Textured Biomaterial Surfaces Reduce Bacterial Adhesion and Biofilm Formation

Li-Chong Xu, Christopher A. Siedlecki
Department of Surgery, Biomedical Engineering Institute,
The Pennsylvania State University, College of Medicine, Hershey, PA, 17033.

Statement of Purpose: Infection due to bacterial adhesion and biofilm formation are a major problem associated with the use of blood-contacting medical devices. It is difficult to treat biomaterial-centered infections with antibiotics due to the antibiotic resistance of biofilm. Initial bacterial adhesion and colonization on an implanted biomaterial surface is mediated by the surface properties. We hypothesize that specially designed surface texturing would impart resistance to bacterial adhesion and subsequent biofilm formation. In this study, polyurethane biomaterials were fabricated with the sub-micro textured surfaces so that the accessible contact area of the bacterial cells with the surface would be reduced and would lead to a reduction of bacterial adhesion, and subsequent inhibition in biofilm formation.

Methods: The poly(urethane urea) (PUU) (Biospan MS/0.4) surfaces were textured with ordered arrays of pillars using a soft lithography two-stage replication molding technique.[1] Two patterns were used: round pillars with diameter and separation of 400 nm and 400 nm (400/400) or 500 nm and 1000nm (500/1000). Strains *S. epidermidis* RP62A and *S. aureus* Newman were cultured in media for 24 hrs and collected by centrifuge at 1360 g for 10 min. The pellet was re-suspended in PBS and the concentration of bacteria was measured by a spectrophotometer at 600 nm. Bacterial adhesion and biofilm formation on PU surfaces were carried out with a rotating disk system (RDS) producing well-defined dynamic flow conditions across the PUU surfaces.[1] The test solutions contain the bacteria ar a concentration of 10^8 cfu/ml and other components as desired. Bacteria attached on PU surfaces were fixed in 2.5% glutaradlehyde and labeled with Hoechst. Bacterial adhesion was counted under a fluorescent optical microscope. To observe the biofilm formation, samples were run in RDS system for either 2 or 5 days, and stained with FITC conjugated wheat germ agglutinin. Biofilm was observed under a fluorescent microscope.

Results / Discussion:

Bacterial adhesion on PUU surfaces with shear: The variation in bacterial adhesion to smooth and 400/400 nm PUU under shear was assessed across a low shear stress range (0-13 dyn/cm^2) in PBS or dilute plasma/serum as illustrated in Fig. 1. A reduction in bacterial adhesion on textured surfaces was generally observed for both strains in PBS with a reduction rate of ~80% for *S. epidermidis* and ~ 60% for *S. aureus* (Fig. 1a and 1c). An increase in adhesion was observed for *S. epidermidis* in 25% plasma solution at near-static condition (~0.2 dyn/cm^2) on textured surface, however, bacterial adhesion significantly decreased with further shear increases. Due to clumping by coagulase positive *S. auresu* in normal plasma, adhesion of *S. aureus* was run in serum (clotting factors removed from plasma) and the reduction in bacterial adhesion varied over the range of 40-75%. A second textured PUU surface with 500/1000 nm pattern was tested in same conditions and similar results were observed (data not shown). Results suggested that the reduction in bacteria accessible contact area to surfaces was efficient in inhibition of bacterial adhesion.

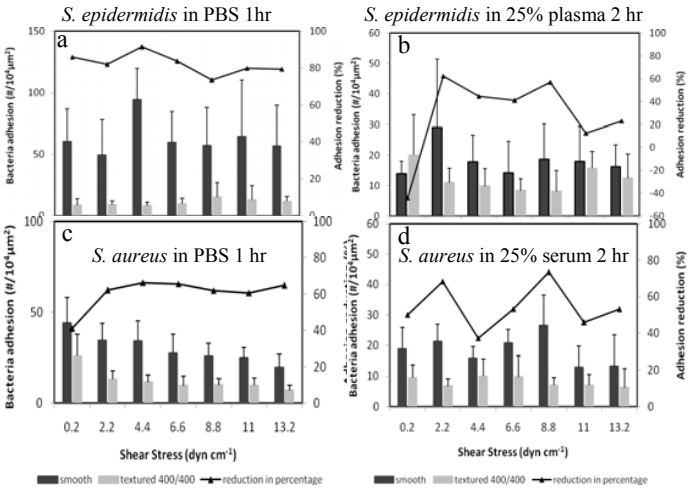

Figure 1. Bacteria adhesion on smooth and textured (400/400) PUU surfaces with shear.

Biofilm formation on PUU surfaces. Biofilms were observed on smooth PUU surfaces after long term incubation at low shear, while only individual bacteria or small bacterial clusters were observed on textured PUU (Fig. 2). Results strongly suggested that the reduction in bacterial adhesion is associated with a reduction in biofilm formation.

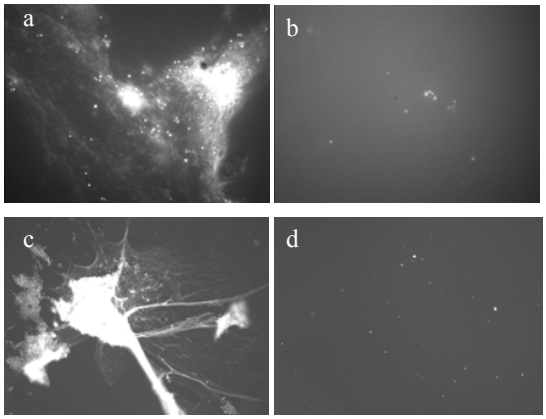

Figure 2. Optical fluorescence images of smooth (a, c) and textured (b, d) PUU surfaces showing biofilm formation. (a, b) *S. epidemidis* RP62A in 25% plasma for 2 days and (c, d) *S. aureus* in 25% serum for 5 days.

Acknowledgement:
Financial support from a Dept of Surgery Feasibility Grant, Hershey Medical Center, Penn State College of Medicine.

Reference:
1. Milner, K.R., Snyder, A.J., Siedlecki, C.A., J Biomed Mater Res. 200, 76A, 561-570