Bacterial Adhesion to Submicron Textured Biomaterials

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Statement of Purpose: Biomaterial-centered infections are extremely prevalent and have a negative impact on human health. Surface modification of polymeric biomaterials may reduce incidence of infection by reducing bacterial adhesion. To date, most of these modifications have involved surface chemistry changes either through changing the chemistry of the polymer backbone or by surface-specific modification using different strategies. In a previous study[1], we have shown that addition of sub-micron sized architecture onto the surface of a poly(urethane urea) (PUU) led to reductions in blood platelet adhesion. *Staphylococcus epidermidis* is a common bacterial strain found in biomaterial-centered infections and is similar in size to blood platelets. We hypothesized that sub-micron structures would also reduce bacterial adhesion to poly(urethane urea) (PUU) . In this study, a rotating-disk system was used to assess bacterial adhesion to PUU materials under dynamic flow.

Methods: Biospan MS/0.4 (Polymer Technology Group) segmented PUU was used in creating the samples. The surface topographies on the PUU were created using a soft lithography two-stage replication molding technique. Arrays of 400 nm pillars with 400 nm spaces between and of 700 nm pillars with 700 nm spaces were fabricated. Briefly, a master pattern was created on a silicon wafer via stepper lithography, from which a poly(dimethyl-siloxane) (PDMS) negative mold was cast. PUU replicas with uniform surface chemistry were prepared by casting into the PDMS mold. Bacterial adhesion onto these surfaces was compared to a smooth control prepared using a similar process on an unexposed silicon wafer.

Strain RP26A of *Staphylococcus Epidermidis* was used in this study. The bacterial colonies were inoculated in tryptic soy broth, and cultured 24hr in an orbital shaker at 37˚C. The bacteria was centrifuged at 1360g for 10min and resuspended in phosphate buffered saline (PBS).

The rotating disc system (RDS, Pine Instruments) produces conditions where the wall shear stress varies linearly with radial distance from zero in the center of the PUU sample while the flux of bacteria is uniform across the PUU. Prior to rotation, bacteria were injected into PBS test suspension using a 30-gauge syringe to minimize aggregation. The RDS was rotated for 1 hour at 431 rpm, producing shear stresses from 0-18 dyn/cm^2. Samples were rinsed with PBS and fixed overnight in 2.5% glutaraldehyde. Adherent bacteria were stained with Hoechst 33258 and enumerated using fluorescent microscopy.

Results: Figure 1 illustrates bacterial adhesion at shear stresses ranging from 0 to 18 dyn/cm^2 with bacteria presented as colony forming units/mm^2. The 400 nm textures showed nearly constant adhesion across the entire shear stress range, with a small decrease at higher shear. While the three textures show similar levels of bacterial adhesion at higher shear stress, at 0 dynes/cm^2 the 400 nm PUU has lower adherence compared to the other samples. Bacterial aggregates were prevalent on the smooth PUU, scarce on the 700 nm PUU, and absent on 400 nm PUU.

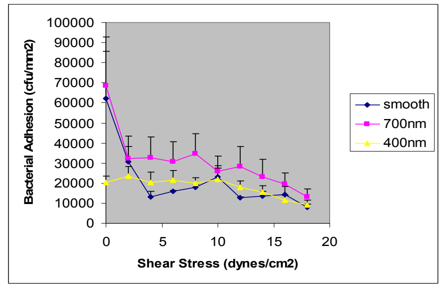

Figure 1. Bacterial adhesion assessed on smooth, 700 nm and 400 nm PUU. Illustrating bacterial adhesion from PBS onto polyurethane under shear-stress conditions, with n=3. All samples displayed decreasing adhesion with increasing shear, however, the smooth and 700 nm samples had larger adhesion at the lowest shear stresses.

SEM analysis showed that bacteria were able to fit between the pillars on the 700 nm samples (Figure 2).

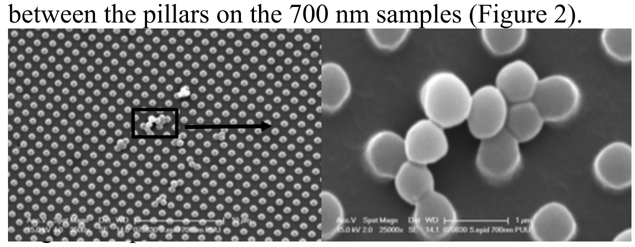

Figure 2: SEM images of bacterial adhesion to 700 nm texture polymer

These data show that the surface properties of the textured polyurethanes affect bacterial adhesion. *S. epidermidis* adhesion varied with shear stress for both the smooth and 700 nm textured materials. However, the 400 nm textured materials showed lower adhesion at low shear and less dependence on shear stress. Bacterial aggregates formed on the smooth samples at low shear stress while no aggregates were found on the 400 nm sample. The high adhesion on the 700 nm samples appears to arise from bacteria becoming lodged in the interpillar gaps.

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References: 1. Milner et al. JBMRA, 2006; 76A:561