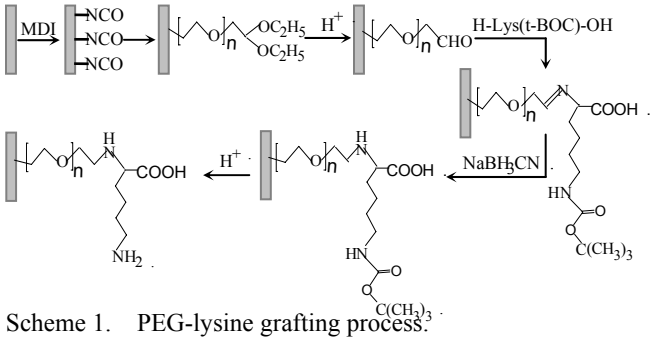
A clot lysing surface: lysine immobilized on polyurethane through a PEG spacer.

Hong Chen[1,2], Xiaoyang Hu[1], Dan Li[1],Yanxia Zhang[1], W. Glenn McClung[3], John L. Brash[3]
[1]School of Materials Science and Engineering, [2]Biomedical Materials and Engineering Center, Wuhan University of Technology, P.R.China, [3]School of Biomedical Engineering, McMaster University, Canada

Polyurethanes are widely used in the areas of biomedicine and bioengineering due to their excellent mechanical properties. Unfortunately, unmodified polyurethanes are not biocompatible and tend to initiate thrombogenic reactions when in contact with blood. It is thus desirable to improve the biocompatibility of polyurethanes by eliminating or reducing unfavorable biological responses such as biofouling and thrombogenic reactions, and to promote favorable specific biological reactions such as fibrinolysis.

Previous work has demonstrated that surfaces prepared using a coating technique to incorporate high densities of conjugated lysine in which the ε-amino groups are free, could bind substantial quantities of plasminogen from plasma almost to the exclusion of all other proteins [1]. Plasminogen bound in this manner was readily converted to plasmin in the presence of tissue plasminogen activator (tPA) and readily dissolved "nascent" clots formed around them in plasma [2].

PEO-grafted surfaces have been shown to be particularly effective in minimizing protein adsorption and platelet adhesion. Immobilization of biomolecules through a PEG spacer is believed to stabilize the biomolecule and enhance its bioactivity. In this study, we conjugated lysine through a PEG spacer to a "base" polyurethane, leaving the ε-amino group of the lysine free. The grafting procedure is shown in Scheme 1.



Scheme 1. PEG-lysine grafting process.

Methods: Methylene diphenyl diisocyanate (MDI) was reacted with the urethane bonds on the surface of films of a "base" polyurethane to incorporate isocyanate groups as described previously [3]. PEG-grafted polyurethane was prepared by treating the NCO-functionalized polyurethane films with 2,2'-diethoxy ethyl poly(ethylene oxide) for 24h. The resulting films were then incubated in 1 N HCl to produce aldehyde-PEG modified surfaces. After rinsing, the surfaces were treated with a 5 mg/ml H-Lys(t-BOC)-OH solution in PBS for 5h under nitrogen, after which 0.05g $NaBH_3CN$ was added and reacted for 3h. The discs were then immersed in a 25% trifuoroacetic acid solution to remove the tBOC protecting group on lysine. Similarly, unprotected lysine was conjugated to PU through a PEG spacer to produce a PU-PEG-Lysine. The ability of the surfaces to dissolve plasma clots formed around them after adsorption of plasminogen (from plasma) and t-PA was assessed by following absorbance of the plasma at 405 nm vs time after recalcification [2].

Results and Discussion: Data for clot formation and dissolution on the lysine-modified surfaces, a polyurethane control, and normal human plasma (no surface) are shown in Figure 1. The polyurethane control and plasma samples show "typical" clot formation: a steep rise in the absorbance versus time curve (increased plasma turbidity) followed by a plateau region (fully-formed clot).

In contrast, the PEG-(ε-lysine) surface while allowing clot formation to occur (initial increase in absorbance), shows a rapid return of the absorbance to baseline, clearly indicating clot dissolution and the presence of surface bound plasmin. The PU-PEG-lysine surface was unable to dissolve clots in this assay, showing that lysine must be attached to the surface in a manner that leaves its ε–amino group free to bind plasminogen and t-PA.

From plasminogen adsorption experiments (data not shown), the PU-PEG-(ε-lysine) surface adsorbed less plasminogen than previously observed for a "coated" lysine-derivatized PU [2], but was still able to dissolve incipient clots in the plasma recalcification assay.

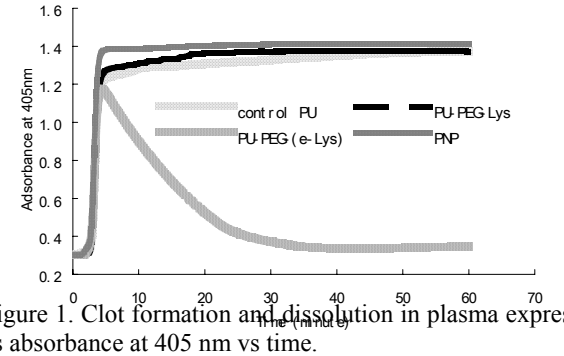


Figure 1. Clot formation and dissolution in plasma expressed as absorbance at 405 nm vs time.

References:

1. WG McClung, DL Clapper, S-P Hu, JL Brash. J Biomed Mater Res, 2000, 49:409–414.
2. WG McClung, DL Clapper, S-P Hu, JL Brash. Biomaterials, 2001, 22 :1919-1924.
3. C Freij-larsson, B Wesslen. J Appl Polym Sci. 1993, 50:345-352.

Acknowledgements

Work supported by National Natural Science Foundation of China (No.20574055, 90606013, 20634030), Program for Excellent Talents in Hubei Province [2005ABB020], and Canadian Institutes of Health Research.