Long-Term and Broad-Spectrum Biofilm Resistant Surface Modification

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Statement of Purpose: Most surface modifications on implantable medical devices using anti-fouling polymers such as polyethylene glycol or polyphosphorylcholine do not achieve long-term performance in complex media such as serum or blood. Our previous research involving a novel surface modification to apply betaine polymers on medical devices demonstrated dual resistance of thrombus accumulation and microbial adhesion (1). The modification provides a highly packed betaine surface coverage on medical devices, including those with complex shape, roughness and multiphase defects. This research further challenged these betaine-modified substrates with long-term serum incubation and tested biofilm resistance over a broad spectrum of microorganisms. The mechanical and chemical stability of the surface was also investigated.

Methods: *Materials:* Carbothane® extrusions (10 Fr by 3 cm) with 20% $BaSO_4$ (medical grade polyurethane used for dialysis catheters), modified with Semprus betaine polymers.

Serum exposure: Samples were exposed for periods up to 90 days in 50% fetal bovine serum (FBS) at 37˚C with weekly exchanges.

Static biofilm assay: Samples were exposed to 50% FBS for 18 hours at 37 ˚C with agitation, rinsed, and transferred to individual conical tubes containing an inoculum of 1e5 CFU/ml bacteria suspended in media appropriate for the test microorganism. Samples were agitated for 24 hrs at 37˚C followed by a PBS rinse, sonication, and quantitative plating. Log reductions in adherent bacteria were calculated as difference on control and modified materials. ANOVA analysis was performed to determine statistically significant differences (P-value<0.05). Test microorganisms included *S. aureus* ATCC 25923 and MRSA clinical isolates.

Biofilm flow assay: A modified CDC biofilm reactor system was utilized for growing biofilms under shear stress using *Escherichia coli* (ASTM E2562 – 07). Briefly, samples were seeded with1e6CFU/ml followed by nutrient flow of M63 for a period of 24 hours. Sonication and quantitative plating was used to assess adherent bacteria. Log reductions were calculated as previously described.

Mechanical and chemical stability: The friction co-efficient was measured using lubricity assessments with a standard Harland Medical model that measures force to pull through fixed clamps after hydration including after dynamic cycling. The samples were also challenged by tensile stretching and flexural bending, and the surface was characterized by optical microscopy, SEM, and ATR-FTIR.

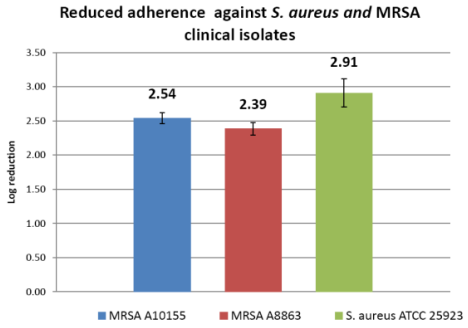

Figure 1. Reduced adherence against MRSA and *S. aureus* (static biofilm assay).

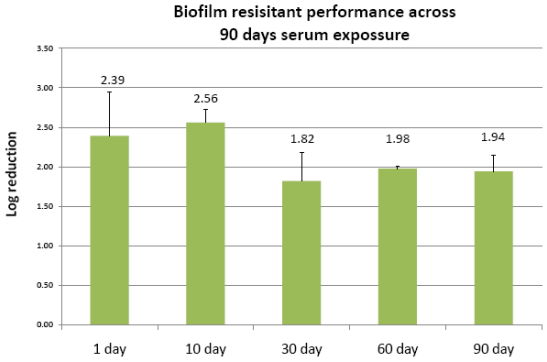

Figure 2. *E. coli* biofilm challenge after serum exposure (biofilm flow assay).

Results: Modified materials exhibited greater than 2 Log reduction relative to unmodified substrate after 24 hr serum preincubation for freshly harvested clinical MRSA isolates (Figure 1). Results from biofilm testing of Semprus modification demonstrated no statistically significant change in performance from one to 90 days of 50% FBS exposure (Figure 2). It was found that a surface modified with betaine-based polymers showed a 24% reduction in mean drag force up to 50 cycles compared with the first 10 cycles, demonstrating that lubricity is maintained after substantial abrasive challenge. The samples showed morphological and chemical consistence before and after the mechanical challenges.

Conclusions: Betaine-modified medical device surfaces demonstrated a broad spectrum reduction in microbial adhesion after long-term serum exposure up to 90 days. In addition, the modification was effective in reducing adherent bacteria of clinical MRSA isolates. The surface maintains both mechanical and chemical stability after mechanical deformation and abrasion.

References: 1. Z. Zhang, et al., Annual Meeting of the Society for Biomaterials; 2010 Apr 21-24; Seattle, WA.