Electronic Supplementary Information for

Nanopatterned Antimicrobial Enzymatic Surfaces Combining Biocidal and Fouling Release Properties

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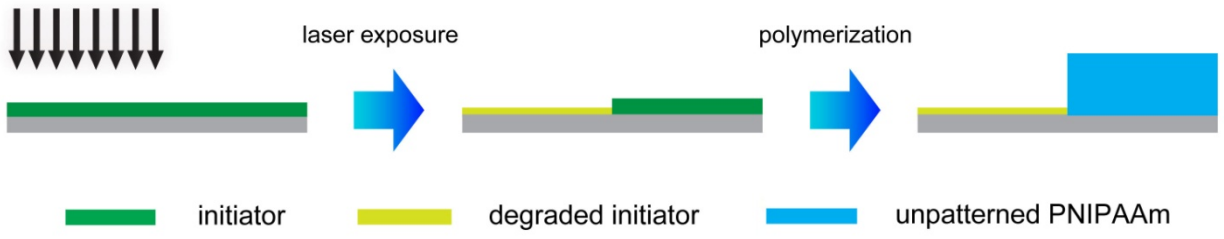
1. Materials and bacteria strains

N-isopropylacrylamide (NIPAAm), Cu(II)Br_2 (98% pure), 1,1,4,7,7--pentamethyldiethylenetriamine (PMDETA, 99% pure) and ascorbic acid (reagent grade, 20-200 mesh) were purchased from Sigma-Aldrich (St. Louis, MO). The monomer was recrystallized twice from a benzene/hexane mixture and then dried under vacuum before use. The atom transfer radical polymerization (ATRP) initiator (3-trimethoxysilyl) propyl 2-bromo 2-methylpropionate was purchased from Gelest (Morrisville, PA) and stored under dry condition until use. Silicon wafers and glass coverslips (size=25 × 50 mm, thickness=0.13 mm) were purchased from University Wafer and VWR, respectively. Unlabeled lysozyme and fluorescein isothiocyanate (FITC) -labeled lysozyme (FITC-lysozyme, 4 mg/mL in phosphate-buffered saline (PBS)) were obtained from Sigma-Aldrich (St. Louis, MO) and Nanocs (New York, NY), respectively, and stored at -20°C before use.

Escherichia coli K12 (ATCC 29425) and *Staphylococcus epidermidis* (ATCC 14990) were received as lyophilate from the American Type Culture Collection (Bethesda, MD) and stored as frozen stock aliquots in Difco nutrient broth (NB) +20% glycerol at -80°C. Experimental stock cultures were maintained on NB slants and were stored at 4°C for up to 2 weeks. A single colony from the slants was incubated in 50 mL of NB and grown overnight with shaking at 37°C. After growth, the bacterial culture was centrifuged at a relative centrifugal force of 11,952 × g for 10 min at 4°C. The pellet was then suspended in 0.85% NaCl (for *E. coli*) or phosphate buffered saline (PBS) (for *S. epidermidis*). This washing procedure was repeated twice. The final concentrations of *E. coli* and *S. epidermidis* were $\sim 1 \times 10^8$ cells/mL and $\sim 3 \times 10^7$ cells/mL, respectively, as measured using a hemocytometer (C-chip CYTO Corp, Sunnyvale, CA) and phase contrast microscopy (Axioimager, Carl Zeiss Microimaging, Inc.) through a 40X objective.

2. Preparation of a macroscopically patterned surfaces

To examine the adsorption of lysozyme under conditions similar to those employed for backfilling, we prepared a sample in which half of the surface was degraded initiator and half was unpatterned PNIPAAm as shown in **Scheme S1**.



Scheme S1 Schematic depiction of the procedure for preparation of a surface composed of macroscopic areas with degraded initiator and unpatterned PNIPAAm.

3. X-ray photoelectron spectroscopy (XPS)

The elemental compositions of degraded initiator, unpatterned PNIPAAm, and nanopatterned PNIPAAm surfaces before and after adsorption of lysozyme were examined by XPS (see **Table S1**). After incubation in lysozyme solution (5 mg/mL in PBS) at 37°C for 2 h, we found (i) no obvious changes on unpatterned PNIPAAm surface due to the protein-resistance of PNIPAAm; (ii) appearance of nitrogen and marked changes of carbon, oxygen and silicon on degraded initiator surface, indicating the adsorption of lysozyme; (iii) slight increase of nitrogen and oxygen on the nanopatterned PNIPAAm surface, suggesting the adsorption of lysozyme.

Table S1 Comparison of elemental composition before and after adsorption of lysozyme. Data consist of the mean ± standard error (n=3).

Surface	Adsorption of Lysozyme	C (%)	N(%)	O(%)	Si(%)
Degraded initiator	before	27.8±0.5	N.D.	38.3±0.8	33.5±0.7
	after	43.5±1.2	9.1±0.6	23.0±0.5	24.1±0.8
Unpatterned PNIPAAm	before	76.9±0.8	12.2±0.6	10.7±0.3	N.D.
	after	76.5±0.4	12.3±0.5	10.9±0.7	N.D.
Nanopatterned	before	76.3±0.9	11.8±0.6	10.7±0.5	1.3±0.06

PNIPAAm	after	75.0±1.0	12.4±0.4	11.4±0.5	1.1±0.04

4. Wettability

To evaluate the wettability of the nanopatterned PNIPAAm surfaces before and after adsorption of lysozyme at different temperatures, the contact angle was measured in water at 25°C and at 45°C using the captive air bubble method (**Fig. S1**). The nanopatterned PNIPAAm surfaces exhibit higher hydrophobicity at 45°C, as do unpatterned PNIPAAm surfaces.[1] The introduction of lysozyme did not significantly alter the thermally responsive wettability of the PNIPAAm surface, suggesting that lysozyme is restricted primarily to the regions between PNIPAAm lines.

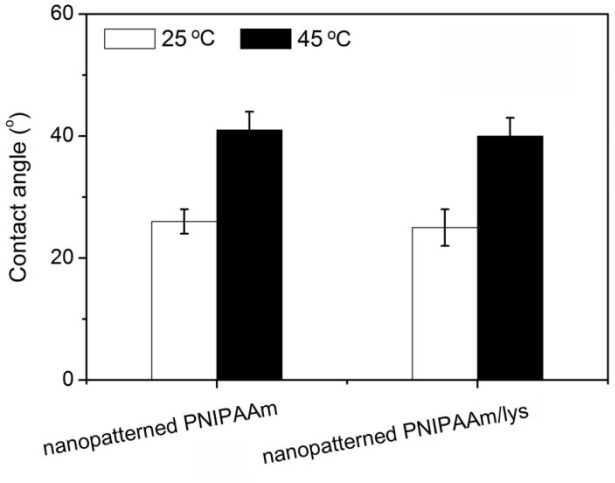


Fig. S1 Captive air bubble contact angles at 25°C and 45°C for nanopatterned PNIPAAm surfaces before and after adsorption of lysozyme. Data consist of the mean ±standard error (*n*=6)

5. Lysozyme adsorption at different temperatures

Fluorescence intensities after the adsorption of FITC-lysozyme on nanopatterned PNIPAAm and control surfaces (degraded initiator and unpatterned PNIPAAm) under identical conditions are summarized in **Fig. S2.** As the incubation temperature increases from 25°C to 37°C, the fluorescence intensity of nanopatterned PNIPAAm surfaces increases ~1.7 fold, while no significant changes in fluorescence intensity are observed on the control surfaces. The thermo-responsive protein adsorption behavior of nanopatterned PNIPAAm surface results from the conformational change of PNIPAAm chains as suggested by our previous report.[2]

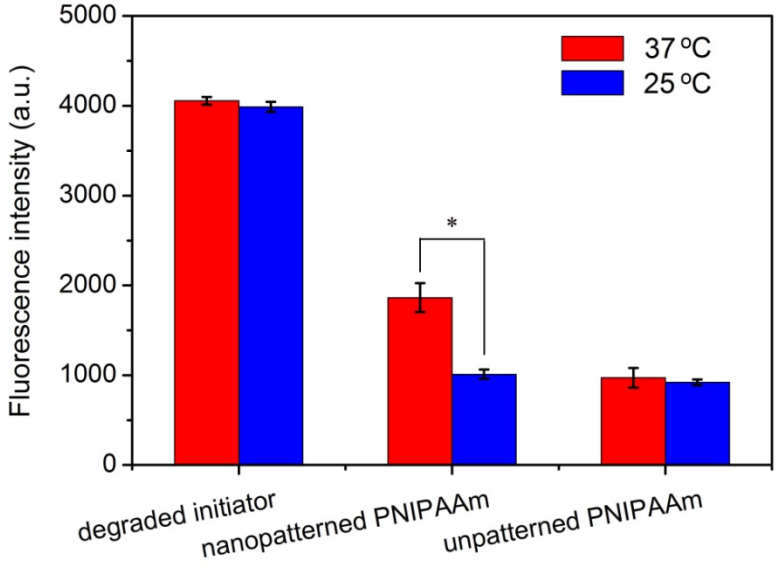


Fig. S2 Fluorescence intensity of the degraded initiator, nanopatterned PNIPAAm and unpatterned PNIPAAm surfaces after adsorption of FITC-lysozyme (1 mg/mL in PBS) at 37°C or 25°C for 2 h. Data consist of the mean ±standard error (n=6, * $p<0.05$)

6. Attachment, killing and release of bacteria on unpatterned PNIPAAm surfaces

We tested the attachment and release of bacteria and killing efficacy of unpatterned PNIPAAm surfaces (**Fig. S3**). The unpatterned PNIPAAm surfaces did not exhibit significant biocidal activity themselves (the killing efficiency is less than 10%), but did show bacterial release capability (the bacterial release ratio is 77.7±3.4% and 74.2±2.7% for *E. coli* and *S. epidermidis*, respectively).

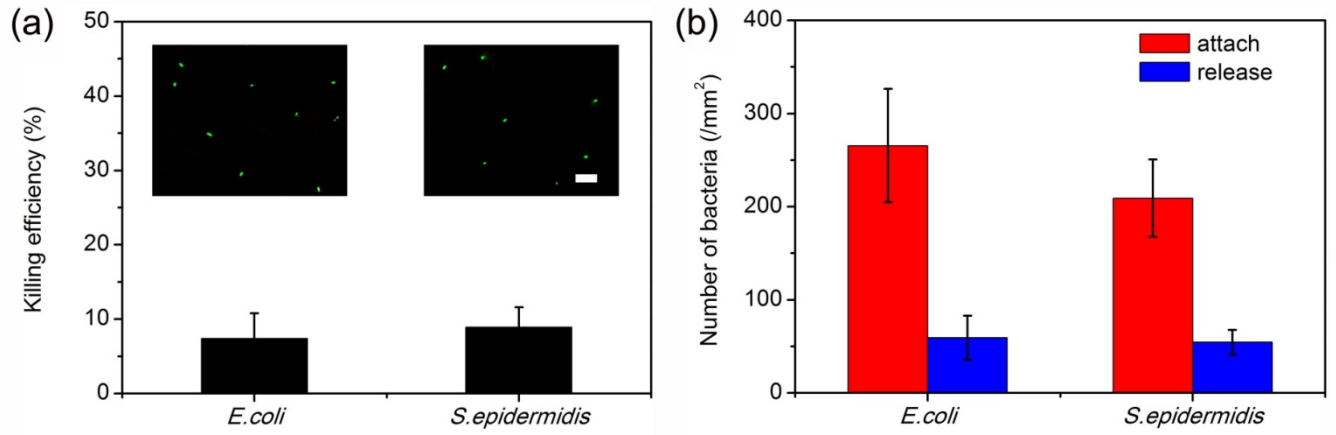


Fig. S3 (a) Killing efficiency of unpatterned PNIPAAm surfaces against *E. coli* and *S. epidermidis.* The surfaces were incubated in suspensions of *E. coli* (at 37°C) or *S. epidermidis* (25°C) for 2 h. The killing efficiency was defined as the ratio of amount of dead bacteria and amount of total bacteria attached on the surfaces at 37°C or 25°C. Typical fluorescence microscopy images of attached bacteria are shown as insets. Green staining indicates live bacteria, and red staining indicates dead bacteria. The scale bar is 10 μm. (b) Attachment and detachment of *E. coli* and *S. epidermidis* on unpatterned PNIPAAm surfaces. The surfaces were incubated in suspensions of *E. coli* at 37°C or *S. epidermidis* at 25°C for 2 h and the average number of attached cells was determined (attach). Then the surfaces were rinsed with 0.85% NaCl in water at 25°C (*E. coli*) or PBS at 37°C (*S. epidermidis*) and the remaining cells were counted (release). Error bars represent the standard deviation of the mean (n=3).

7. SEM study of sequential biocidal activity and fouling-release

We used SEM to observe the morphology of attached bacteria on the nanopatterned PNIPAAm/Lys surface before and after rinsing with cold water for two cycles (**Fig. S4**). After rinsing with 0.85% NaCl in water and ultrapure water at 25°C, there were still some bacteria and debris on the surface, which might have covered the underlying lysozyme and impeded the conformational change of PNIPAAm chains, leading to the degraded biocidal activity and bacterial release ability when the surface was used after several cycles.

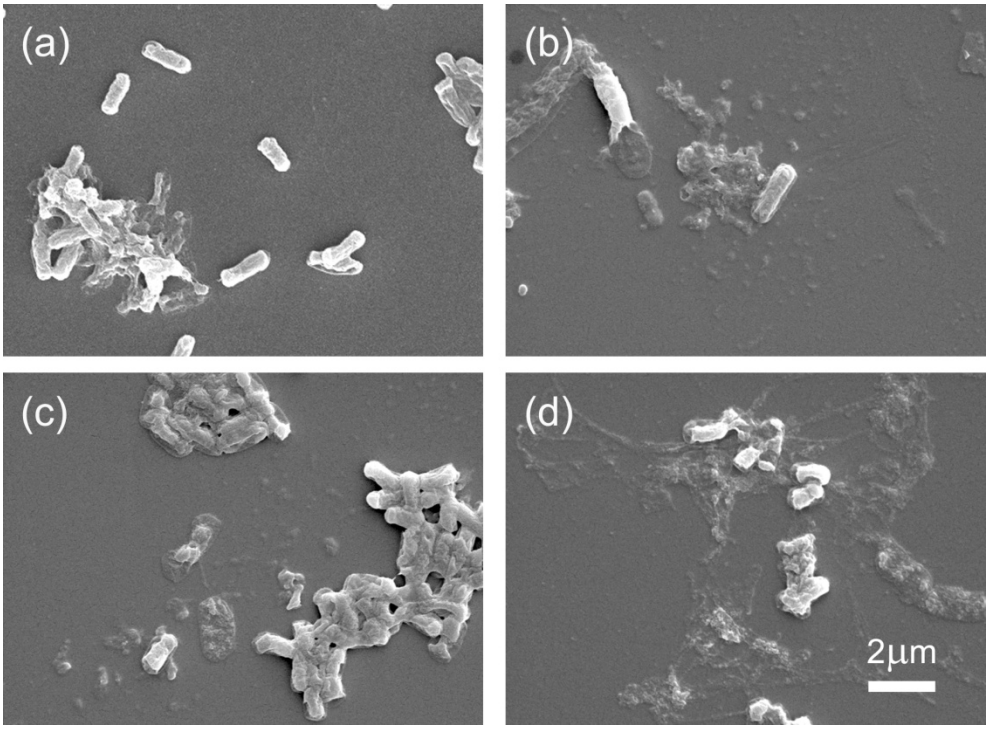


Fig. S4 SEM images of (a) attached *E. coli* on a nanopatterned PNIPAAm/Lys surface after incubation in suspensions of *E. coli* at 37 °C for 2 h; (b) remaining bacteria and debris on the same surface after rinsing with 0.85% aqueous NaCl and ultrapure water at 25°C; (c) attached *E. coli* on the same surface

after re-incubation in suspensions of *E. coli* at 37 °C for 2 h; and (d) remaining bacteria and debris on the same surface after re-rinsing with 0.85% aqueous NaCl and ultrapure water at 25°C.

References

1 Q. Yu, J. Cho, P. Shivapooja, L. K. Ista and G. P. Lopez, *ACS Appl. Mater. Interfaces,* 2013, **5**, 9295-9304.

2 Q. Yu, P. Shivapooja, L. M. Johnson, G. Tizazu, G. J. Leggett and G. P. Lopez, *Nanoscale,* 2013, **5**, 3632-3637.