**Antimicrobial-tolerant bacterial biofilms are inhibited by Sharklet microtopography**

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**Statement of Purpose:** Medical device-associated infections are a leading cause of hospital-acquired infections (HAI) despite improved patient care practices. Device surfaces present an optimal substrate for attachment of bacteria that form biofilms. A biofilm is an adherent community of microorganisms encased in a polymeric substance. Biofilm related infections are particularly fearsome due to inherent antimicrobial tolerance and persistence against host immunity. Therefore innovative approaches are needed that prevent biofilm formation. A bio-inspired surface microtopography, called Sharklet, provides a novel strategy for biofilm prevention in that it does not use toxicity or chemical agents. It has been shown to reduce microbial attachment and biofilm formation of several bacterial pathogens[1,2]. In this study, the microtopography was directly compared to current antibacterial strategies of coating medical devices with antibiotics to prevent biofilm infection.

**Methods:** Thermoplastic polyurethane (TPU) samples with either smooth (SM) or Sharklet micropatterned (MP) surfaces were fabricated by thermal embossing against metal molds. Additional smooth samples were adsorbed with a combination of minocycline (15 mg/ml) and rifampin (30 mg/ml) by immersion for 1 h followed by drying and rinsing excess antibiotic from the surface[3]. Antimicrobial efficacy of the antibiotic-treated surfaces was tested using a Kirby-Bauer assay. Rectangular samples (25 mm x 75 mm) were sterilized in 0.3% $H_2O_2$ then placed in the drip-flow biofilm reactor (BioSurfaces Technologies, Bozeman, MT) at a 25° decline. An overnight culture of *Pseudomonas aeruginosa* strain *bifA*[4] was diluted 1:100 in 200 ml of fresh growth media. The inoculum was dripped onto the test and control surfaces for 48 h at approximately 25˚C. The biofilm biomass was quantified by obtaining three 8 mm punches and placing them in 1 ml of Dey-Engley neutralization buffer plus 2 µg/ml of proteinase K (Qiagen, Venlo, Limburg). The samples were vortexed and sonicated for 10 m each before and after being incubated at 37˚C for 30 m to disperse all viable cells from the biofilm. The colony forming units (CFU) from the resulting buffer were quantified. The mean CFU log-reductions (LR) from 3 individual experiments, each containing multiple sample replicates, were used to compare microtopography performance. Statistical significance was evaluated using a single t-test or ANOVA of the LRs.

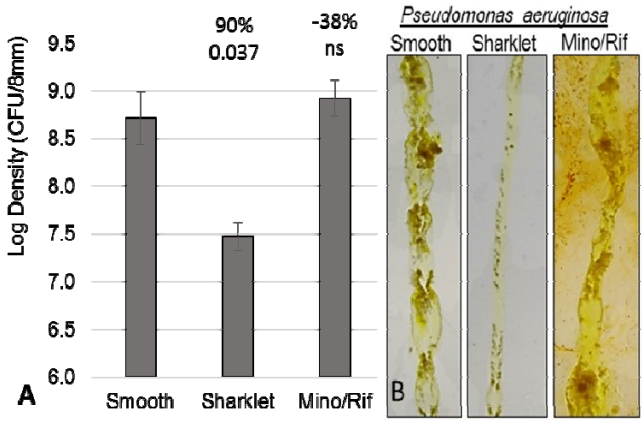


**Figure 1. Minocycline/rifampin fails to inhibit biofilm.** *P. aeruginosa* biofilms were grown for 48 h in a drip flow reactor on each of the surfaces and quantified via dilution plating (A) and evaluated qualitatively (B).

**Results:** The antimicrobial-coated surfaces exhibited 8 mm zones of inhibition in the Kirby-Bauer assay. In the drip flow reactor, the Sharklet microtopography significantly reduced *P. aeruginosa* biofilm accumulation by 90% ($p = 0.037$) (Fig. 1A) compared to the smooth control. Conversely, minocycline/rifampin (Mino/Rif)-treated smooth surfaces failed to limit biofilm accumulation compared to either smooth or Sharklet microtopography. Large colonies of *P. aeruginosa* biofilm are evident on the smooth and minocycline/rifampin surfaces but absent on the Sharklet microtopography (Fig. 1B).

**Conclusions:** The Sharklet microtopography prevents the formation of *P. aeruginosa* biofilms in the drip flow biofilm model. These data suggest that the Sharklet microtopography is more appropriate for implementation on medical devices commonly infected with biofilm-forming bacteria than popular antimicrobials.

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