Platelet adhesion and activation on nanotextured polyurethane

K.R. Milner, H. Yamanka, K.A.V. Zubris, S.Z. Jones, A.J. Snyder and C.A. Siedlecki.
Pennsylvania State University, Milton S Hershey Medical Center, Hershey, PA.

Introduction: Polyurethanes are frequently used as blood-contacting materials because of their acceptable hemocompatibility. It has recently been demonstrated that sub-micron surface topographies may be used to reduce platelet adhesion to polyurethane and thereby possibly further reducing surface thrombosis. In this study the activity and procoagulant state of platelets adhered to smooth and textured polyurethane are compared. Preliminary results from time-varying fluid flow conditions are also presented, possibly indicating further reductions in platelet adhesion.

Methods: Ordered sub-micron topographies were fabricated in biomedical polyether(urethane urea) (PUU: Biospan MS/0.4) via a two-stage soft lithography replication molding technique. Master patterns were fabricated by Stepper lithography: pillars with 700nm width and separation; and pillars with 400nm width and separation. Pillar height was ~650nm. Molds were prepared by casting silicon elastomer over the masters. Smooth molds were also prepared. Replicas of the master patterns were fabricated by spin casting PUU in to the mold to a thickness of ~100μm.

Bovine blood was drawn into 3 U/ml heparin anticoagulant. Platelet-rich and -poor plasma was prepared by centrifugation and combined to form platelet-rich plasma samples (PRP) at physiological concentration (2.5×10^8 platelets/ml). PUU samples were cut to 15mm dia. with a steel die, mounted on a rotating disk system (RDS) and rotated at 238rpm for 2hr, exposing platelets to wall shear stress ranging linearly from 0-10dyn/cm^2 along the disk radius. PRP was replaced with buffer and platelet adhesion and procoagulant state were determined.

For platelet adhesion, samples were fixed and labeled for immunofluorescent microscopy. Variation in adhesion with shear was determined by counting adherent platelets at 1mm incremental radial distances. Image analysis software was used to assess platelet circularity and spreading. Time-varying fluid flow was simulated by superimposing a lateral wobble of ~100μm to the RDS shaft. This would be expected to generate a small, non-zero shear stress at the center of the PUU sample and possibly modest disturbance of the boundary layer at the sample surface, likely leading to a loss of laminar flow.

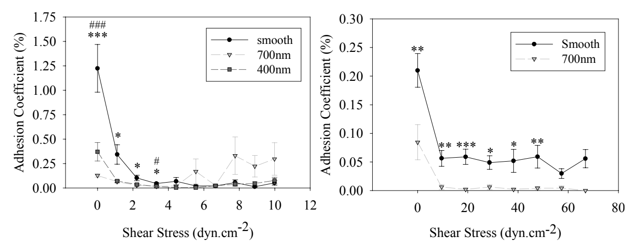
For platelet procoagulant state, a cloning ring was placed over the PUU exposing a 0-8dyn/cm^2 shear stress range. Bovine blood was drawn into sodium citrate, platelet-poor plasma was prepared (PPP) and recalcified, and a PPP aliquot was incubated for 30min in the cloning ring, contacting platelets adhered to the PUU. PPP aliquots were then assayed for FXa and meizothrombin production via chromogenic substrates and compared to PPP standard curves activated by Russell's viper venom and ecarin respectively. Both FXa and meizothrombin are intermediate steps in the coagulation cascade leading to thrombin and fibrin production, and are potentiated by platelet membrane cofactors.

Results / Discussion: Previous results[1] indicated platelet adhesion to 700 and 400nm pillar PUU was reduced compared to smooth at low shear stress <5dyn/cm^2 (Fig 1a). On smooth, 700 and 400nm PUU, mean platelet circularity (±SD) was calculated as 0.75±0.19 0.78±0.15 and 0.81±0.14 respectively and mean platelet area was 4.8±3.4, 5.4±3.0 and 4.3±2.2μm^2. No statistically significant differences were found, suggesting that platelet pseudopod formation and spreading, both measures of activation, are comparable on smooth and textured PUU. Preliminary results assaying two components of the coagulation cascade reveal no statistically significant difference in procoagulant state of platelets adhered to smooth or textured PUU samples (Fig 2), further supporting the finding that platelets adhered to textured PUU have a response comparable to those adhered on smooth PUU. Preliminary results of platelet adhesion to 700nm and smooth PUU under non-steady state fluid flow revealed a significant reduction in adhesion to 700nm PUU in a 0-50dyn/cm^2 range, greater than that observed under steady-state flow.

Conclusions: These results demonstrate that surface texture may act to reduce platelet adhesion to PUU at low shear stress. Platelets that do adhere to the PUU appear to have similar activity based on circularity, spreading and procoagulant potential. Preliminary results indicate further improvements may be achieved in non-steady state flow such as *in vivo* pulsatile conditions.

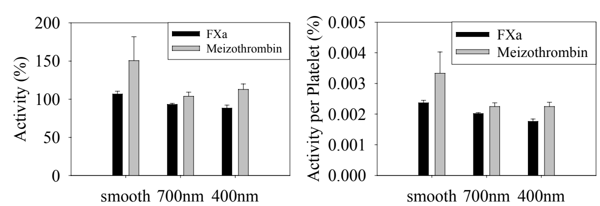
References:

1. Milner KR. ASAIO J. 2005;51:578-584; J. Biomed. Mater. Res. A (in press)



(a) (b)

Fig 1: Variation in platelet adhesion with shear stress from (a) 0-10dyn/cm^2 with uniform flow and (b) 0-67dyn/cm^2 with time-varying flow



(a) (b)

Fig 2: Activity of FXa and meizothrombin on smooth and textured PUU in (a) 0-8dyn/cm^2 shear stress range and (b) normalized to estimated adherent platelets as percentage of standard curve from activated PPP