Lysine-PEG modified polyurethane: effect of PEG spacer length on plasminogen capture and platelet adhesion.

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Introduction: Modification with poly(ethylene glycol) (PEG) is the most widely used approach to bioinertness due to the excellent resistance of PEG to protein adsorption and cell adhesion [1]. PEG has also been used as a spacer to couple bioactive moieties to surfaces, thus potentially exerting both "bioinert" and "bioactive" functions [2,3]. From this perspective, the aim of our work is to develop the concept of a fibrinolytic surface on which PEG is used as a spacer to immobilize lysine such that the ε-amino group is free to capture plasminogen in blood contact. We showed previously that these surfaces suppress nonspecific protein adsorption and are able to bind plasminogen from plasma. However, the rate of plasminogen uptake was relatively slow due to the protein repellent properties of the PEG and the enhanced mobility of the chain-end conjugated lysines [4]. In the work reported here we investigated the balance between the rate of plasminogen uptake and suppression of nonspecific protein adsorption using PEG spacers of molecular weight 300 and 1000. We also studied the interactions of platelets with these surfaces.

Experimental: The PEG (MW 300 and 1000) and lysine modified polyurethane surfaces were prepared as described [4]. The adsorption of fibrinogen (buffer) and plasminogen (plasma) were measured by radiolabeling. Surfaces were also incubated in plasma and the adsorbed proteins eluted and examined by SDS PAGE and immunoblotting. Quantitative studies of fibrinogen adsorption and platelet adhesion from whole blood were conducted under flow conditions (300 s^{-1} wall shear rate) as described [5]. To assess clot lysing activity, the surfaces were incubated in plasma and treated with t-PA. They were then incubated in recalcified plasma and absorbance at 405 nm measured over time [6].

Results and Discussion: Fibrinogen adsorption from buffer was greatly reduced on the PEG grafted surfaces (no lysine) compared to the control polyurethane. The PEG-Lys surfaces were also fibrinogen resistant though less so than the PEG alone. The lysine-derivatized surface with PEG300 as spacer (PEG300-Lys) adsorbed plasminogen from plasma more rapidly than the PEG1000-Lys, although the ultimate adsorbed quantities were the same (Figure 1). The suppression of adsorption to the surfaces modified with PEG alone (no lysine) was very low. These results suggest that the optimum spacer length for protein resistance and plasminogen binding on the PEG-Lys surfaces is relatively short.

The immunoblot data on the PEG300 and PEG300-Lys series (not shown) indicated that most of the proteins nonspecifically adsorbed on the PU surface were suppressed on the modified surfaces. Moreover only the PU-PEG300-Lys blot showed clear evidence of plasminogen adsorption with a strong band at ~94 kDa , again indicating the specific affinity of ε-amino free lysine for plasminogen.

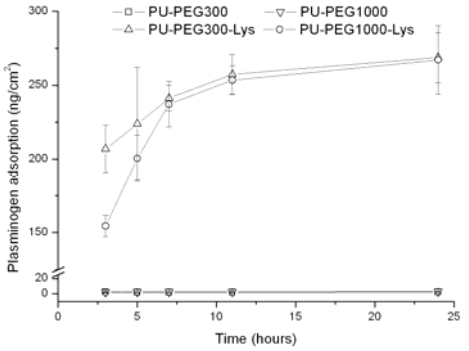

Figure 1. Plasminogen adsorption from plasma as a function of time (mean±S.D., n=3).

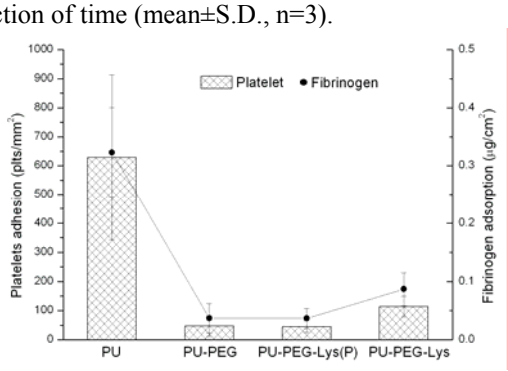

Figure 2. Fibrinogen adsorption and platelet adhesion from whole blood (mean±S.D., n=3).

The modified surfaces showed much lower fibrinogen adsorption and platelet adhesion from whole blood than the PU control (Figure 2) and the levels on the PEG-Lys surfaces were similar to those on PEG (no lysine). The platelet resistance of the lysine surfaces may be attributed to the PEG spacer. Platelet adhesion was strongly correlated with fibrinogen adsorption.

Clot lysis was more rapid on the PU-PEG300-Lys surface (~20 min) than on the PU-PEG1000-Lys (~40 min). Thus the surface with the greater plasminogen binding capacity (at 2h) showed more rapid clot lysis.

Conclusions: When used both as a spacer and a protein repelling agent, PEG of relatively short chain may be optimal. More generally the PEG-lysine surface system with dual "bioinert" and "bioactive" functions appears to be a promising approach to surfaces of improved hemocompatibility.

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