Immobilization of Polyethylene Glycol-Corn Trypsin Inhibitor on Polyurethane for Inhibition of Contact Factor Activation upon Blood Contact

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Introduction: Blood-contacting materials initiate coagulation via the contact factor pathway. Activation of factor (F)XII to FXIIa, which is the initial step in this pathway, leads to fibrin and thrombus formation. In this study, we used corn trypsin inhibitor (CTI), a 12.5 kDa protein which specifically inhibits FXIIa[1] in combination with PEG (for general protein resistance) to modify the surface of a polyurethane. In previous work using gold as a model substrate, we studied PEG-CTI immobilization using two methods: sequential (PEG immobilization followed by CTI conjugation), and direct (preparation of a PEG-CTI conjugate, which was then immobilized on the surface).[2] We showed that the PEG-CTI combination was more effective when attachment was sequential. In this study, we applied these methods for PEG-CTI attachment to polyurethane as a substrate that would have applicability to blood-contacting medical devices.

Methods: Primary amino groups of CTI were converted to thiol using Traut's reagent (product: thiol-CTI). Polyurethane discs (Tecothane®) were functionalized with NCO groups by reaction with methylene-bis-(4-phenyl-isocyanate) (MDI). Amino-PEG-maleimide was used to graft polyethylene glycol via the NCO groups (product: PU-PEG-MAL). Surfaces were then incubated with thiol-CTI (product: PU-PEG-CTI). PU and PU-NCO surfaces were also incubated with thiol-CTI as controls. Water contact angles and uptake of ^{125}I-labeled CTI were measured to verify surface modification. Fibrinogen (Fg) adsorption from buffer and plasma was determined to assess resistance to nonspecific adsorption. A chromogenic substrate assay was used to measure the ability of the surfaces to inhibit FXIIa. Plasma clotting times were used to assess anticoagulant activity.

Results and discussion: The water contact angle (Table 1) decreased on modification of PU with PEG. CTI uptake was the highest on PU-NCO followed by PU-PEG-MAL and PU. PU-PEG-CTI showed significantly lower Fg adsorption compared with the other CTI surfaces (Fig 1) indicating the effect of the PEG. FXIIa inhibition was highest on the PU-PEG-CTI surface (Fig 2). Despite having lower CTI density than PU-NCO-CTI, PU-PEG-CTI showed significantly higher FXIIa inhibition, suggesting optimal "presentation" of CTI on this surface. Two factors may contribute to this behavior. First, the orientation of CTI may be more optimal when it is attached to PEG via the thiol groups, and second, the spacer effect of PEG may increase CTI reactivity. Prolonged clotting times were observed on PU-PEG-CTI compared with controls, again suggesting that CTI was able to inhibit FXIIa on this surface.

Table 1: Water contact angles and CTI densities. (mean±SD, n>3).

Surface	Contact angle (degrees)	CTI density (μg/cm^2)
PU	94±5	N/A
PU-CTI	92±3	0.16±0.03
PU-NCO	85±3	N/A
PU-NCO-CTI	73±5	0.53±0.23
PU-PEG-MAL	46±3	N/A
PU-PEG-MAL-CTI	55±4	0.22±0.03

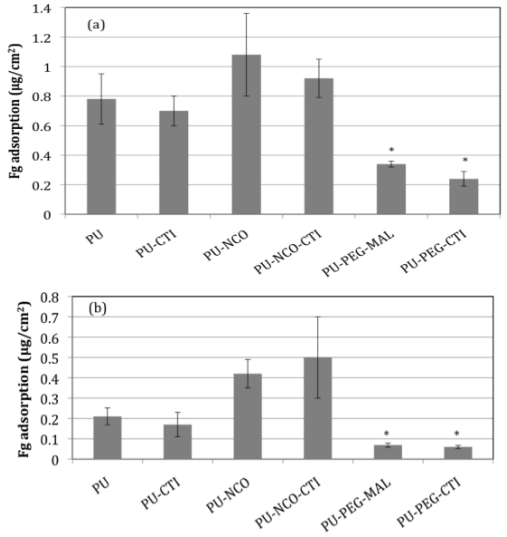

Figure 1: Fg adsorption (3 h) from: (a) buffer, (b) plasma. Data are mean±SD (n=3). *p≤0.05 (vs other surfaces).

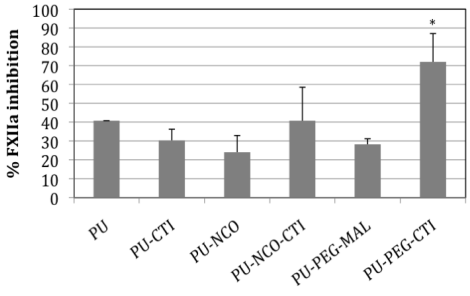

Figure 2: FXIIa inhibition (chromogenic substrate assay). Data are mean±SD (n=3). * p≤0.05 (vs other surfaces).

Conclusions: Of the three CTI surfaces investigated, the PU-PEG-CTI surface exhibited the greatest FXIIa inhibition and protein resistance. Modification with PEG-CTI may be a useful approach to prepare thromboresistant materials.

References:

1. Hojima Y et al. Thrombos. Res. 1980;20:149.
2. Alibeik S et al. Proceedings SFB, Seattle, 2010.

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