Immobilization of corn trypsin inhibitor for inhibition of the contact factor pathways on blood-contacting materials

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Introduction: Blood-contacting materials trigger blood coagulation by activating the contact factor pathway. The initiating step is the activation of factor (F) XII to FXIIa, leading ultimately to thrombin generation and fibrin formation. Previous attempts to prevent biomaterial-induced coagulation have mainly targeted the later stages of the blood coagulation pathway. For example, heparin, which mainly targets FXa and thrombin, and hirudin, which inhibits thrombin, have been immobilized on the surface of biomaterials to render them non-thrombogenic. Because FXIIa is the initiator of biomaterial-induced clotting, we modified commercial catheter surfaces with corn trypsin inhibitor (CTI)[1], a 12.5 kDa protein that specifically inhibits FXIIa.[2] In the work reported here, different methods of surface modification with CTI were developed in an attempt to optimize anticoagulant properties. Starting with gold as a model substrate amenable to detailed surface characterization, we made modifications using combinations of polyethylene glycol to minimize non-specific protein adsorption and CTI to inhibit FXIIa.

Methods: Using Traut's reagent, the primary amino groups of lysine residues in CTI were converted to thiols. The CTI-thiol was then immobilized on gold-coated silicon wafers using three different methods:

Method 1: PEG-NHS ester disulfide (MW=1100) was first immobilized on the gold-coated surface and then reacted with amino-PEG-maleimide (MW=2000). CTI-thiol was then conjugated to the PEG-modified gold surface.

Method 2: PEG-NHS ester disulfide was first immobilized on the gold-coated surface. CTI-thiol was conjugated to amino-PEG-maleimide and the conjugate was then reacted with the PEG-modified gold.

Method 3: CTI-thiol was conjugated to amino-PEG-maleimide. After reacting the PEG-CTI conjugate with PEG-NHS ester disulfide, the final product was then immobilized on the gold-coated surface.

Water contact angles and ellipsometry were used to confirm surface modifications. CTI densities on surfaces were determined using ^{125}I-labeled CTI or PEG-CTI conjugates. The abilities of the surfaces to bind and inhibit FXIIa were assessed using a chromogenic substrate assay, while the procoagulant activities of the surfaces were compared using a clotting assay.

Results and discussion: The water contact angle decreased upon PEG attachment to gold and, as expected, increased with CTI conjugation (Table 1). Surfaces prepared by method 3 showed the highest contact angle. CTI density was also highest on the method 3 surface. The lower CTI densities for methods 1 and 2 likely reflect the protein resistance of the pre-immobilized PEG layer.

All of the CTI-modified surfaces exhibited greater FXIIa inhibitory capacity than the control surfaces (Fig 1). Surfaces prepared by methods 1 and 3 had highest FXIIa inhibitory capacities, likely reflecting the higher densities of CTI on these surfaces. The PEG-CTI surfaces also exhibited less procoagulant activity than the controls as evidenced by longer clotting times (Fig 2). Method 1 surfaces had the least procoagulant activity, suggesting that CTI is optimally deployed when immobilized by this method.

Table 1: Water contact angles and CTI densities of control and PEG-CTI surfaces. Data are mean±SD (n>3).

Surface	Contact angle (degrees)	CTI density (μg/cm^2)
Au (control)	56±5	N/A
Au-PEG-OH (control)	30±2	N/A
Method 1	34±1	0.07±0.01
Method 2	36±2	0.05±0.002
Method 3	48±3	0.24±0.007

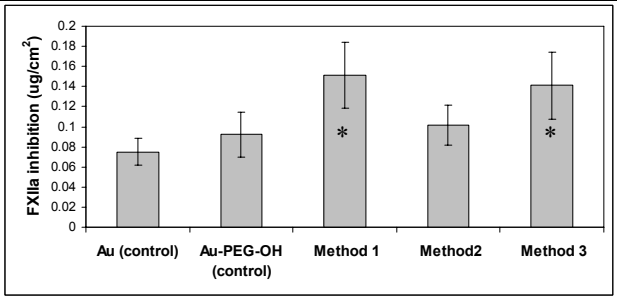

Figure 1: FXIIa inhibitory capacity (chromogenic substrate assay). Data are mean±SD (n=3). * p≤0.1 vs. control surfaces.

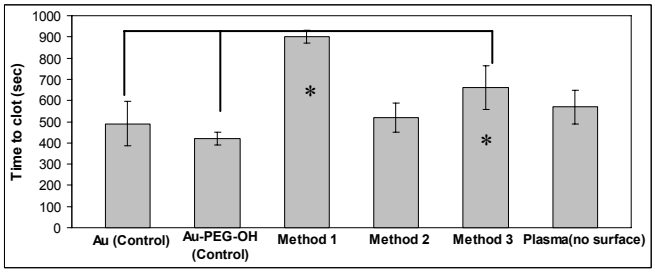

Figure 2: Procoagulant activity. Data are mean±SD (n=3). *p≤0.05 vs control surfaces.

Conclusions: These studies confirm our previous observation[1] that PEG-CTI modified surfaces show reduced procoagulant activity because of their capacity to inhibit FXIIa. We now show that the activity of immobilized PEG-CTI can be optimized depending on the method of attachment. Immobilized CTI may be useful to render blood-contacting biomaterials non-thrombogenic.

References:

1. Yau J. MASc thesis, McMaster University.
2. Hojima Y et al. Thrombos. Res. 1980;20:149.

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