Engineered Substratum Topography to Disrupt Bacterial Biofilm Formation

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Statement of Purpose:
Biological adhesion on synthetic surfaces is of great concern in the successful development of biomaterials, ultrafiltration, and underwater vessels. The general consensus for micro and macrofouling on substratum topography has been that organisms preferentially attach to randomly roughened surfaces. In the case of bacteria, specific surface properties that have been associated with bacterial biofilm fouling include hydrophobicity, surface charge, and roughness[1]. However, existing literature is contradictory on what type of substratum will most effectively disrupt bacterial attachment. Previously we have successfully demonstrated engineered micro-topographies in polydimethylsiloxane elastomer (PDMSe) that inhibit settlement of algae spores and various larvae (barnacles and tubeworms) of marine organisms[2]. These topographies are unique in that they have clearly defined surface structures that are tailored to the critical dimensions of the fouling organism. Our most successful topography, a biomimetic structure based on the skin of fast-moving sharks (Sharklet AF^{TM}, University of Florida, Gainesville, FL), has been evaluated for its ability to disrupt and prohibit biofilm formation. In this study, a common bacteria associated with nosocomial infections in medical devices, *Staphylococcus aureus*, was evaluated for its tendency to attach and form biofilms on PDMSe surfaces.

Methods:
The topographically modified PDMSe surface was tested against a smooth PDMSe surface. The engineered topography was designed and fabricated with microprocessing techniques[2] at a 2µm critical dimension to complement the micron-sized cells of *S. aureus*. All sampes were then statically exposed to 10^7 CFU/mL of *S. aureus* in growth medium, with five replicates of each sample type (smooth and Sharklet AF^{TM}) removed on days 2, 7, 14, and 21 for characterization. Scanning electron microscopy (SEM) was used to image samples for each time period.

Results/Discussion:
All SEM images were analyzed for area coverage using image analysis software. Images were shown to an expert in the field to qualify the existence of bacterial biofilms. Mean values of percent area coverage of bacteria on all PDMSe surfaces were calculated and statisically analyzed. Smooth PDMSe samples showed significant increases in bacteria coverage for each time point, with the first evidence of biofilm on day 14 samples. The Sharklet AF^{TM} samples showed significantly lower values of percent area coverage for each time point, with biofilm not appearing until day 21. Percent area coverage values on day 21 samples were 77% for smooth and 35% for Sharklet AF^{TM}. Even on day 21, biofilm colonies covered only isolated areas on Sharklet AF™ samples, with little to no evidence of biofilm colonies or bacteria cells in other areas.

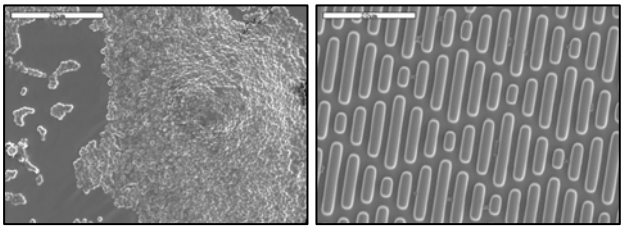

Figure 1. Scanning electron micrographs of *S. aureus* after 14 days of growth on (left) smooth PDMSe (20µm scale bar) and (right) Sharklet AF™ PDMSe (20µm scale bar).

While cells were able to grow in the region between the topographical features, bacteria did not grow over the top of the riblet features until day 21, implying that these features may be responsible for impeding bacterial accretion and subsequent biofilm formation. These results suggest a correlation between the defined micro-scale topography and the bacteria's ability to settle and form subsequent biofilm colonies on the surface.

Conclusions:
S. aureus grown on PDMSe surfaces for up to 21 days responded to an engineered surface micro-topography with non-random, clearly defined features tailored to the critical dimensions of the cells. The elicited response disrupted bacterial adhesion and biofilm formation on the topographically modified surface, a significant development in the pursuit of a non-toxic solution to bacterial biofilm infections related to medical implants. Future studies will include dynamic flow conditions to further evaluate the adhesion mechanism of bacteria on these engineered surfaces.

References:
1. Characklis WG. Biofilms, Wiley, New York, 1990.
2. Carman ML. Biofouling 2006: 22: 11-21.