Tissue Plasminogen Activator–Containing Polyurethane Surfaces for Fibrinolytic Activity

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Introductions: Major reasons for the failure of blood-contacting devices are coagulation and thrombus formation. One approach to preventing clot formation on implanted biomaterials is to design the surface to take advantage of the natural fibrinolytic or clot-dissolving capacity of the body [1]. Tissue plasminogen activator (t-PA) is a serine protease sequestered by endothelial cells that cleaves plasminogen to generate plasmin, the clot-dissolving enzyme [2]. In the present work, we developed a simple process that can be carried out under mild conditions for loading t-PA into polyurethane (PU) surfaces. This approach may have potential for the development of surfaces which can lyse clots that begin to form on them.

Methods: Poly(dimethylaminoethyl methacrylate) (PDMAEMA)-modified PU surfaces were prepared by free radical graft polymerization on double bond-modified surfaces [3]. The amino groups on the resulting PU-*g*-PDMAEMA surfaces were then quaternized with iodomethane or 1,6-diiodohexane or dichlor-*p*-xylene. Finally, these materials (PU-CH_3I, PU-$I(CH_2)_6I$, PU-Cl) were treated with t-PA in tris-buffered saline (TBS, pH 9.0) to give t-PA-loaded PU surfaces. The t-PA content of the surfaces was determined by radiolabelling. The activity of the bound t-PA was measured by a plasma clotting-dissolution assay and a chromogenic substrate assay (S-2251^{TM}).

Results: Since the quaternized surfaces are positively charged and t-PA (pI 6.5-7.5) is negatively charged at pH 9, it is expected that t-PA will be taken up by the quaternized PU surfaces via electrostatic interactions at this pH. Uptake on the PU-Cl, PU-CH_3I and PU-$I(CH_2)_6I$ surfaces was 6.27, 4.59, and 5.97 μg/cm^2, respectively, viz. ~14-fold, 10-fold and 13-fold greater than on the unmodified PU (adsorption of t-PA from 0.3 mg/mL solutions in TBS). These data show that PU surfaces having high t-PA content were obtained.

Data on the release of t-PA from the materials in contact with plasma are shown in Figure 1. The rates of release were in the order PU-CH_3I > PU-$I(CH_2)_6I$ > PU-Cl. From the shape of the release curves it is clear that slow release of t-PA would continue beyond the 48-hour time frame.

The enzymatic activity of t-PA released from the quaternized PU surfaces at different times was measured using a chromogenic assay. After 6 days, the activity of t-PA released from the PU-Cl, PU-CH_3I and PU-$I(CH_2)_6I$ surfaces was approximately 74%, 58% and 59% that of a control t-PA sample, suggesting that the enzymatic activity was largely preserved in the surface-associated state over this time period.

To determine whether the bound t-PA retained its fibrinolytic activity, surfaces that had been incubated for 48 h in plasma (Figure 2) were used in a clot formation-lysis assay. The PU-CH_3I surface, with the highest t-PA release rate, showed the highest fibrinolytic activity and the PU-Cl surface, with the lowest release rate, the lowest activity. These data suggest that the fibrinolytic activity of t-PA was maintained on all three of the quaternized surfaces.

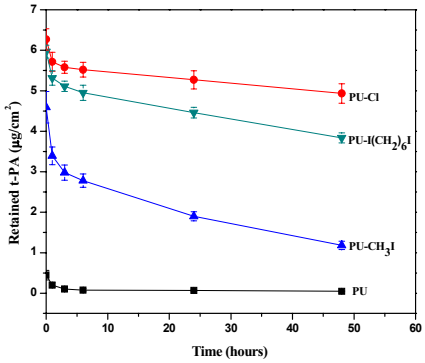

Figure 1. Release of t-PA in contact with human plasma. Data are means ± standard error (n = 3).

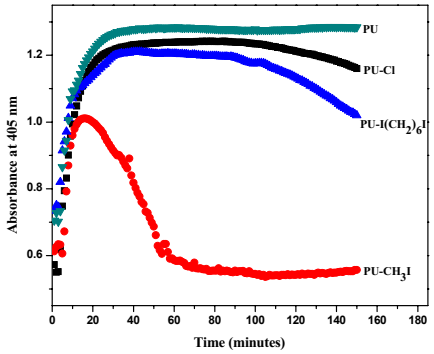

Figure 2. Clot formation-dissolution on PU surfaces.

Conclusions: t-PA immobilization on modified PU surfaces via electrostatic interactions gave high uptake with long term retention of fibrinolytic activity. Of the three modified surfaces studied, the most rapid release of t-PA into plasma and the most rapid lysis of surface-adjacent clot occurred on the surface modified with PU-CH_3I as the quaternizing agent. The fibrinolytic behavior of these t-PA containing polyurethanes may make them interesting candidates as materials for minimizing thrombus formation in blood-contacting devices.

References: [1] McClung WG *et al.* J. Biomed. Mater. Res. 2003; 66A: 795–801.
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