Lysine-poly(HEMA) modified polyurethane surface with high lysine density and fibrinolytic activity

Shasha Wang[1], Dan Li[1], Hong Chen[1*], John L. Brash[1,2]
[1]State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan, PR China.
[2]School of Biomedical Engineering, McMaster University, Hamilton, Ontario, Canada.

Introduction: Surface modification with bioactive agents capable of inhibiting enzymes in the coagulation cascade is a widely used strategy for improving blood compatibility. Polyethylene glycol (PEG) has been used as a spacer to couple these bioactive moieties to surfaces because of its chain flexibility and excellent protein resistance [1]. We have developed the concept of a fibrinolytic surface in which PEG is used as a spacer to immobilize lysine such that the ε-amino group is free to capture plasminogen and tPA in contact with blood [2-4]. However, the surface density of PEG achievable by "grafting to" was limited due to steric hindrance; the density of lysine was correspondingly limited. Herein, we introduce a simple, generic approach for surface modification of polyurethane (PU), in which a unique monomer, methacryloyl isothiocyanate, is used to introduce C-C double bonds. These can then be used to initiate the graft polymerization of appropriate monomers ("grafting from"). The approach is exemplified in this work using 2-hydroxyethyl methacrylate (HEMA). Like PEG, poly(HEMA) has been shown to be protein repellent, and the hydroxyl side chains provide abundant active termini for attachment of lysine. The surface lysine density, suppression of nonspecific protein adsorption and clot lysing efficiency of poly(HEMA)-Lys modified PU are reported.

Methods: The preparation of poly(HEMA)-modified PU surface and the immobilization of lysine to the hydroxyl terminus of poly(HEMA) were described in [5] and [3], respectively. Surface lysine density was determined by a colorimetric method using 4-nitrobenzaldehyde as reagent. The adsorption of fibrinogen from buffer was measured by radiolabeling. To assess clot lysing activity, the surfaces were incubated in plasma (adsorption of plasminogen) and treated with t-PA (formation of plasmin). They were then incubated in recalcified plasma and absorbance at 405 nm (as a measure of clot formation) was recorded over time [4].

Results and Discussion: Fibrinogen adsorption from buffer was greatly reduced on the poly(HEMA) surface compared to the control PU. The poly(HEMA)-Lys surface was also fibrinogen resistant though less so than the poly(HEMA) (Figure 1). Lysine density was 2.62 nmol/cm^2 on the PU-poly(HEMA)-Lys surface, much higher than on a comparable PU-PEG-Lys surface (0.76 nmol/cm^2) reported previously [4]. This is presumably due to the higher density of hydroxyl groups available for reaction with lysine. It is expected that the high density of lysine should result in increased adsorption of plasminogen from plasma and increased fibrinolytic potential. Typical data on clot lysis after exposure to plasma (adsorption of plasminogen) are shown in Figure 2. The control surfaces showed typical clot formation curves in which plateaus were reached and maintained indicating a fully formed, stable clot. In contrast, for the PU-poly(HEMA)-Lys surface the absorbance increased and then returned to baseline, indicating that the clot formed and then was lysed by the action of surface localized plasmin. It is important to note that the clot dissolved much more rapidly (20 min) on the PU-poly(HEMA)-Lys than on the PU-PEG-Lys surface reported previously (40 min [3]).

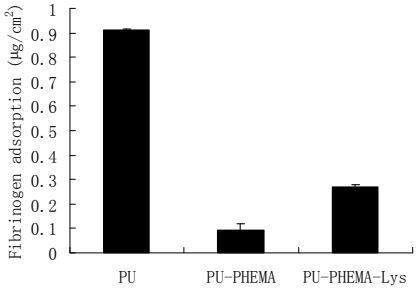

Figure 1. Fibrinogen adsorption from buffer on modified and unmodified PU surfaces (mean±SD, n=3).

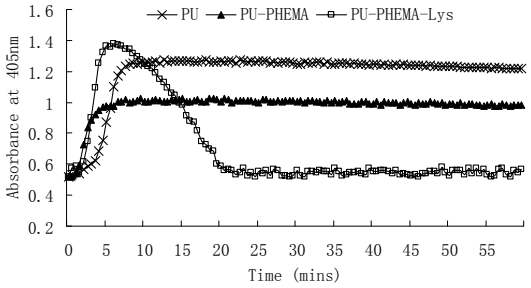

Figure 2. Clot formation in plasma expressed as absorbance at 405 nm vs. time.

Conclusions: Protein resistant PU surfaces having high lysine density were prepared by surface grafting of HEMA and subsequent reaction with lysine. With increased plasminogen binding capacity, these surfaces showed more rapid clot lysis than the corresponding PEG-lysine system. This method of modification provides a generic approach for preparing bioactive polyurethane surfaces of high activity.

Acknowledgments: The work was supported by the National Natural Science Foundation of China (20920102035, 20974086, 20634030) and the Ministry of Science and Technology of China (2008CB617510).

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