of $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4$ on a Ti implant that effectively eliminated biofilm within a short time using 808 nm NIR light. The hybrid coating devitalized bacteria rapidly because of the synergistic of photothermal and photocatalysis effect. Compared to $\text{Bi}_2\text{S}_3/\text{Ti}$, $\text{Bi}_2\text{S}_3@\text{Ag}_3\text{PO}_4/\text{Ti}$ exhibited higher photocatalytic performance and higher ROS yields. The introduction of Ag_3PO_4 NPs reduced the bandgap of $\text{Bi}_2\text{S}_3/\text{Ti}$ and promoted the separation of photogenerated carriers. Meanwhile, the enlarged surface area was beneficial for photocatalytic interface reactions. In addition, the coating exhibited good cell viability compared to pure Ti. By modifying the surface of bone implants with the NIR-activated nontoxic photocatalyst, we achieved rapid biofilm elimination in situ within 15 min. We hope this practicable strategy of antibiofilm coating might offer new insight on constructing NIR-activated photocatalysts, which will be applied clinically.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Bi₂S₃@Ag₃PO₄ nanorod arrays, biofilm elimination, bone implants, heterostructures, near-infrared photocatalysts

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