Supporting Information

Smart Photothermally-Activated Antibacterial Surfaces with Thermally-Triggered Bacteria-Releasing Properties

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S1 Experimental Section

S1.1 Pretreatment of Substrates

Gold-coated silicon wafers (80 nm of gold deposited on a 10 nm chromium adhesion layer, Guangzhou Institute of Semiconductor Materials, China) were cut into square chips of approximately 0.5 cm × 0.5 cm in size. The stainless steel chips (SS, 316 L, 12 mm in diameter) were obtained from Suzhou Nuder New Material Technology Co., Ltd. Poly(dimethylsiloxane) (PDMS) films were prepared using Sylgard silicon elastomer kit from Dow Corning (Midland, MI, USA) and cut into small disks (0.6 cm in diameter). The polycaprolactone (PCL, Mn = 80000, Sigma-Aldrich) electrospun nanofiber mat (PCL mat) was prepared using an electrospinning machine according to literature procedure.¹

The gold surfaces were washed with ethanol and cleaned by 30 min UV-ozone treatment. Then the surfaces were thoroughly immersed in a mixture solution of ammonia, hydrogen peroxide, and deionized water (NH₃·H₂O/H₂O₂/H₂O = 1:1:5, v/v/v) for 10 min at 75°C, rinsed with abundant deionized water and dried under a stream of nitrogen. The PDMS discs were immersed in a “piranha solution” (H₂SO₄:H₂O₂ = 7:3, v/v; caution: piranha solution reacts violently with organic materials and should be handled carefully!) for 30 s at 40°C and then thoroughly rinsed with deionized water and dried in a vacuum oven. The cleaned SS chips were treated with a “piranha solution” for 30 min at room temperature and then thoroughly rinsed with deionized water and dried under a stream of nitrogen. The PCL mats were cleaned by deionized water and ethanol and then dried under a stream of nitrogen.
S1.2 Surface Characterization

The chemical composition of the sample surfaces was determined using an ESCALAB 250 Xi X-ray photoelectron spectrometer (XPS, Thermo Scientific, USA). Thicknesses of TA/Fe complex layer and PNIPAAm layer of Au-TA/Fe and Au-TA/Fe-PNIP surfaces were measured using an M-88 spectroscopic ellipsometer (J. A. Woollam Co., Inc.). Six replicates were measured for each surface. The surface morphology of the sample surfaces was observed using atomic force microscopy (AFM, Bruker multimode 8system, Bruker Co., Ltd.) and scanning electron microscopy (SEM, S-4700, Hitachi). The water contact angles of sample surfaces were measured at 25°C and 40°C with an SL200C optical contact angle meter (Solon Information Technology Co., Ltd.). Six replicates were measured for each surface.

S1.3 Photothermal Properties of Surfaces

A continuous-wave NIR laser (808 nm, Hi-Tech Optoelectronics Co., Ltd.) was used in all experiments. To determine the photothermal properties of the sample surfaces, completely dry samples or samples immersed in PBS were exposed to NIR laser at different power densities for desired times, and the surface temperature was recorded using an infrared thermal imaging camera (FOTRIC 225-4, FOTRIC. Inc.).

S1.4 Bacterial Strains

*Escherichia coli* (E. coli, ATCC-700926) and *Methicillin resistant S. aureus* (MRSA, USA 3000) were used in our experiments. Prior to the experiments, the bacteria were incubated in Luria-Bertani broth medium (LB, Sigma-Aldrich) and tryptone soy broth (TSB, Solarbio), respectively, grown overnight under shaking at 37°C, and harvested
during the exponential growth phase via centrifugation. The supernatant was then discarded, and the cell pellet was re-suspended in PBS. The final concentration of bacteria was adjusted to approximately $1 \times 10^7$ cells/mL before use.

**S1.5 Live/Dead Staining Assay**

A standard live/dead staining assay was performed using LIVE/DEAD® BacLight™ Bacterial Viability Kits (Invitrogen, USA) to examine the viability of the bacteria attached on the sample surfaces. The staining solution contained a 1:1 mixture of Syto 9 (3.34 mM) and propidium iodide (PI, 20 mM). After bacterial culture and laser irradiation, 20 μL of a staining solution were dropped onto the surfaces. After incubating for 15 min in the dark, the surfaces were gently rinsed with sterile water and dried under a low-pressure stream of dry nitrogen. The bacteria attached to the surfaces were examined using a fluorescence microscope (IX71, Olympus, Japan) with a 40 × objective, and images of 15 randomly chosen fields of view were captured. For each type of surface, three replicates were examined, and the density of the adherent bacteria was analyzed using Image-Pro Plus software.

**S1.6 Colony Counting Assay**

Briefly, the surfaces were incubated in 0.5 mL of bacterial suspension (E. coli or MRSA, $1 \times 10^7$ cells/mL in PBS) at 37°C for 2 h. They were then gently rinsed with sterile water to remove loosely attached cells and salts. After completion of the attachment experiment, the sample surfaces were transferred to centrifuge tubes filled with PBS and then centrifuged at $5 \times 10^3$ rpm for 5 min to detach the attached cells from the surfaces. The PBS containing the detached cells were appropriately diluted with PBS
and placed on gelatinous Luria agar plates (Luria nutrient medium containing 1.5 wt% agar) and incubated at 37°C for 18 h. The number of viable cells was then determined in colony-forming units. Ideally, each surviving cell should develop into a distinct colony after incubation, thus providing a direct measure of bacterial viability.

**S1.7 Scanning Electron Microscopy**

To observe the morphologies of the attached bacteria, after bacterial attachment experiment and laser irradiation, the sample surfaces were gently rinsed with sterile water to remove unattached cells, fixed in 2.5% glutaraldehyde solution for 2 h, dehydrated in a series of ethanol solutions (30-100%), and air-dried. Before characterization, the samples were sputter coated with a 5-nm layer of gold. The bacteria attached on the surfaces were observed via scanning electron microscope (SEM, S-4700, Hitachi, Japan) at an accelerating voltage of 15.0 kV.

**S1.8 Statistical Analysis**

Each experiment was performed at least three times, and the data are expressed as the mean ± standard deviation (SD). Data were compared using Student’s t-test and p-values less than 0.05 were considered significant.
S2 Supporting Results

S2.1 Surfaces Characterization

Figure S1. Thickness of Au-TA/Fe surface with increasing deposition numbers of TA/Fe complex film. Data are presented as the mean ± SD (n = 3).

Figure S2. Thickness of PNIPAAm layer of Au-TA/Fe-PNIP surfaces grafted with PNIPAAm-NH₂ with different molecular weight. Data are presented as the mean ± SD (n = 3).
Figure S3. Surface temperature of Au surface and a series of Au-TA/Fe surfaces under NIR irradiation (2.2 W/cm²) in a dry state (I, II, III, IV and V means that the deposition number of TA/Fe complex film is 2, 4, 6, 8, and 10, respectively).

Figure S4. (a) Water contact angle of Au-TA/Fe-PNIP surfaces grafted with PNIPAAm-NH₂ with different molecular weight at 25°C and 40°C. Data are presented as the mean ± SD (n = 3). (b) Surface temperature of the Au-TA/Fe-PNIP surfaces grafted with PNIPAAm-NH₂ with different molecular weight under NIR irradiation (2.2 W/cm²) in the dry state.
**Figure S5.** XPS high-resolution (a) Fe$_{2p}$ spectrum of Au-TA/Fe surface and (b) N$_{1s}$ spectrum of Au-TA/Fe-PNIP surface.

**Table S1.** Elemental composition of Au-TA/Fe and Au-TA/Fe-PNIP surfaces

<table>
<thead>
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<th>Surface</th>
<th>Elemental composition (%)</th>
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<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Au-TA/Fe</td>
<td>64.0</td>
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<tr>
<td>Au-TA/Fe-PNIP</td>
<td>71.6</td>
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</table>

**Figure S6.** Root-mean-square (RMS) roughness values of different surfaces. Data are presented as the mean ± SD (n = 3).
Figure S7. (a) Changes in surface temperature of different surfaces under NIR irradiation (2.2 W/cm²) in the dry state. Corresponding thermal images are shown on the right. (b) Change in temperature of the Au-TA/Fe-PNIP surface under alternating ON/OFF NIR irradiation (2.2 W/cm²) in the dry state over five cycles. A single cycle consisted of irradiation for 3.5 min followed by cooling in room air.
**S2.2 Optimization of NIR Irradiation Condition**

**Figure S8.** Evaluation of bactericidal activity of the Au-TA/Fe-PNIP surfaces under NIR irradiation with different conditions. (a) Representative fluorescence images of attached bacteria (*E. coli*) on Au-TA/Fe-PNIP surfaces under NIR irradiation with different laser power density for 5 min. (b) Representative fluorescence images of attached bacteria (*E. coli*) under NIR irradiation (2.2 W/cm²) for different time. The attached bacteria were stained with a mixture of Syto 9 (green) and PI (red). The corresponding killing efficiencies of the surfaces are summarized in (c) and (d), respectively. Data are presented as the mean ± SD (*n* = 3).
S2.3 Biocidal Activity of Different Surfaces without NIR Irradiation

Figure S9. Representative fluorescence images of attached bacteria on different surfaces without NIR irradiation. The surfaces were incubated in suspensions of either *E. coli* or MRSA at 37°C for 2 h, followed by being stained with a mixture of Syto 9 (green) and PI (red).
S2.4 Storage Stability Test

**Figure S10.** Comparison of surface temperatures of the Au-TA/Fe-PNIP surface before and after storage in air or in PBS for 10 days under NIR irradiation (2.2 W/cm²) in the dry state.

**Figure S11.** Comparison of water contact angles at 25°C and 40°C of the Au-TA/Fe-PNIP surface before and after storage in air or in PBS for 10 days. Data are presented as the mean ± SD (n = 3).
**S2.5 Broad Applicability Test**

![Typical photographs of different substrates before and after modification.](image)

**Figure S12.** Typical photographs of different substrates before and after modification.

**Table S2.** Water contact angles at 25°C and 40°C and final surface temperatures after NIR irradiation (2.2 W/cm² for PDMS and SS; 0.9 W/cm² for PCL mat) in PBS for 2 min of different substrates coated with TA/Fe-PNIP hybrid films. Data are presented as the mean ± SD (n = 6).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Water contact angle (°)</th>
<th>Temperature (°C)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>40°C</td>
</tr>
<tr>
<td>PDMS</td>
<td>72.4 ± 2.0</td>
<td>99.2 ± 1.7</td>
</tr>
<tr>
<td>SS</td>
<td>13.4 ± 2.8</td>
<td>26.8 ± 2.9</td>
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| PCL mat   | N.D.                    | N.D.             | 47
Figure S13. Typical photographs of *E. coli* colonies re-incubated on agar plates after being detached from different substrates coated with TA/Fe-PNIP hybrid films with/without NIR irradiation (2.2 W/cm² for PDMS and SS; 0.9 W/cm² for PCL mat).

Figure S14. Representative fluorescence images of attached *E. coli* on different substrates coated with TA/Fe-PNIP hybrid films before and after release.

References