Studying the Synergistic Effect of Coatings and Nitric Oxide release on Platelet Adsorption

Kagya A Amoako [1, 3, 5], Hari S Sundaram[3], Ahmed Suhaib[5], Yuting Li[3], Jules Lin[1], Shaoyi Jiang[3], Keith E Cook[1, 2, 3]
[1]Department of Surgery, University of Michigan – Ann Arbor
[2]Biomedical Engineering, University of Michigan – Ann Arbor
[3]Chemical Engineering, University of Washington – Seattle
[4]Department of Chemistry, University of Michigan – Ann Arbor
[5]Cook Lab, University of Michigan– Ann Arbor

Statement of Purpose: Surfaces of blood-contacting medical applications are chemically and functionally different from healthy blood vessels. Nonspecific protein adsorption to such surfaces followed by platelet activation lead to poor device function and subsequent acute inflammatory response. Resistance to nonspecific protein adsorption, cell/bacterial adhesion, and biofilm formation is critical for the development and performance of biomedical and analytical devices. Significant efforts have been made in the development of biocompatible and bioactive materials for antifouling surfaces, but much of the work either focus on antifouling coatings or nitric oxide (NO) rich surfaces, and not both. In contrast to the separated approaches, the endothelium uses, among other mechanisms, NO secretion and an antifouling surface to prevent coagulation. In this preliminary study, low fouling polycarboxybetaine (pCB), polysulfobetaine (pSB), random copolymer (R3) and polymethoxyethylacrylate (PMEA) were grafted onto polypropylene (PP) and polydimethylsiloxane (PDMS) membranes and tested for platelet adsorption under flow conditions using platelet rich plasma (PRP). The effect of NO release from pCB-grafted surfaces on platelet adsorption was also evaluated.

Methods: A 3.0 x 0.5 inch microporous PP (0.1 µm porosity, Sterlitech membranes Inc., Kent WA) and Sylgard 184 PDMS membranes (Dow Corning, Elizabethtown KY) were surface grafted with pCB using "graft-to" immobilization approach. To test for antifouling, the membranes were held in a bioreactor flow chamber, which allowed PRP flow (0.3L/min) over their coated surfaces and NO gas (100 ppm, 0.6L/min) on the opposite surface. PRP was prepared from sheep blood by centrifugation at 730 rpm for 20 min for PRP supernatant. The packed cells were then centrifuged at 2750 for 15 min for platelet poor plasma (PPP) supernatant. PPP was re-suspended in PRP to achieve a final concentration of $1x10^8$ cells/mL. PRP was re-circulated for 8hrs with and without NO flow. Platelet adsorption was quantified using the lactate dehydrogenase (LDH) assay (Cayman Chemicals Ann Arbor, 10008882). Blood draws were conducted in accordance with UCUCA standards.

Results: A model of pCB/pSB-grafted surfaces, which illustrates electrostatically induced hydration of polymeric surfaces to reduce nonspecific protein and platelet fouling is show in Fig. 1. Preliminary fouling results on such surfaces indicate that: 1) surface grafted membranes reduce platelet adsorption and fibrinogen fouling (Figs. 2A and 3A), 2) NO secretion at membrane surface reduce platelet adsorption (Fig. 3B), and 3) the synergistic effect of coating and NO on platelet adsorption is additive (Fig. 3B). Adsorbed platelet distribution shown in Fig. 2B also reflect quantitative platelet adsorption data trend in Fig. 2A.

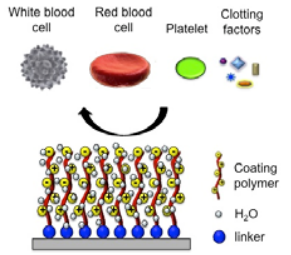

Figure 1: Resistance to nonspecific adsorption of plasma proteins, platelets, and cells involved in surface-induced clot formation.

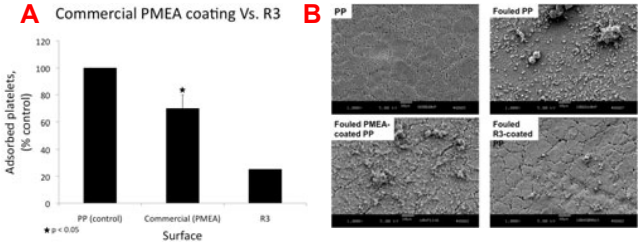

Figure 2: Adsorbed platelets on uncoated PP, PP coated with PMEA, and PP coated with R3 surface, as measured by LDH activity (A), and SEMs of PP surface, and platelet adsorption on uncoated PP, PP coated with PMEA, and PP coated with R3 (B).

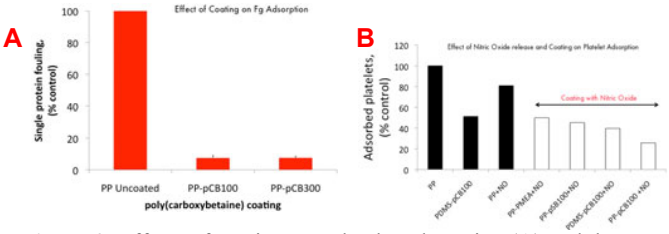

Figure 3: Effects of coatings on platelet adsorption (A) and the synergistic effects of coatings and nitric oxide release on platelet adsorption (B).

Conclusion: Zwitterionic polymers, especially pCB and pSB, have been applied to a wide range of biomedical and engineering materials. pCB , pSB and R3 random copolymer modified surfaces have shown high resistance to nonspecific protein fouling. Our current challenge now is testing these coatings against more complex biological media, ultimately blood, for longer flow durations. The preliminary data herein show a promising approach for meeting these challenges.

Future direction: Several optimizations in the grafting process such as increasing graft density and graft stability testing is, however, needed to achieve super-low fouling on PP and PDMS. Further testing along with NO is also needed.

Reference: Shaoyi Jiang et al. Advanced Materials 2010; 22:920–932.