Electronic Supplementary Information

Regenerable Smart Antibacterial Surfaces: Fully Removal of Killed Bacteria via a Sequential Degradable Layer

Yangcui Qu,[a] Ting Wei,[a] Jian Zhao,[b] Shuaibing Jiang,[a] Peng Yang,[b]* Qian Yu,[a]* Hong Chen[a]

[a] State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, 199 Ren'ai Road, Suzhou, 215123, P. R. China

[b] Key Laboratory of Applied Surface and Colloids Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710119, China

*Corresponding author: Qian Yu, Peng Yang

E-mail address: yuqian@suda.edu.cn; yangpeng@snnu.edu.cn.

S1. Materials and Methods

Materials. Gold-coated silicon wafers (80 nm of gold deposited on a 10-nm chromium adhesion layer), silicon wafers [p-doped, (100)-oriented, 0.45 mm in thickness, 100 mm in diameter, one side polished, Guangzhou Institute of Semiconductor Materials, China] were cut into square chips of approximately 0.5 cm × 0.5 cm in size. Stainless steel chips (SS, 316 L, 12 mm in diameter) were obtained 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). Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 4H_2O$) and glucose were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Potassium hydrogen carbonate (AR) was from Shanghai Zhanyun Chemical Co. (Shanghai, China). β-cysteamine (C_2H_7NS, 95%) was from Sigma-Aldrich. Lysozyme (from hen egg white), tris (2-carboxyethyl) phosphine (TCEP) were purchased from Sigma. Vitamin C (Vc) was purchased from the Apollo Scientific. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, pH = 7.4, sterilized) was obtained from Solarbio. All other solvents, which were of analytical reagent grade, were from Sinopharm Chemical Reagent Co. (Shanghai, China). All aqueous solutions were prepared in 18.2 MΩ·cm purified water from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Escherichia coli (*E. coli,* ATCC-700926), *Staphylococcus aureus* (*S. aureus,* ATCC-6538), 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 nutrient bouillon medium (NB, Sigma-Aldrich), 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 1×10^7 cells/mL before use.

Surface pre-treatment. Briefly, gold-coated wafers were washed with acetone and then treated with ozone plasma for 30 min. After washing with deionized water and ethanol, the gold coated wafers were immersed in a mixture of ammonia ($NH_3 \cdot H_2O$), hydrogen peroxide (H_2O_2), and deionized water ($NH_3 \cdot H_2O : H_2O_2 : H_2O = 1:1:5$ v/v/v) for 10 min at 75 °C and then rinsed with deionized water and dried under nitrogen. The gold slides were immersed in a β-cysteamine solution (20 mM in ethanol) overnight at room temperature. The surfaces were then rinsed with ethanol and deionized water to remove physically adsorbed molecules, and dried under nitrogen to achieve amino-functionalized surfaces (Au-NH$_2$). The cleaned silicon wafers were first treated with a freshly prepared "piranha solution" ($H_2SO_4 : H_2O_2 = 7:3$, v/v; **Caution: *piranha solution reacts violently with organic materials and should be handled carefully!***) at 90 °C for 2 h, rinsed with abundant deionized water and dried under a stream of nitrogen. Then the freshly cleaned samples were immersed in an anhydrous toluene solution of 3-aminopropyltriethoxysilane (99%, APTES) (2% by volume) for 12 h at 80 °C, subsequently the samples were washed with toluene, acetone and water and dried under a stream of nitrogen to achieve amino-functionalized surfaces (Si-NH$_2$). The PDMS discs were immersed in a "piranha solution" for 30 s at 40 °C. After washing with deionized water and drying in a vacuum oven, the freshly prepared hydrophilic silanol-covered PDMS (PDMS-OH) discs were immersed in a solution containing APTES (5% by volume), deionized water (5% by volume) and absolute ethanol (90% by volume). After treatment at room temperature for 24 h, the discs were thoroughly rinsed with ethanol, toluene, and acetone, dried in a vacuum oven, and kept at 60 °C for 5 h to form the amino-terminated silanized surfaces (PDMS-NH$_2$). The cleaned SS chips were treated with a "piranha solution" for 30 min at room temperature and were then thoroughly rinsed with deionized water and

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dried under a stream of nitrogen. The chips were immersed in an anhydrous toluene solution of APTES (2% by volume) for 12 h at 80 °C, followed by being washed with toluene, water and acetone to remove the physic-attached APTES and being dried in nitrogen flow to achieve amino-functionalized SS surfaces (SS-NH$_2$).

Bactericidal assays. *Live/Dead staining assay:* A standard live/dead staining assay was performed using LIVE/DEAD® *BacLight*^{TM} 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 (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 ImageJ software (National Institutes of Health).

Scanning electron microscopy (SEM): 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* field emission scanning electron microscope (FESEM, S4700, Hitachi, Japan) at an accelerating voltage of 15.0 kV.

Colony counting assay: Briefly, the surfaces were incubated in 0.5 mL of bacterial suspension (*E. coli*

or *S. aureus* 1×10^7 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 5×10^3 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.

S2. Statistical Analysis

Each experiment was performed independently at least in duplicate and quantified at least in triplicate. The results are expressed as the mean ± standard error for each sample. The statistical analysis was performed using Origin Pro 8.6 software.

S3. Supporting Results

S3.1 Surface morphology
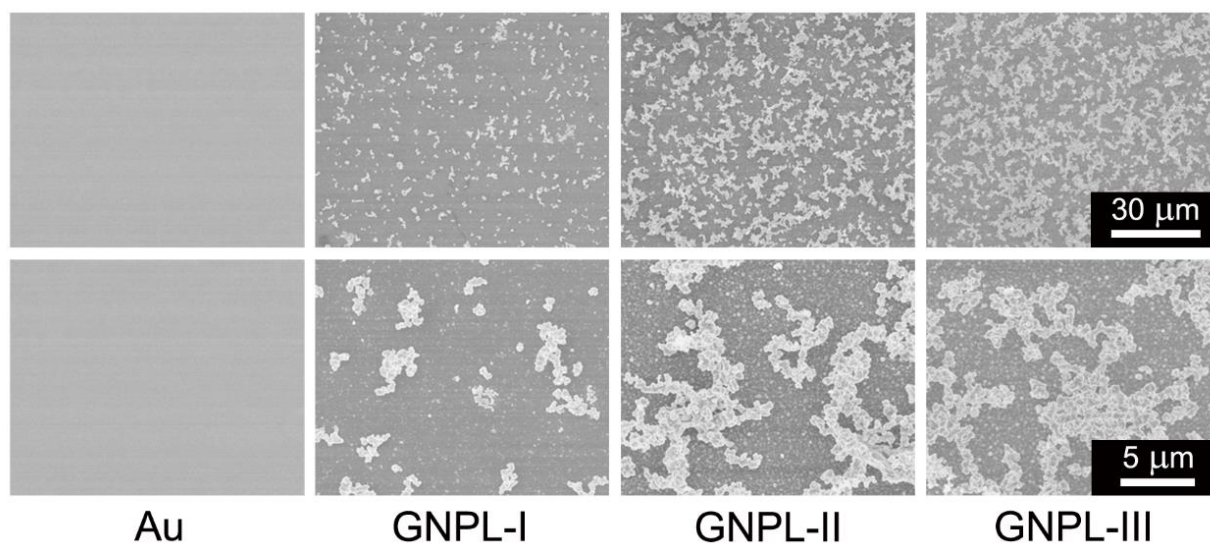
Fig. S1 Representative SEM images of unmodified Au surface and GNPL surfaces prepared using different volumes of GNP plating solution (I, II, and III means that the volume of GNP plating solution is 150 μL, 300 μL, and 500 μL, respectively).
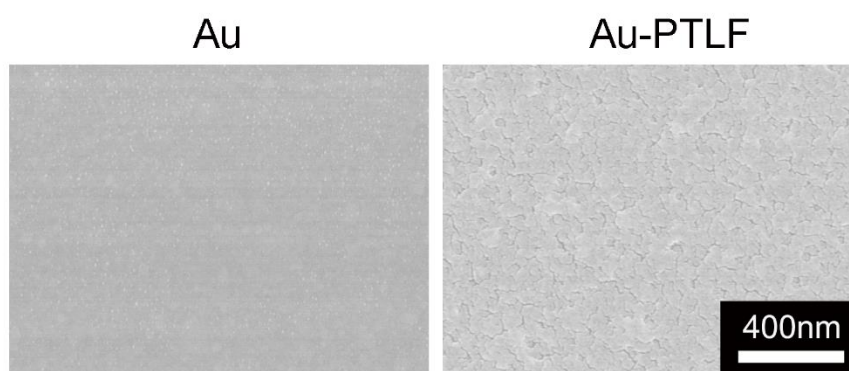

Fig. S2 Representative SEM images of Au surfaces before and after being coated with PTLF.

S3.2 Surface elemental compositions

Table S1 Elemental composition of a GNPL surface before and after being coated with PTLF.

Surface	Elemental composition (atom%)			
	Au	C	N	O
GNPL	37.0	42.7	6.0	14.3
GNPL-PTLF	N.D.	43.0	32.4	25.0

S3.3 Photothermal properties
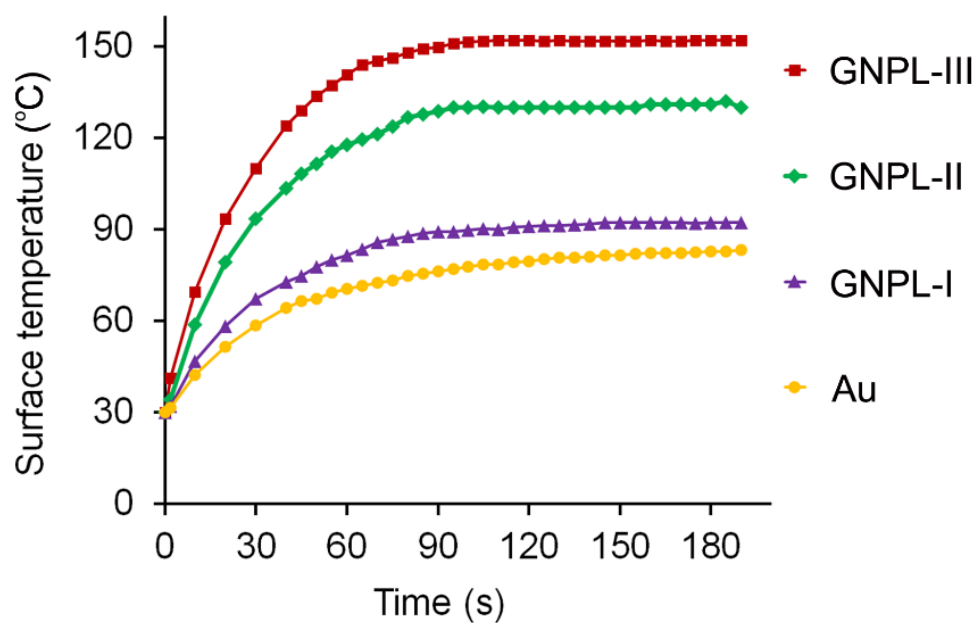
Fig. S3 Comparison of photothermal properties of a smooth gold surface and a series of GNPL surfaces prepared using different volumes of GNP plating solution under NIR laser irradiation (2.3 W/cm^2) in a dry state.

S3.4 Bactericidal activity evaluated via Live/Dead staining assay
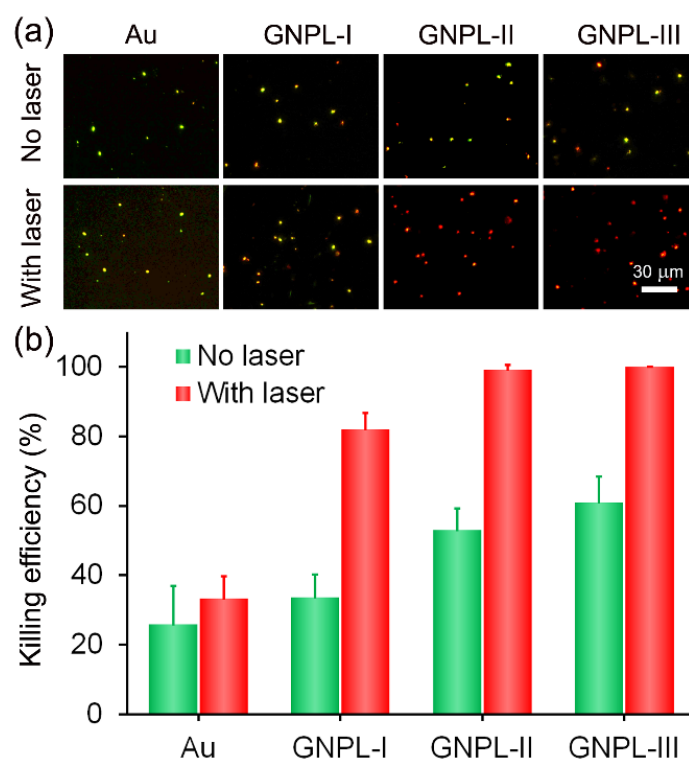
Fig. S4 Evaluation of bactericidal activity of different surfaces with/without NIR laser irradiation (2.3 W/cm^2, 5 min). (a) Representative fluorescence images of attached bacteria on different surfaces exposed to live/dead stains. The corresponding killing efficiency is shown in (b). Error bars represent the standard deviation of the mean ($n = 3$).
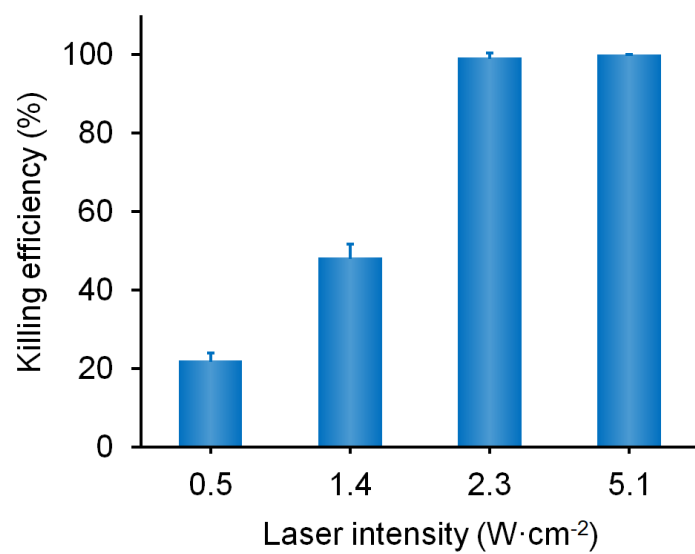
Fig. S5 Effect of laser power density on the photothermal biocidal activity of GNPL-PTLF surface against *E. coli*. The irradiation time is 5 min. Error bars represent the standard deviation of the mean (*n* = 3).

S3.5 Vc-induced degradation of PTLF
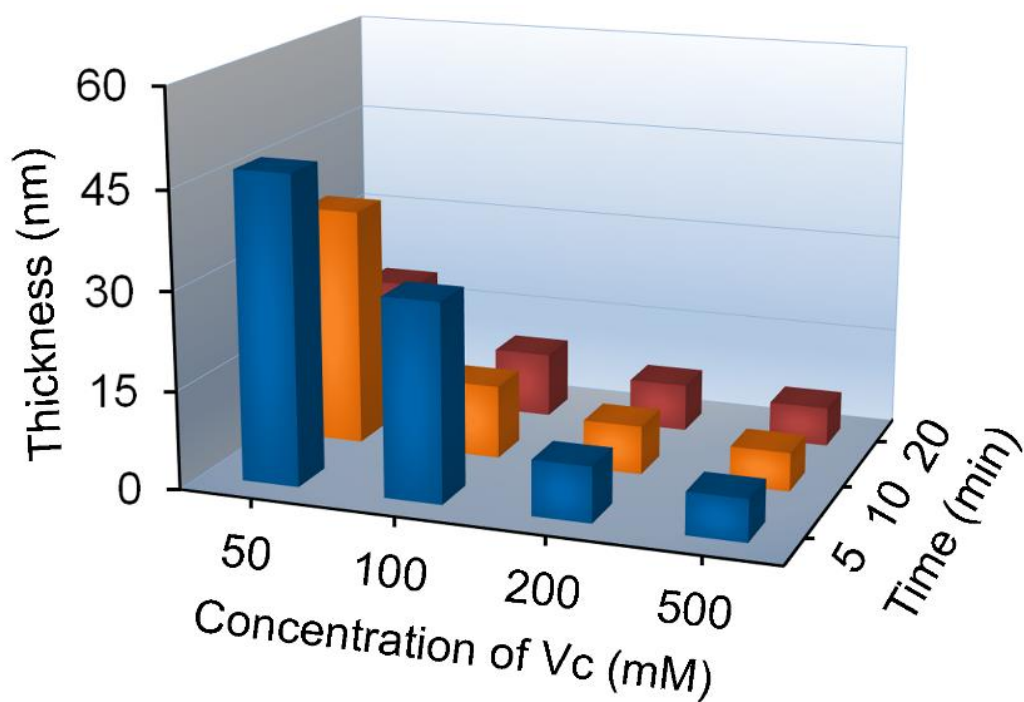
Fig. S6 Thicknesses of Au-PTLF surface after treatment of Vc solution with different concentration of

(50, 100, 200 and 500 mM) and different incubation time (5, 10 and 20 min).
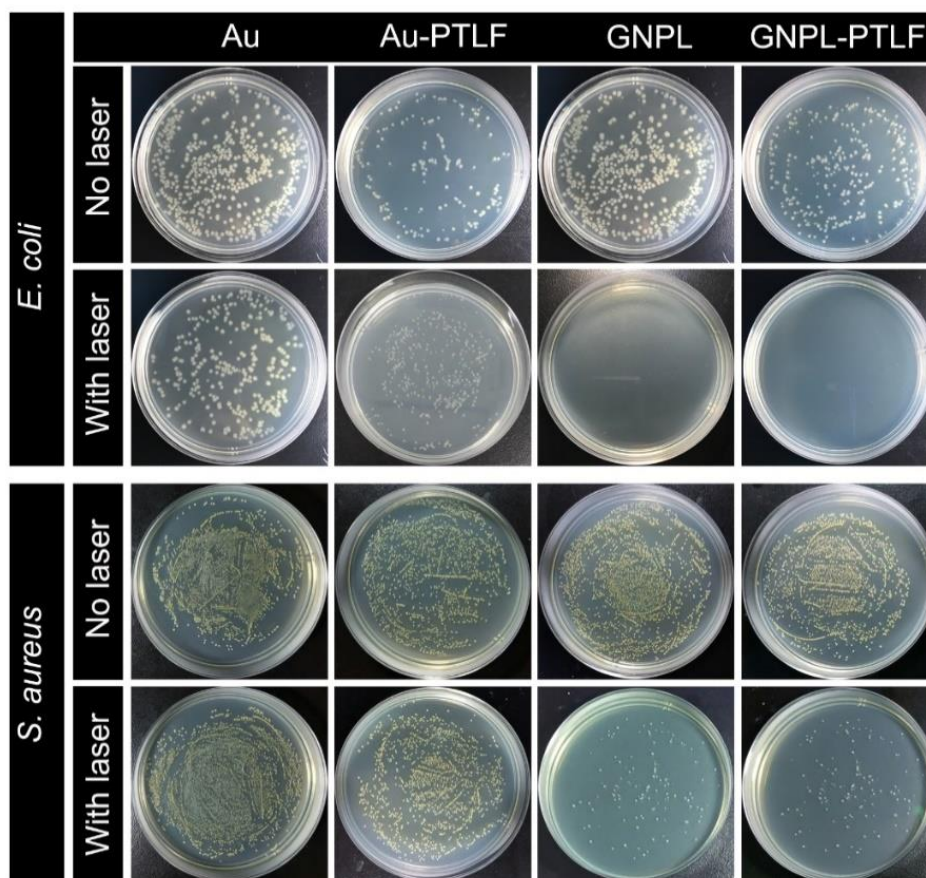
S3.6 Bactericidal activity evaluated via colony counting assay

Fig. S7 Typical visual images of bacterial colonies re-incubated on agar plates after being detached from surfaces with/without NIR laser irradiation (2.3 W/cm^2, 5 min).

S3.7 Vc-triggered bacteria-releasing abilities of GNPL-PTLF surface
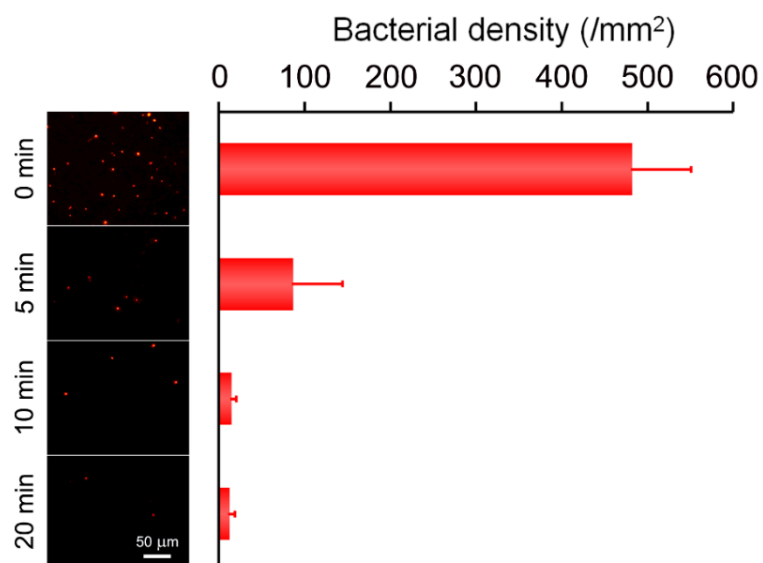
Fig. S8 Representative fluorescence images of attached bacteria of GNPL-PTLF surface before and after incubation in 100 mM Vc for 5, 10 and 20 min. The corresponding bacterial density is shown on right. Error bars represent the standard deviation of the mean ($n = 3$).
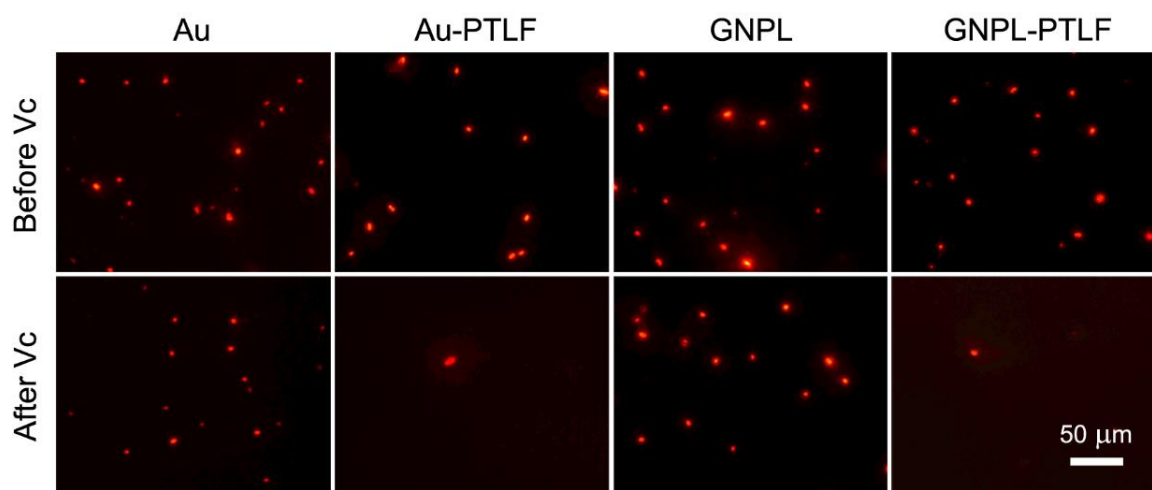

Fig. S9 Representative fluorescence images of attached bacteria on different surfaces before and after incubation in 100 mM Vc for 10 min.

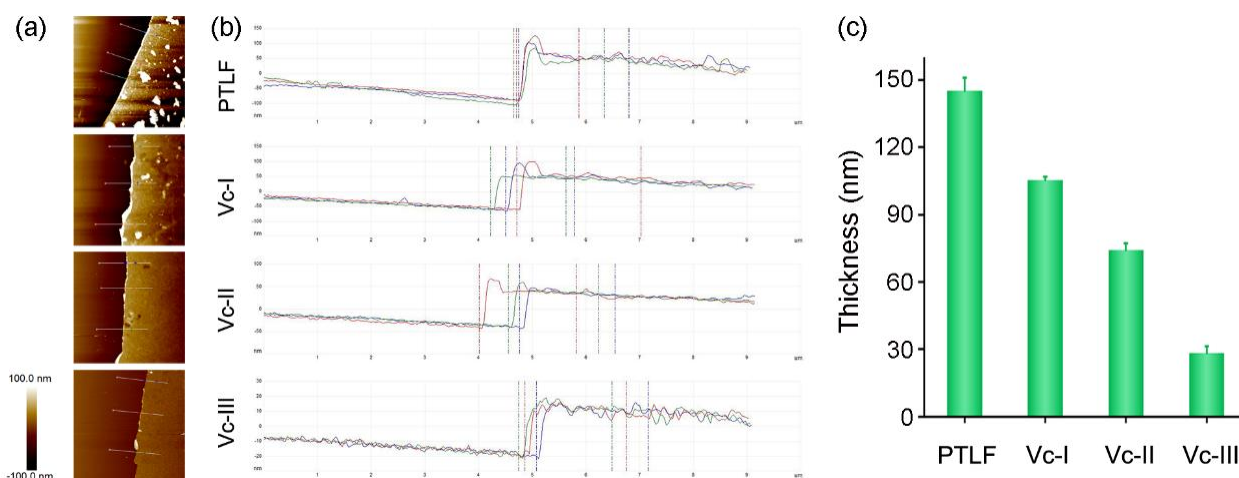## S3.8 Sequentially degradation of PTLF

Fig. S10 (a) AFM images of scratches on Au-PTLF surfaces before and after Vc treatment (where Vc-I, Vc-II, Vc-III represents Au-PTLF surfaces treated with (I) 50 mM Vc for 15 min, then treated with (II) 250 mM Vc for another 30 min, and finally treated with (III) 500 mM Vc for another 30 min, respectively). The corresponding section analysis and PTLF layer thickness is shown in (b) and (c), respectively. Error bars represent the standard deviation of the mean ($n = 3$).

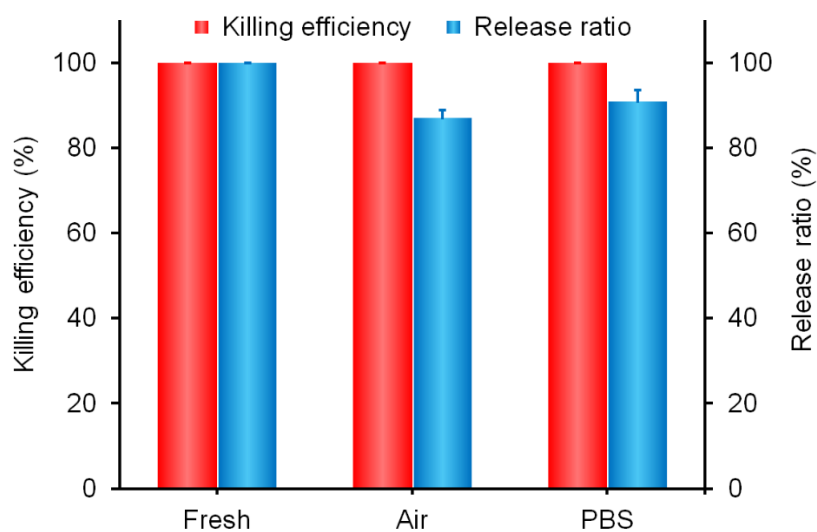**S3.9 Storage stability and long-term effectiveness of GNPL-PTLF surface**

Fig. S11 Comparison of killing efficiency and bacterial release ability of GNPL-PTLF surfaces against *E. coli* before and after storage in air or in PBS for 14 days. Error bars represent the standard deviation of the mean ($n = 3$).
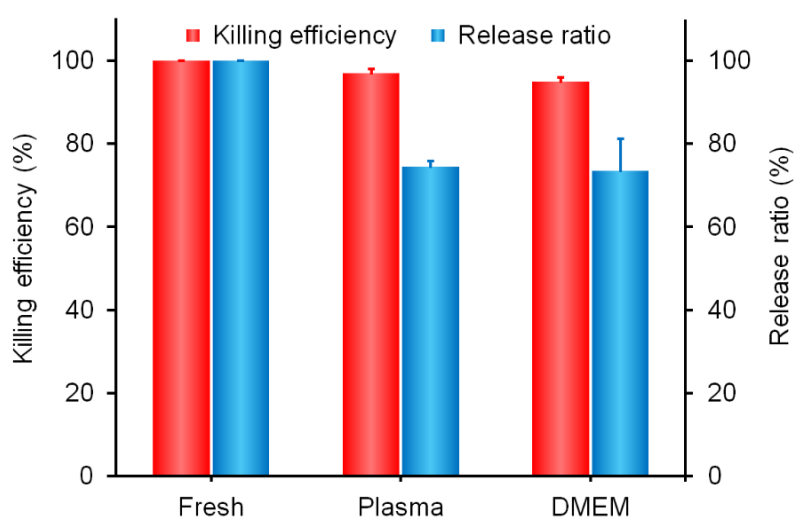

Fig. S12 Comparison of killing efficiency and bacterial release ability of GNPL-PTLF surfaces against *E. coli* before and after incubation in human plasma or in cell culture medium (DMEM) for 14 days. Error bars represent the standard deviation of the mean ($n = 3$).

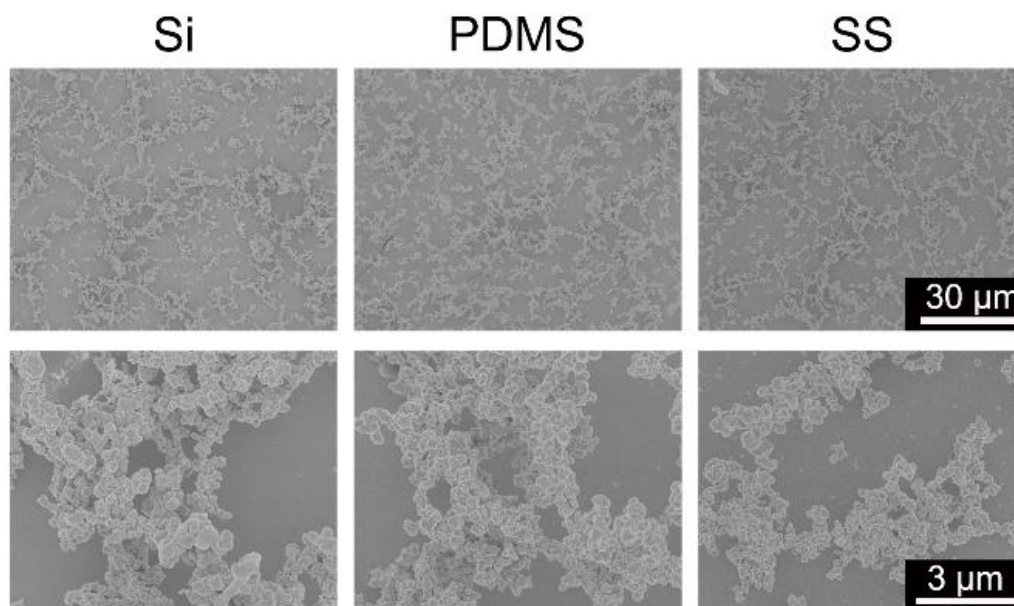**S3.10 Surface properties and antibacterial performances of GNPL and GNPL-PTLF surfaces on different substrates**

Fig. S13 Representative SEM images of different substrates coated with GNPL.

Table S2 Water contact angles of GNPL and GNPL-PTLF surfaces on different substrates. Data are mean ± standard error ($n = 6$).

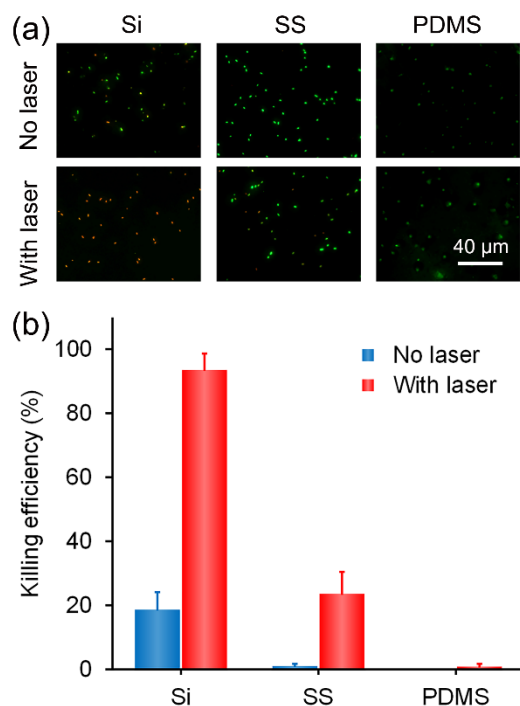| Substrate	Water contact angle (º)		
	Pristine	GNPL	GNPL-PTLF
Au	77.7 ± 1.1	135.0 ± 10.3	121.0 ± 1.5
Si	28.3 ± 3.2	113.9 ± 3.6	109.5 ± 1.1
PDMS	109.3 ± 1.7	145.1 ± 1.1	113.3 ± 5.8
SS	24.0 ± 1.7	85.2 ± 1.8	87.6 ± 9.9

Fig. S14 Evaluation of bactericidal activity of different substrates with/without NIR laser irradiation (2.3 W/cm², 5 min). (a) Representative fluorescence images of attached bacteria on different surfaces exposed to live/dead stains. The corresponding killing efficiency is shown in (b). Error bars represent the standard deviation of the mean ($n = 3$).
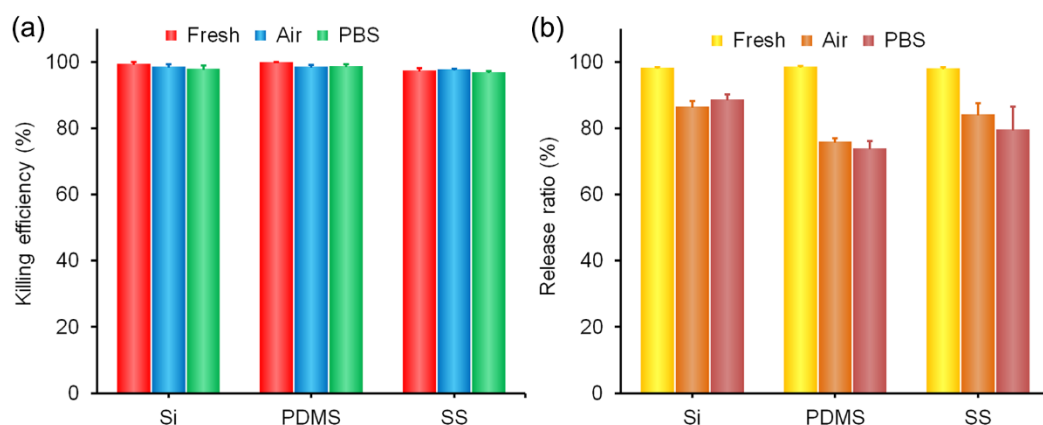

Fig. S15 Comparison of (a) killing efficiency and (b) bacterial release ability of different substrates coated with GNPL against *E. coli* before and after storage in air or in PBS for 14 days. Error bars represent the standard deviation of the mean ($n = 3$).
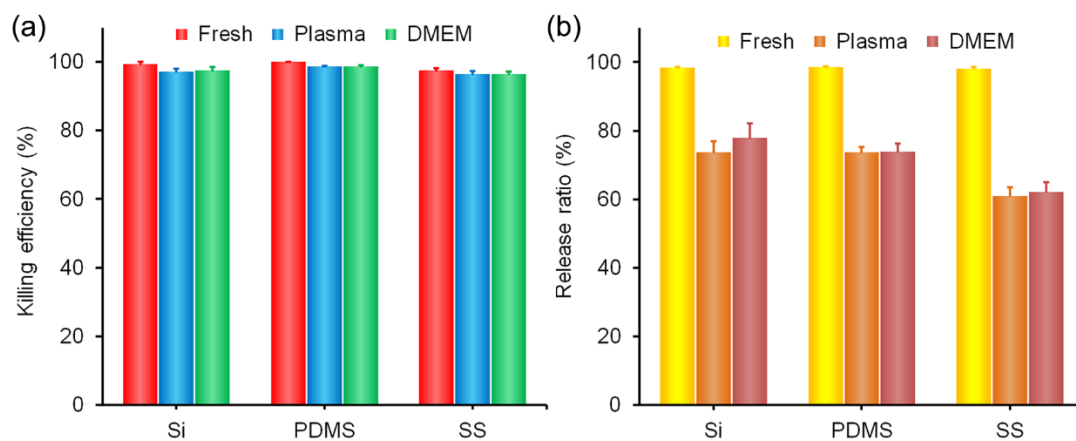
Fig. S16 Comparison of (a) killing efficiency and (b) bacterial release ability of different substrates coated with GNPL against *E. coli* before and after in human plasma or in cell culture medium (DMEM). Error bars represent the standard deviation of the mean ($n = 3$).