Platelet Adhesion to Nanotextured Polyurethane under Pulsatile Flow

K.R. Milner, A.J. Snyder and C.A. Siedlecki
Pennsylvania State University, Milton S Hershey Medical Center, Hershey, PA.

Statement of Purpose: Blood-contacting medical devices run the risk of thrombosis occurring at the blood-material interface and subsequent thromboembolism. The initial step in thrombosis is protein adsorption to the material that may i) mediate adhesion and subsequent activation of blood platelets to which other platelets may aggregate and ii) activate the intrinsic component of the coagulation cascade producing fibrin polymer strands. These events are intertwined with fibrin strands stabilizing the platelet aggregate, activated platelets acting as cofactors for the coagulation cascade, and the coagulation cascade producing the platelet agonist thrombin. It has been previously demonstrated that sub-platelet sized textures on the material surface reduce platelet adhesion under low, uniform shear stress. The textures are hypothesized to restrict the surface area accessible to platelets, thereby reducing the probability of platelets contacting adsorbed adhesive ligands and improving hemocompatibility. In this study, platelet adhesion to textured polyurethane is assessed under physiologically relevant pulsatile flow.

Methods: A two-stage replication molding technique was used to create sub-platelet sized surface topographies on a biomedical polyether(urethane urea) (PUU). Master templates of ordered arrays of sub-micron pillars were fabricated via Stepper lithography on 6" dia. silicon wafers. Pillars had 700nm width and separation or 400nm width and separation with ~650nm height. Non-textured substrates were used a controls. Molds were prepared by casting silicone elastomer over these masters. Replicas were prepared by spin casting Biospan MS/0.4 PUU in to the molds to a thickness of ~100μm.

Three parallel plate flow chambers were fabricated with 25.4mm chamber length, 8mm width and 200μm height. Samples of smooth, 700nm and 400nm textured PUU were mounted on glass microscope slides in the chambers. Silicone tubing was connected to the chamber inlets and outlets. Platelet-rich and platelet-poor plasma (PRP and PPP) were prepared by centrifugation of bovine blood containing 3U/ml heparin anticoagulant. PRP and PPP were combined to yield a physiological concentration of $2.5x10^8$ platelets/ml. The three chambers were positioned in parallel and a three-channel, programmable peristaltic pump was used to prime the chambers with PBS. The pump was programmed with two pulsatile waveforms for peak wall shear stress in the chambers of 1 or 10dyn/cm^2 (Fig1a). Reynolds numbers at 1 and 10dyn/cm^2 were 0.94 and 9.4 respectively, ensuring laminar flow. Entrance lengths were 18μm and 183μm; negligible compared to chamber length. PRP was drawn through the chambers from a stock sample for either 60min (1dyn/cm^2) or 30min (10dyn/cm^2). Outlet PRP was collected from each of the three chambers for flow cytometric evaluation of platelet activity via binding of annexin-V. Chambers were rinsed with PBS and 1% PFA. Adherent platelets were enumerated following immunofluorescent labeling for platelet integrin $\alpha_{IIb}\beta_3$. The experimental setup is shown in Fig 1b.

Results/Discussion: The two-stage molding process replication efficiency has been previously demonstrated for fabricating 700nm and 400nm pillars in to PUU from master templates of scale comparable to practical medical devices (Fig2). These sub-micron textures have been demonstrated to reduce adhesion of platelets exposed to non-pulsatile, uniform shear stress <5dyn/cm^2 (Milner KR. J Biomed Mater Res A. 2006;76:561-570). Under physiological pulsatile shear stress, it is unclear how platelet adhesion to textured polyurethanes varies with maximum and minimum shear values and with waveform period. The three-channel system described here allows platelet adhesion to smooth, 700nm and 400nm PUU to be simultaneously assessed in parallel from a single PRP sample. Initial tests assessing non-adherent platelets via flow cytometry indicate minimal activation by passage through the chambers and flow loop.

Conclusions: It has been demonstrated that ordered arrays of sub-platelets sized PUU pillars act to reduce the adhesion of platelets exposed to low, uniform wall shear stress. Additionally, platelets adherent to smooth and textured PUU showed comparable activation as determined by morphology and coagulation cascade propagation. The system presented here assesses platelet adhesion under more physiologically relevant pulsatile fluid flow in preparation for *in vivo* investigations. Ongoing work is focusing on fabricating PUU tubes containing sub-micron textures for use as inlet and outlet cannulae of pediatric ventricular assist devices.

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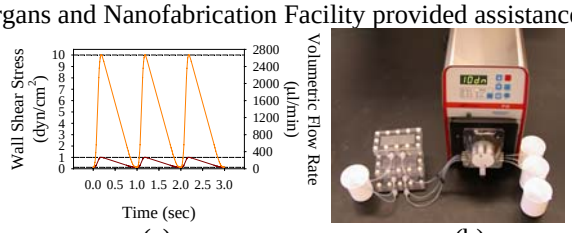
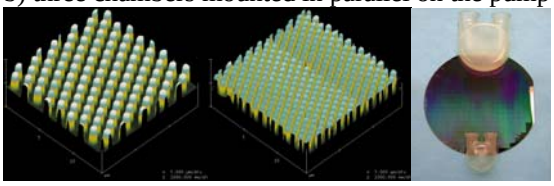


(a) (b)

Figure 1. Experimental setup showing a) waveforms with 1sec period and peak shear stress of 1 or 10dyn/cm^2 and b) three chambers mounted in parallel on the pump



(a) (b) (c)

Figure 2. 15 x 15 μm atomic force microscope images of a) 700nm and b) 400nm with c) a 6" silicon master alongside 70cc and 15cc PUU ventricular assist devices