Influence of Polyethylene Oxide Spacer on Anticoagulant Properties of Immobilized Antithrombin-Heparin Complex

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Introduction: Upon exposure of materials to blood, rapid protein adsorption and platelet adhesion leads to thrombosis. Such blood-surface interactions can be controlled by modifying materials with protein and cell resistant agents such as polyethylene oxide (PEO), and with molecules that provide anticoagulant or antithrombotic activity. Immobilization of heparin to inhibit thrombin is one such approach. As an alternative, we have developed a covalent antithrombin-heparin (ATH) complex that has many advantages over heparin[1]. Surfaces based on ATH immobilization on model gold substrates using linker and spacer molecules, including PEO, have demonstrated its superior bioactivity[2]. In the present work ATH and PEO were immobilized on polyurethane (PU) as a more practical substrate. The use of an initial PEO layer provides resistance to nonspecific protein adsorption (as well as spacer functions), but can limit the uptake of ATH and subsequent binding of antithrombin (AT), the target protein in blood contact. To study this effect ATH-PEO surfaces having a range of PEO molecular weights were investigated. AT binding was measured to assess anticoagulant potential.

Methods: Polyurethane (PU) films were cast from solutions of Tecothane® in dimethyl formamide. NCO groups were introduced by reaction with methylene-bis-(4-phenyl-isocyanate). PEO was grafted by reaction with the NCO groups. The PEO molecular weight was varied from 300 to 4600. To attach ATH or heparin to the PU-PEO, surfaces were reacted with N,N'-disuccinimidyl carbonate and triethylamine in anhydrous acetonitrile. The resulting N-hydroxysuccinimide (NHS) functional chain ends were then available to interact with amino groups in the ATH and heparin. Uptake of ATH and adsorption of AT were measured using radioiodination methods. Labelled AT was added to citrated human plasma at 5% of the physiological concentration.

Results and Discussion: Modification of the surfaces was monitored via water contact angle. Immobilization of PEO caused a large decrease in angle and grafting of ATH to PU-PEO caused a smaller increase. Uptake of ATH on PU-PEO surfaces with various PEO molecular weights is shown in Figure 1. The PU control showed the greatest uptake; however, subsequent treatment with SDS caused almost complete elution showing that the ATH was only physically adsorbed. For the PU-PEO surfaces, the greatest density of ATH was achieved on the surface with the lowest MW PEO (300). SDS treatment removed only small quantities of ATH from these surfaces, suggesting that attachment occurred by reaction with the NHS terminal groups on PEO to form covalent bonds. In blood contact, antithrombin adsorption is required for the anticoagulant effect of the heparin component of ATH. As shown in Figure 2, the ATH surfaces demonstrate significantly greater antithrombin adsorption than control surfaces or analogous surfaces modified with heparin, thus again showing the superiority of ATH over heparin. AT adsorption on ATH surfaces decreased as PEO molecular weight increased, whereas resistance to nonspecific protein is known to increase. Thus an optimal balance may exist between these apparently conflicting effects of anticoagulant protein uptake and resistance to coagulant protein binding.

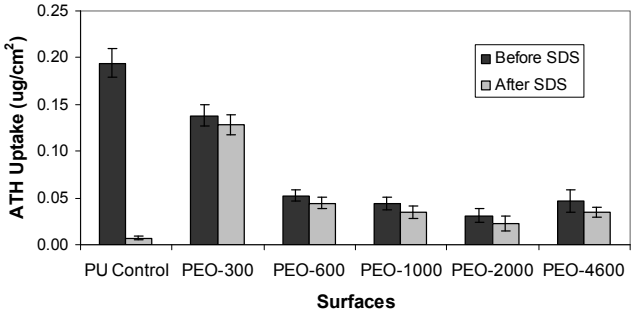

Figure 1. Uptake of ATH on surfaces before and after SDS treatment. Data are mean ± SD, n =3.

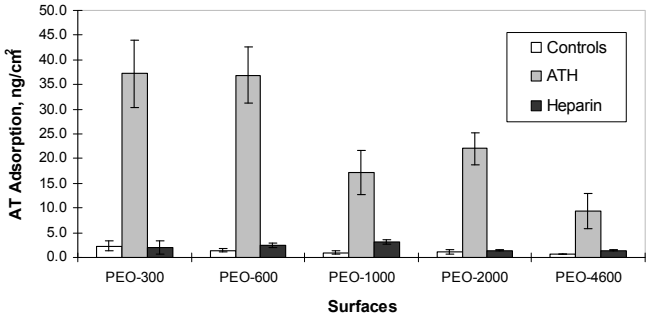

Figure 2. Antithrombin adsorption from plasma (2 h). Data are mean ± SD, n = 3.

Conclusions: Techniques to modify polyurethane surfaces sequentially with PEO and an antithrombin-heparin complex have been developed. Surfaces with PEO of lower molecular weight showed greater ATH uptake and adsorbed greater quantities of antithrombin from plasma. Additional characterization and bioactivity studies on these surfaces, including platelet interactions and clotting assays are underway.

References:

1. Patel S. et al. Thrombos Res. 2007;120:151.
2. Sask KN et al. Trans Soc Biomat. 2009;31:439.

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