Bio-Inspired, Engineered Microtopographies Reduce Platelet Adhesion and Activation on Blood-Contacting Materials

[a]Chelsea M. Kirschner, [a]Rhea M. May, [a]Ethan E. Mann, [a]Jay Fraser, [b]Christopher A. Siedlecki, [a,c,d]Anthony B. Brennan and [a]Shravanthi T. Reddy

[a]Sharklet Technologies, Inc., Aurora, CO, USA, [b]Departments of Bioengineering and Surgery, The Pennsylvania State University, Hershey Pennsylvania, USA, [c]Department of Materials Science and Engineering and [d]J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL USA

Statement of Purpose: Nearly six million central venous catheters (CVCs) are inserted each year in the United States (US) and as many as 67% of these patients develop catheter-related thrombosis (CRT), a complication associated with thromboembolic conditions – the leading cause of in-hospital mortality in the US[1]. Platelet adhesion and activation are key events in thrombus or clot formation on blood-contacting biomaterials. Thus understanding the complex interactions between biomaterial surface properties and platelets is important for developing vascular access devices that limit thromboembolic events.

Medical-grade poly(urethanes) are frequently used in blood-contacting medical devices due to their desirable mechanical properties and high level of hemocompatability. Moreover, it has been shown that sub-platelet-sized micropatterns reduce platelet adhesion[2]. Based on this evidence, we hypothesized that bio-inspired, antifouling Sharklet™ (SK) microtopographies replicated in biomedical thermoplastic poly(urethane) (TPU) reduce both platelet adhesion and activation compared to smooth (SM) controls.

Methods: Smooth and micropatterned samples were fabricated in Tecoflex® TPU (Lubrizol, EG85A) by hot embossing. Briefly, films were created by melting TPU pellets at 185°C on the bottom platen of a Carver press and then pressing against a SM or micropatterned mold for 2 min at 185°C and 40 Mpa. This process produced microtopographies with features protruding from the surface in a SK pattern (Fig. 1c) with an average height of ~3 μm and width and spacing of ~2 μm.

For each of three biological replicates, three 12 mm discs of each film were mounted around the perimeter of a 100 mm Petri dish. Platelet-rich plasma (PRP, Bonfils Blood Bank), derived from human, citrate-treated blood, was diluted to ~1x10^6 platelets/μL in phosphate buffered saline (PBS) supplemented with 10 mM $CaCl_2$. For both adhesion and activation experiments, samples were dynamically exposed to this PRP solution for 2 h at 37°C while rotating at 80 RPM. Samples were subsequently rinsed with PBS and fixed with 1% paraformaldehyde for 1 h. To quantify adhesion, platelets were immunostained for the $\alpha_{IIb}\beta_3$ integrin and the average area covered by platelets for each sample was measured using confocal microscopy. Platelet spreading was measured as an indicator of platelet activation. To quantify activation, samples were stained for F-actin to visualize morphology and the average platelet area was measured using confocal microscopy.

Results: Hot embossing biomedical TPU produced micropatterned and SM controls with identical surface chemistries to facilitate the evaluation of the influence of surface microtopography on platelet adhesion and activation. SK microtopographies reduced platelet adhesion by 86%, p=0.007 (Fig. 1) compared to SM controls.

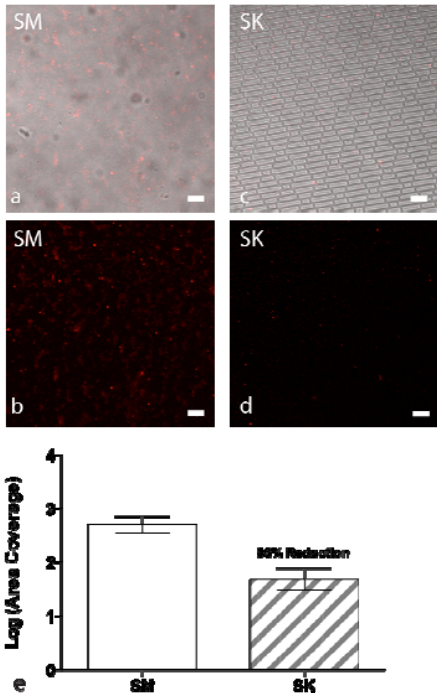

Figure 1. Representative images of platelet (red) adhesion on SM (a-b) and SK (c-d) samples. e) Quantification of immunofluorescent images revealed that the SK microtopography reduced the average area covered by platelets by 86%, p=0.007 compared to SM. Scale bars, 10 μm.

Similarly, platelet activation as measured by average individual platelet area was reduced significantly (68%, p=0.002) on the SK pattern compared to SM.

Conclusions: Here, we demonstrate that the application of engineered surface microtopographies to blood-contacting medical devices such as CVCs could further improve blood-biomaterial interactions and thus hemocompatibility without the application of anticoagulant coatings. Incorporating this technology into medical devices could also improve patient safety by reducing in-hospital morbidity, mortality and treatment costs.

References: [1] Burns KEA. Can J Anesth. 2008;55:532-541. [2] Milner KR. JBMR Part A. 2006;76A:561-570.

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