Polyurethane Modified with an Antithrombin-Heparin Complex (ATH) via PEO: Effect of PEO End Group on ATH Immobilization and Subsequent Antithrombin and Fibrinogen Binding

Kyla N. Sask,[1] Leslie R. Berry,[2] Anthony K. C. Chan,[1,2] John L. Brash,[1]
[1]School of Biomedical Engineering, [2]David Braley Research Institute, McMaster University, Hamilton, Ontario, Canada.

Introduction: Polyurethanes are frequently used in medical device applications that involve blood contact. Like most materials, proteins rapidly adsorb to their surface resulting in thrombosis and other adverse effects. A common method to reduce protein adsorption is to graft hydrophilic polymers such as polyethylene oxide (PEO) to polyurethane (PU). Bioactive molecules can also be tethered to functionalized PEO surfaces to provide anticoagulant activity and improved blood compatibility. These two strategies must be balanced to maintain PEO's protein repellent effect as well as the activity of the anticoagulant. A covalent antithrombin-heparin (ATH) complex developed in our lab has demonstrated potential as an anticoagulant surface modifier (alternative to heparin)[1]. ATH immobilized on gold showed higher antithrombin (AT, the target protein for heparin) selectivity than analogous heparinized surfaces; however the need to increase uptake on PEO modified surfaces was evident[2]. The goal of the present work was to optimize ATH attachment and increase subsequent AT binding to PEO modified PU, while achieving low nonspecific adsorption of other proteins. The use of a hetero-bifunctional PEO was introduced and compared with a typical dihydroxy PEO to attach ATH and heparin.

Methods: Tecothane® PU films were functionalized with NCO groups by reaction with methylene-bis-(4-phenyl-isocyanate). PEO was grafted to NCO via chain end groups; dihydroxy PEO by reaction with OH (PEO-OH) and carboxy-amino-PEO by reaction with NH_2 (PEO-COOH). To introduce N-hydroxysuccinimide (NHS) groups for attachment of ATH and heparin, surfaces were reacted with either N,N'-disuccinimidyl carbonate and triethylamine (conversion of PEO-OH) or 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) and NHS (conversion of PEO-COOH). Surfaces were characterized by water contact angle and fibrinogen (Fg) adsorption from PBS (^{125}I-labeling). ATH uptake from PBS was evaluated by ^{125}I-labeling. After adsorption, surfaces were treated with SDS and the remaining ATH was measured to assess strength of binding. In addition AT and Fg adsorption from plasma were measured simultaneously (^{125}I-AT, ^{131}I-Fg) as an indication of heparin bioactivity and nonspecific protein adsorption, respectively.

Results and Discussion: PU modification with PEO using both functionalization methods was confirmed by decreases in water contact angle and a reduction in Fg adsorption from PBS of over 70% compared to PU. ATH uptake was high on PU and PU-NCO surfaces and much lower on PEO modified surfaces (Figure 1). Following treatment with SDS, most of the ATH was removed from PU, PEO-OH and PEO-COOH indicating relatively weak attachment. On the PU-NCO, and on the PEO surfaces functionalized with NHS a high proportion of ATH remained after SDS, likely indicating covalent attachment through amino groups on ATH. Data on adsorption from plasma (Figure 2) showed the highest amounts of Fg on PU-NCO and the lowest on PU-PEO. Although the PU-NCO-ATH surface had the highest density of ATH (Fig 1), it adsorbed Fg and AT in similar molar amounts (Fig 2). Both of the PEO-ATH surfaces showed a strong preference for AT over Fg, whereas the PEO-heparin surfaces "preferred" Fg and bound relatively small amounts of AT. Work is ongoing to investigate the effect of PEO end group as a function of PEO molecular weight. An optimum balance between resistance to nonspecific adsorption and selectivity for AT binding is expected to result in increased anticoagulant activity.

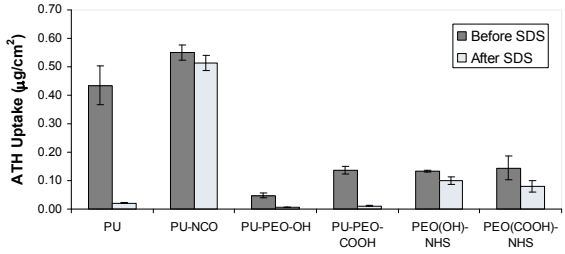

Figure 1. ATH uptake from PBS before and after SDS treatment. Data are mean ± SD, n =3.

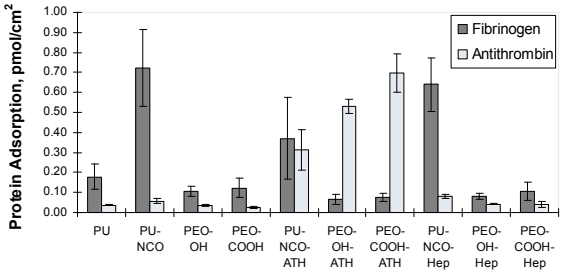

Figure 2. Fg and AT adsorption from plasma (2 h) to various surfaces. Data are mean ± SD, n ≥3.

Conclusions:
PU surfaces were modified with PEO having different end groups for attachment of ATH or heparin. In contact with plasma the PEO-ATH surfaces adsorbed AT in preference to Fg, indicating strong biospecificity of the heparin moiety and anticoagulant.

References:
1. Klement P. et al. Biomaterials. 2002;23:527-535.
2. Sask KN. et al. Acta Biomater. 2010;6:2911-2919.

Acknowledgements:
Work supported by NSERC (Canada) and the Canadian Institutes of Health Research (CIHR).