Antifouling Topographies to Combat Microbial Biofilms

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Primary CNF Tools Used: Heidelberg mask writer DWL2000, ABM contact aligner, Plasma-Therm UNAXIS 770 deep silicon etcher, MVD 100

Abstract:

Bacteria can adhere and develop multicellular structures (known as biofilms) on medical devices, which are the primary cause of hospital-acquired infections (HAI) and biofouling. Once mature biofilms are established, they are hard to eradicate, leading to the persistence of biofilm-mediated medical challenges. In this project, we aim to develop a strategy to address these challenges using biocompatible shape memory polymers (SMPs) with defined surface topography. The surface topography is introduced onto the surface of SMPs using photolithography and soft lithography. This strategy can prevent bacterial adhesion and remove mature biofilms on-demand, and therefore, providing prolonged antifouling properties. By challenging these new antifouling surfaces with *Pseudomonas aeruginosa* biofilms, these topographies demonstrate 99.9% biomass reduction compared to the static and flat controls. Similar potent antifouling effects are also observed against biofilms of *Staphylococcus aureus* and a uropathogenic strain of *Escherichia coli*. The data included in this report has been published in the Journal of ACS Applied Materials and Interfaces [1].

Summary of Research:

To fabricate well-defined surface topography on SMPs, we used poly(dimethylsiloxane) (PDMS) surfaces with complementary topography as molds (Figure 1). These PDMS surfaces were created using photolithography and soft lithography by following the protocol described by Hou, et al. [2]. In detail, the configuration of the patterns was designed using the software L-Edit (Tanner Research, Monrovia, CA, USA) to create hexagonal shapes with side length (L) of 5, 10, 15, 20, 30, 40, or 50 μm and inter-pattern distance (D) of 2, 5, 10, 15, or 20 μm.

This design was first written onto a photomask using a photomask writer (Heidelberg Mask Writer - DWL2000) and then transferred onto silicon wafers coated with photoresist S1813 using the ABM contact aligner. The hexagonal holes on silicon wafers were etched to obtain approximately 10 μm deep features using a Plasma-Therm Unaxis 770 deep silicon etcher.

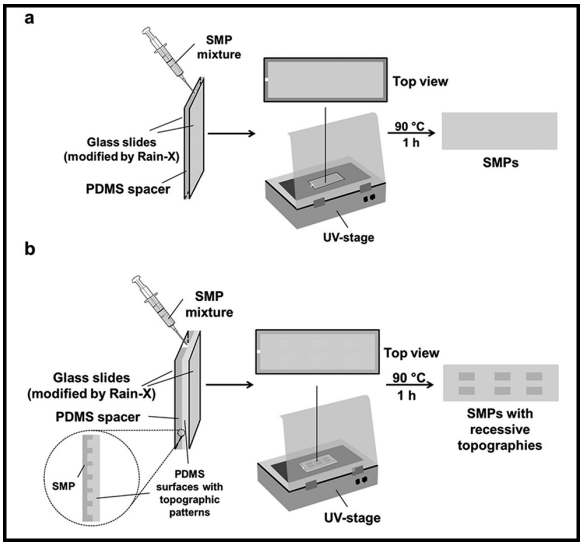

Figure 1: Schematic illustration of the process for preparing SMPs without (a) or with (b) surface topography.

To minimize PDMS residues in each round of soft lithography, silicon wafers were coated with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)trichlorosilane (FOTS) prior to use as templates. Silicon elastomer mixtures (base : cure agent (wt/wt) = 10 : 1; Sylgard® 184, Sigma-Aldrich, St. Louis, MO, USA) were applied onto the silicon wafers and polymerized at 60°C for 24 h. PDMS surfaces with recessive hexagonal patterns were then gently peeled off and used as molds.

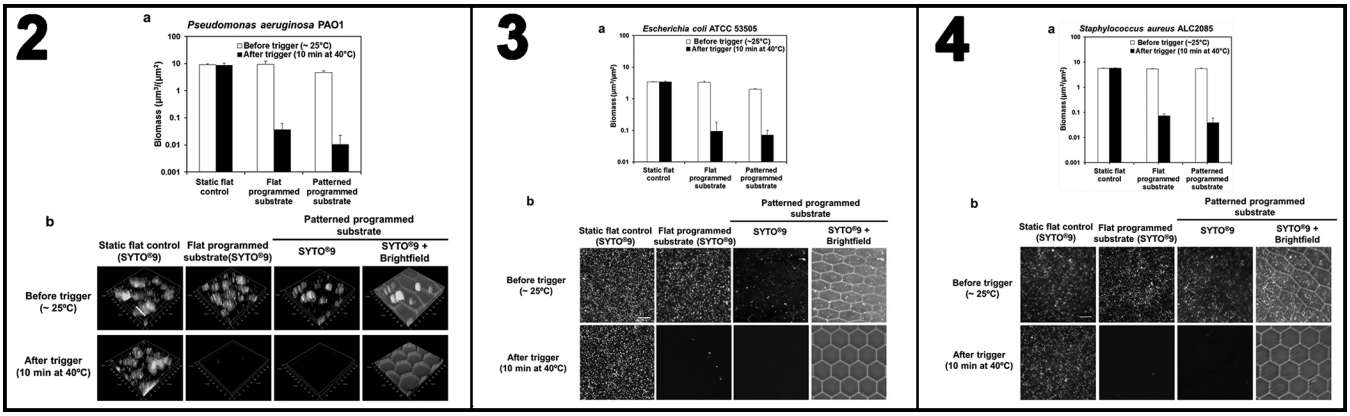

Figure 2, left: *Effects of static flat control and programmed substrates on the biofilm formation and mature biofilms of* P. aeruginosa *PAO1. Figure 2 shows the biomass (a) and representative fluorescence images (b) of* P. aeruginosa *PAO1 biofilms on different surfaces before and after trigger (10 min incubation at 40°C) (Bar = 50 μm) [1]. (Reproduced with permission from the Journal ACS Applied Materials and Interfaces.)* ***Figure 3, middle:*** *Effects of static flat control and programmed substrates on the biofilm formation and mature biofilms of* E. coli *ATCC53505. Figure 3 shows the biomass (a) and representative fluorescence images (b) of* E. coli *ATCC53505 biofilms on different surfaces before and after trigger (10 min incubation at 40°C) (Bar = 50 μm) [1]. (Reproduced with permission from the Journal ACS Applied Materials and Interfaces.)* ***Figure 4, right:*** *Effects of static flat control and programmed substrates on the biofilm formation and mature biofilms of* S. aureus *ALC2085. Figure 4 shows the biomass (a) and representative fluorescence images (b) of* S. aureus *ALC2085 biofilms on different surfaces before and after trigger (10 min incubation at 40°C) (Bar = 50 μm) 1]. (Reproduced with permission from the Journal ACS Applied Materials and Interfaces.)*

The SMP used in this study was synthesized by mixing t-butyl acrylate (tBA), poly(ethylene glycol) n dimethacrylate (PEGDMA), and photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) (wt:wt:wt = 9:1:0.04). Flat or topographically patterned substrates were prepared by injecting the mixture between two glass slides with a 1 mm thick PDMS spacer using a syringe, being pre-polymerized under 365 nm UV irradiation for 10 min, followed by a thermal post-cure for 1 h at 90°C to maximize the conversion of monomers [3] (Figure 1). We programmed the shape change of SMPs by incubating dog bone shaped flat or topographically patterned substrates at 50°C for 5 min and then gradually stretching using a manual stretcher to 1.5 times of the original length. This temporary shape was fixed via approximately 5 min cooling at room temperature. We triggered shape recovery by incubating these SMP substrates with their temporary shape in pre-warmed 0.85% NaCl for 10 min at 40°C. Flat static control (samples that do not undergo shape change when heated) were also prepared.

By comparing the biomass of 48 h *P. aeruginosa* PAO1 on the three different surfaces that are flat static controls, flat and topographically patterned programmed surfaces, recessive hexagonal patterns were found to significantly prevent biofilm formation with 50.9 ± 7.2% and 51.9 ± 7.3% reduction in biomass compared to that on flat programmed substrates and static flat control, respectively ($p < 0.001$ for both, one way ANOVA adjusted by Tukey test; Figure 2a). No significant difference was found between static flat controls and flat programmed substrates (both around 9 $\mu m^3/\mu m^2$; $p = 0.93$). Shape recovery induced movement of recessive hexagonal patterns triggered more profound effects on removing the established biofilms. For instance, the biomass on topographically patterned programmed substrates was 0.01 ± 0.01 $\mu m^3/\mu m^2$ after shape recovery. This represents a 469-fold reduction of biomass compared to the biomass before shape recovery (4.7 ± 0.7 $\mu m^3/\mu m^2$), and 909-fold reduction compared to the 48 h *P. aeruginosa* PAO1 biofilm biomass (9.1 ± 0.8 $\mu m^3/\mu m^2$) on static flat controls without topographic patterns and shape change. These results were obtained by quantifying fluorescence images using COMSTAT4 (Figure 2b). Similar results were observed in preventing the biofilm formation and removing mature biofilms of two other microorganisms, *E. coli* and *S. aureus* (Figures 3 and 4).

In summary, we developed new antifouling surfaces based on shape memory triggered changes in surface topography. This strategy was found effective for the prevention and removal of established biofilms of multiple species. Future studies are needed to understand the underlying mechanism and develop biocompatible polymers for *in vivo* use with tailored temperature and duration of heating to achieve multiple cycles of shape change.

References:

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