On-Demand Removal of Bacterial Biofilms via Shape Memory Activation
Huan Gu,‡ Sang Won Lee,‡ Shelby Lois Buffington,‡ James H. Henderson,‡ and Dacheng Ren*‡§⊥∥

‡Department of Biomedical and Chemical Engineering, §Syracuse Biomaterials Institute, ∥Department of Civil and Environmental Engineering, and ⊥Department of Biology, Syracuse University, Syracuse, New York 13244, United States

Supporting Information

ABSTRACT: Bacterial biofilms are a major cause of chronic infections and biofouling; however, effective removal of established biofilms remains challenging. Here we report a new strategy for biofilm control using biocompatible shape memory polymers with defined surface topography. These surfaces can both prevent bacterial adhesion and remove established biofilms upon rapid shape change with moderate increase of temperature, thereby offering more prolonged antifouling properties. We demonstrate that this strategy can achieve a total reduction of Pseudomonas aeruginosa biofilms by 99.9% compared to the static flat control. It was also found effective against biofilms of Staphylococcus aureus and an uropathogenic strain of Escherichia coli.

KEYWORDS: surface topography, shape memory polymer, temperature-responsive material, biofilm, antibiofouling surfaces

Bacteria can colonize both biotic and abiotic surfaces and form biofilms that are multicellular structures with extracellular polymeric substrates secreted by the attached cells.1 Biofilm cells are difficult to eradicate compared to their planktonic counterparts due to enhanced resistance to antimicrobials and mechanical forces.2 The grand challenge of biofilms has motivated the search for new strategies for biofilm prevention and removal.3

In the past decade, surface topography has been well studied for its potential in controlling bacterial adhesion and biofilm formation.4−9 For example, topographic lines, static nanohair like structures and patterns arranged in diamond arrays have been embossed onto poly(dimethylsiloxane) (PDMS) surfaces to mimic cilia and the topographies on shark skin, respectively,7−9 for biofilm control. In addition to static features of surface chemistry and topography, bacterial adhesion can also be prevented by stimuli-responsive antifouling surfaces, such as those triggered by pH, temperature, salt concentration, electrical potential, light, magnetic field, and the surrounding media.10−14 Movements of or near the substrate surfaces such as these provide an additional level of control that can disperse attached cells.

Inspired by the natural systems and previous research,15 we aimed to engineer novel antifouling surfaces that can be programmed to remove well-established biofilms. We hypothesized that by changing the shape and dimension of topographic patterns, the biofilm structure can be disrupted, leading to biofilm dispersion. To test this hypothesis, we evaluated the effects of dynamic change in surface topography on biofilms formed on shape memory polymer (SMP) surfaces with recessive hexagonal patterns. SMPs are a class of materials that can memorize a permanent shape through physical or chemical cross-linking, be manipulated and fixed in a temporary shape via an immobilizing transition, such as vitrification or crystallization, and subsequently recover to the permanent shape as the result of a triggering event, such as thermal, electrical, light, or solvent activation.16,17 This phenomenon is known as one way shape memory, because activity occurs in one direction. In addition to one way SMPs, there are also two way, triple shape, multishape, and multifunctionality SMPs18 which make SMP with systematically designed surface topography a promising
candidate for the development of novel and programmable antifouling strategies. We chose hexagonal patterns because the static protruding or recessive hexagonal patterns have been found to reduce biofilm formation significantly.\textsuperscript{4,5} Thus, they are good candidates for biofouling control using dynamic topography in this study. Another advantage to use recessive hexagonal patterns is that they can maintain structural integrity under a uniaxial strain of >50%, an important step in creating the temporary shape. 

In this proof-of-concept study, we chose an SMP based on t-butyl acrylate (tBA), poly(ethylene glycol)\textsubscript{4} dimethacrylate (PEGDMA), and photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA), which has one way shape memory around 38.5 °C.\textsuperscript{20} The biocompatibility of this SMP has been validated by its cardiovascular applications.\textsuperscript{21} Using dynamic mechanical analysis (DMA), we confirmed that this SMP has a stepwise decrease in tensile storage modulus at 47.5 °C, with a glassy modulus of about 700 MPa below this temperature and a rubbery plateau of about 1.3 MPa above this temperature (Figure S2), which suggests that this SMP can be deformed at temperatures above 47.5 °C and fixed at temperature below 30 °C. The effective T\textsubscript{g} in 0.85% (wt/vol) NaCl solution was found to be 38.5 °C, which is lower than the dry T\textsubscript{g} (44.3 °C) due to plasticization by water (Figure S3), allowing rapid shape change near body temperature (37 °C).\textsuperscript{20} These shape memory properties of our material are consistent with literature values.\textsuperscript{20}

Stretched SMP surfaces used in this study were found to stably maintain their temporary shape during 48 h incubation at room temperature in sterile LB medium. After 10 min of incubation at 40 °C, the programed SMP shank with a 98.9% recovery to the permanent shape (Figure S1).

Static flat control (prepared without shape memory fixing so as not to change shape when heated to 40 °C) and both flat and topographically patterned programmed surfaces (fixed with a temporary but stable uniaxial strain of >50% so as to contract by ~50% when heated to 40 °C) were prepared (Figure S4). All surfaces were challenged with biofilm formation of 

\textit{Pseudomonas aeruginosa} PAO1, \textit{S. aureus} ALC2085, and uropathogenic \textit{E. coli} ATCC53505 for 48 h at room temperature.

We first studied the effects of static topography on adhesion and biofilm formation by comparing the biomass of 48 h biofilms formed on these three different surfaces. For calculating biomass, 3D information was obtained from a series of z stack biofilm images (1 μm interval), which were then analyzed using the software COMSTAT.\textsuperscript{22} By analyzing the biomass of 48 h biofilms on these three different surfaces, hexagonal recessive patterns were found to significantly reduce microbial biofilm formation. For example, the biomass of \textit{P. aeruginosa} PAO1 biofilms on topographically patterned programmed substrates (before triggered shape recovery) was 50.9 ± 7.2% and 51.9 ± 7.3% of that on flat programmed substrates and static flat control, respectively (p < 0.001 for both, one way ANOVA adjusted by Tukey test; Figure 1a). No significant difference was found between static flat controls and flat programmed substrates (both around 9 μm\textsuperscript{3}/μm\textsuperscript{2}; p = 0.93 one way ANOVA).

After 48 h of incubation, we tested the effects of topographic changes on established biofilms. Upon heating for 10 min at 40 °C, shape recovery induced significant detachment of established biofilms. For example, the biomass on topographically patterned programmed substrates was 4.7 ± 0.7 μm\textsuperscript{3}/μm\textsuperscript{2} and 0.01 ± 0.01 μm\textsuperscript{3}/μm\textsuperscript{2} before and after shape recovery induced changes in surface topography, respectively. This represents a 469-fold reduction of biomass due to the change in substrate topography, and 909-fold reduction comparing to the 48 h biofilm biomass (9.1 ± 0.8 μm\textsuperscript{3}/μm\textsuperscript{2}) on static flat controls without topographic patterns and shape change. Collectively, these data demonstrate up to 99.9% biofilm reduction through combined effects of biofilm inhibition by surface topography and biofilm removal by shape change. Similar effects of biofilm removal were also observed for flat programmed substrates, e.g., the biomass on flat programmed substrates was 9.3 ± 2.9 μm\textsuperscript{3}/μm\textsuperscript{2} and 0.04 ± 0.03 μm\textsuperscript{3}/μm\textsuperscript{2} before and after shape recovery, respectively (231-fold reduction, p < 0.001, one way ANOVA adjusted by Tukey test) (Figure 1a). These results were corroborated by fluorescence images (Figure 1b) and colony forming unit (CFU) assay (Figure S5).

In contrast to the reduction in biomass observed on programmed substrates, the biomass on static flat controls before and after incubation at 40 °C for 10 min was 9.1 ± 0.8 μm\textsuperscript{3}/μm\textsuperscript{2} and 8.5 ± 1.9 μm\textsuperscript{3}/μm\textsuperscript{2}, respectively (p = 0.63, one way ANOVA; Figure 1a). Thus, the aforementioned biofilm removal was indeed caused by shape change rather than temperature change.

Biofilm dispersion was further verified by taking real-time movies. Before triggered shape change by heating for 10 min at 40 °C, \textit{P. aeruginosa} PAO1 biofilms were clearly seen with large cell clusters (Movies S1 and S2). When the shape recovery started, rapid detachment of both cell clusters and individual cells were observed on both flat and topographically patterned programmed substrates (Movies S1 and S2). Most changes in shape occurred in the first 6 min after shape recovery started (Figure 2a,b). Surface coverage by biofilms was 33.0% before
The toxicity of this SMP to bacterial cells has not been evaluated.

P. aeruginosa PAO1 (Figure 3a). Nevertheless, change in surface topology triggered by shape recovery still caused dramatic detachment of both E. coli and S. aureus biofilms (Figures S6a and S7a). For example, the biomass of S. aureus biofilms on topographically patterned programmed substrates was similar to that on static flat control and flat programmed substrates (both around 5.5 $\mu$m$^3/$μm$^2$; $p = 0.22$, one way ANOVA; Figure S7a). Similar effects of biofilm removal were also observed for flat programmed substrates (Table S1). In contrast, incubation at 40 °C for 10 min alone did not show significant effect on the biofilms formed on flat control substrates, showing that biofilm removal from stretched samples was indeed caused by shape recovery. Some representative fluorescence images are shown in Figures S6b and S7b.

To understand the long-term effects of biofilm removal and how fast the remaining cells can reform biofilms, we followed the regrowth of P. aeruginosa PAO1 and E. coli ATCC3505 biofilms at 12, 24, and 48 h after shape recovery triggered biofilm removal, and compared the results with the biomass before shape recovery and the static flat control that underwent the same temperature change but not shape recovery. The data summarized in the Figures S8 and S9 show that, for both species, the biomass on the surfaces that had gone through shape recovery was significantly lower than that before shape recovery and on the static flat control. For example, the biomass of P. aeruginosa PAO1 biofilms on flat program surfaces was $1.6 \pm 0.1$ $\mu$m$^3/$μm$^2$ at 48 h after shape recovery. This is 83.7% ($p = 0.0164$, one way ANOVA adjusted by Tukey test, Figure S8) lower than that before shape change (9.3 ± 2.9 $\mu$m$^3/$μm$^2$) and 89.0% ($p = 0.0022$, one way ANOVA adjusted by Tukey test, Figure S8) lower than that on the static flat control (increased from 9.1 ± 0.8 $\mu$m$^3/$μm$^2$ to 14.7 ± 0.9 $\mu$m$^3/$μm$^2$ during the same period of incubation time). Even stronger effects were found for patterned programed surfaces (additional
biofilm form 49 s before transition to 285 s after transition started (AVI).

Movie S2: Dynamic removal of P. aeruginosa PAO1 biofilm from a flat programmed substrate. This movie shows a P. aeruginosa PAO1 biofilm from 48 s before transition to 280 s after transition started (AVI).

Movie S3: Static flat control. This movie shows a P. aeruginosa PAO1 biofilm from 1 min before to 10 min after temperature transition started (AVI).

**AUTHOR INFORMATION**

**Corresponding Author**
* Dacheng Ren. Phone: 001-315-443-4409. Fax: 001-315-443-9175. Email: dren@sy.edu.

**Funding**

The authors thank the U.S. National Science Foundation (CAREER-1055644, EFRI-1137186, and DGE-1068780), Alfred P. Sloan Foundation (a postdoc fellowship for Huan Gu), and U.S. National Institute of Health (1R21EB025750–01A1) for partial support of this work.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Karin Sauer at Binghamton University for sharing P. aeruginosa PAO1 and S. aureus ALC2085, Dr. Arne Heydorn at the Technical University of Denmark for providing the COMSTAT software, and Dr. Patrick Mather at Syracuse University (currently Bucknell University) for the access to a custom built stretcher. We also thank the Cornell Nanoscale Science & Technology Facility for the access to photolithography facilities.

**REFERENCES**

1. Hall-Stoodley, L.; Costerton, J. W.; Stoodley, P. Bacterial Biofilms: From the Natural Environment to Infectious Diseases. Nat. Rev. Microbiol. 2004, 2, 95–108.

2. Bjarnsholt, T.; Givskov, M.; Høiby, N.; Molin, S. Control of Bacterial Adhesion and Growth on Honeycomb-Like Patterned Surfaces. Colloids Surf., B 2015, 135, 549–555.

3. Chung, K. K.; Burne, R. A.; Sampson, E. M.; Schuchmann, J. F.; Costerton, J. W.; Hall-Stoodley, L. Microtopography on Biofilm Formation of Staphylococcus aureus and S. epidermidis. Sci. Rep. 2016, 6, 29516.

4. Friedlander, R. S.; Vlamakis, H.; Kim, P.; Khan, M.; Kolter, R.; Aizenberg, J. Bacterial Flagella Explore Microscale Hummocks and Hollows to Increase Adhesion. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, 5624–5629.

5. Aizenberg, J. Bacterial Flagella Explore Microscale Hummocks and Hollows to Increase Adhesion. Proc. Natl. Acad. Sci. U.S.A. 2013, 110, 5624–5629.

6. Chung, K. K.; Schuchmann, J. F.; Sampson, E. M.; Burne, R. A.; Antonelli, P. J.; Brennan, B. A. Impact of Engineered Surface Microtopography on Biofilm Formation of Staphylococcus aureus. Biofactors 2007, 2, 89–94.

7. Friedlander, R. S.; Vlamakis, H.; Kim, P.; Khan, M.; Kolter, R.; Aizenberg, J. Bacterial Flagella Explore Microscale Hummocks and Hollows to Increase Adhesion. Proc. Natl. Acad. Sci. U.S.A. 2013, 110, 5624–5629.

8. Chung, K. K.; Schuchmann, J. F.; Sampson, E. M.; Burne, R. A.; Antonelli, P. J.; Brennan, B. A. Impact of Engineered Surface Microtopography on Biofilm Formation of Staphylococcus aureus. Biofactors 2007, 2, 89–94.

9. Grinthal, A.; Aizenberg, J. Hydrogel-Actuated Integrated Responsive Systems (HAIRS): Creating Cilia-Like ‘Hair’ Surfaces. RSC Nanosci. Nanotechnol. 2013, 162–185.
(10) Gu, H.; Ren, D. C. Materials and Surface Engineering to Control Bacterial Adhesion and Biofilm Formation: A Review of Recent Advances. Front. Chem. Sci. Eng. 2014, 8, 20–33.
(11) Xue, L.; Lu, X.; Wei, H.; Long, P.; Xu, J.; Zheng, Y. Bio-Inspired Self-Cleaning PAAS Hydrogel Released Coating for Marine Antifouling. J. Colloid Interface Sci. 2014, 421, 178–183.
(12) Shivapooja, P.; Wang, Q.; Orihuela, B.; Rittschof, D.; Lopez, G.; Zhao, X. Bioinspired Surfaces with Dynamic Topography for Active Control of Biofouling. Adv. Mater. 2013, 25, 1430–1434.
(13) Kirschner, C. M.; Brennan, A. B. Bio-Inspired Antifouling Strategies. Annu. Rev. Mater. Res. 2012, 42, 211–229.
(14) Epstein, A. K.; Hong, D.; Kim, P.; Aizenberg, J. Biofilm Attachment Reduction on Bioinspired, Dynamic, Micro-Wrinkling Surfaces. New J. Phys. 2013, 15, 095018.
(15) Hou, S.; Gu, H.; Smith, C.; Ren, D. Microtopographic Patterns Affect Escherichia coli Biofilm Formation on Poly(dimethylsiloxane) Surfaces. Langmuir 2011, 27, 2686–2691.
(16) Mather, P. T.; Luo, X.; Rousseau, I. A. Shape Memory Polymer Research. Annu. Rev. Mater. Res. 2009, 39, 445–471.
(17) Tseng, L. F.; Mather, P. T.; Henderson, J. H. Shape-Memory-Actuated Change in Scaffold Fiber Alignment Directs Stem Cell Morphology. Acta Biomater. 2013, 9, 8790–8801.
(18) Hu, J. L.; Zhu, Y.; Huang, H. H.; Lu, J. Recent Advances in Shape-Memory Polymers: Structure, Mechanism, Functionality, Modeling and Applications. Prog. Polym. Sci. 2012, 37, 1720–1763.
(19) Wu, H.; Moser, C.; Wang, H. Z.; Holby, N.; Song, Z. J. Strategies for Combating Bacterial Biofilm Infections. Int. J. Oral Sci. 2015, 7, 1–7.
(20) Levering, V.; Cao, C.; Shivapooja, P.; Levinson, H.; Zhao, X.; Lopez, G. P. Urinary Catheter Capable of Repeated On-Demand Removal of Infectious Biofilms via Active Deformation. Biomaterials 2016, 77, 77–86.
(21) Yakacki, C. M.; Shandas, R.; Lanning, C.; Rech, B.; Eckstein, A.; Gall, K. Unconstrained Recovery Characterization of Shape-Memory Polymer Networks for Cardiovascular Applications. Biomaterials 2007, 28, 2255–2263.
(22) Heydorn, A.; Nielsen, A. T.; Hentzer, M.; Sternberg, C.; Givskov, M.; Erbøll, B. K.; Molin, S. Quantification of Biofilm Structures by the Novel Computer Program COMSTAT. Microbiology 2000, 146, 2395–2407.
(23) Levering, V.; Cao, C.; Shivapooja, P.; Levinson, H.; Zhao, X.; Lopez, G. P. Urinary Catheter Capable of Repeated On-Demand Removal of Infectious Biofilms via Active Deformation. Biomaterials 2016, 77, 77–86.