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ScienceDirect
Acta Biomaterialia 5 (2009) 1864–1871
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Lysine-PEG-modified polyurethane as a fibrinolytic surface: Effect of PEG chain length on protein interactions, platelet interactions and clot lysis

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Received 26 November 2008; received in revised form 6 February 2009; accepted 2 March 2009
Available online 9 March 2009

Abstract

Fibrinolytic polyurethane surfaces were prepared by conjugating lysine to the distal terminus of surface-grafted poly(ethylene glycol) (PEG). Conjugation was through the α-amino group leaving the ε-amino group free. Lysine in this form is expected to adsorb both plasminogen and t-PA specifically from blood. It was shown in previous work that the PEG spacer, while effectively resisting nonspecific protein adsorption, was a deterrent to the specific binding of plasminogen. In the present work, the effects of PEG spacer chain length on the balance of nonspecific and specific protein binding were investigated. PEG–lysine (PEG-Lys) surfaces were prepared using PEGs of different molecular weight (PEG300 and PEG1000). The lysine-derivatized surfaces with either PEG300 or PEG1000 as spacer showed good resistance to fibrinogen in buffer. The PEG300-Lys surface adsorbed plasminogen from plasma more rapidly than the PEG1000-Lys surface. The PEG300-Lys was also more effective in lysing fibrin formed on the surface. These results suggest that the optimum spacer length for protein resistance and plasminogen binding is relatively short. Immunoblots of proteins eluted after plasma contact confirmed that the PEG–lysine surface adsorbed plasminogen while resisting most of the other plasma proteins. The hemocompatibility of the optimized PEG–lysine surface was further assessed in whole blood experiments in which fibrinogen adsorption and platelet adhesion were measured simultaneously. Platelet adhesion was shown to be strongly correlated with fibrinogen adsorption. Platelet adhesion was very low on the PEG-containing surfaces and neither surface-bound lysine nor adsorbed plasminogen promoted platelet adhesion.

Keywords: Biocompatibility; Fibrinolytic property; Polyurethane; Protein adsorption; Platelet adhesion

1. Introduction

Major reasons for the failure of devices implanted in the body are the rapid accumulation of proteins on the material surface and the interweaving of subsequent host responses, including blood coagulation, platelet activation and complement activation. Many methods of surface modification have been used to improve the biocompatibility of biomaterials. These can be roughly divided into two strategies: "bioinert" and "bioactive". Modification with poly(ethylene glycol) (PEG) or polyethylene oxide (PEO) is the most widely used approach to bioinertness mainly because of the excellent resistance of PEG to nonspecific protein adsorption and cell adhesion. PEG has also been used as a spacer to couple bioactive moieties to surfaces, thus potentially exerting both "bioinert" and "bioactive" functions [1–5]. In this approach PEG/PEO has the potential not only to inhibit nonspecific protein adsorption but also to move the bioactive moiety away from the surface, making it potentially more effective than if coupled directly [6]. From this perspective, the present paper develops the

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doi:10.1016/j.actbio.2009.03.001

concept of a fibrinolytic surface on which PEG is used as a spacer to conjugate lysine such that the ε-NH_2 is free and exposed to capture plasminogen upon exposure to blood. Plasminogen, the key zymogen of the fibrinolytic pathway, is cleaved by its physiological activator, tissue-type plasminogen activator (t-PA), yielding the enzymatically active form plasmin that lyses fibrin [7].

Brash and co-workers have explored the concept of a clot-lysing surface based on immobilization of lysine [8–11]. In vitro experiments indicated that a lysine-immobilized surface can selectively bind plasminogen from plasma and, when activated by t-PA, the plasmin generated at the surface can lyse fibrin. In the present work, lysine was conjugated to the distal terminus of PEG surface-grafted on polyurethane, such that the ε-amino group was free. It was shown that this surface reduces nonspecific protein adsorption efficiently while binding plasminogen from plasma with some degree of selectivity [3]. When activated by t-PA the adsorbed plasminogen was converted to plasmin, and the fibrinolytic activity of the surface localized plasmin was demonstrated. However, the rate of plasminogen uptake was relatively slow (requiring 7 h to saturate), presumably due to the protein repellent properties of the PEG and the mobility of the terminally conjugated lysines. Thus it appeared that it might be necessary to vary the material properties to achieve an appropriate balance of efficient plasminogen uptake and prevention of nonspecific protein adsorption. It has been reported that the repellent effect of PEG increases with increasing chain length in the range up to a few thousand [12,13]; thus, by optimizing the PEG chain length, it seemed that it might be possible to balance the two effects.

Although the protein-resistant properties of PEG/PEO have been extensively investigated, the effects of PEG properties on the binding of target biomolecules to ligands at the distal terminus when the PEG is used as a spacer have not been considered. In the work reported here, the effect of PEG chain length on plasminogen binding to lysine at the PEG distal terminus was investigated. Platelet adhesion and activation in whole blood under flow conditions and Western blot analysis of adsorbed proteins on these surfaces were also studied.

2. Materials and methods

2.1. Reagents

N,*N*′-disuccinimidyl carbonate (DSC, anhydrous, ⩾95%), 4,4′-methylene-bis-(phenyl-isocyanate) (MDI, anhydrous, 98%), trifluoroacetic acid (TFA), H-Lys(t-BOC)-OH and 4-nitrobenzaldehyde were from Sigma–Aldrich Chemical Co. and used without further purification. Poly(ethylene glycol) ($M_n = 1000$ and $M_n = 300$) was purchased from Sigma–Aldrich Chemical Co. and dried before use. All anhydrous solvents such as toluene, dimethyl formamide (DMF) and acetonitrile were obtained from EMD Chemicals Inc., Triethylamine (TEA, 99%) was obtained from Alfa Aesar Co. Both fibrinogen and plasminogen were purchased from Enzyme Research Laboratories (South Bend, IN). Recombinant tissue plasminogen activator (t-PA) was obtained from Genentech (San Francisco, CA).

2.2. Preparation of surfaces

Tecothane polyurethane (TT-1095A) was from Thermedics (Wilmington, MA), and was extracted (Soxhlet) for 48 h with methanol to remove impurities. Films of this material were cast from a 5% (wt./vol.) solution in DMF, dried in air at 75 °C for 48 h and vacuum dried at 60 °C for 48 h to remove excess solvent. The polyurethane elastomer films were punched into discs, approximately 5 mm in diameter and 0.5 mm thick.

The modified surfaces used in this study consist of "base" polyurethane (PU) with immobilized PEG to which lysine is covalently attached at the distal terminus; the PEG may thus be seen as a spacer for the lysine and as a protein-repelling element. The details of the grafting techniques have been described previously [3]. In brief, PU discs were immersed in a toluene solution containing 7.5% (wt./vol.) MDI and 2.5% (wt./vol.) triethylamine. After stirring at 50 °C for 100 min, PEG-grafted PU surfaces (PU-PEG) were obtained by immersing NCO-functionalized polyurethane surfaces (PU-NCO) in a toluene solution containing 5% PEG (wt./vol.) at room temperature for 24 h. For the covalent conjugation of ε-lysine, the PU-PEG surfaces were first added to an acetonitrile solution containing DSC (0.05 mmol ml^{-1}) and TEA (0.05 mmol ml^{-1}) and stirred at room temperature for 6 h. The resulting surfaces (PU-PEG-NHS) were incubated overnight in phosphate-buffered saline (PBS) pH 8.3 containing 5 mg ml^{-1} H-Lys(t-BOC)-OH to give a PU-PEG-Lys(P) surface. The surfaces with ε-NH-t-Boc groups were deprotected by treatment with 25% TFA for 90 min and subsequently washed with PBS. The resulting surface, with the ε-NH_2 groups of lysine exposed (PU-PEG-Lys), is of the greatest interest in terms of its fibrinolytic potential. To study the effect of PEG spacer length on the balance between "repulsion" and "attraction" of plasminogen, two PEGs, with molecular weights of 300 and 1000, referred to as PEG300 and PEG1000 respectively, were used.

2.3. Graft density

The lysine graft densities were determined by reacting the surface amino groups with 4-nitrobenzaldehyde to form imines, and subsequent hydrolysis to liberate the 4-nitrobenzaldehyde [14]. Typically, nine discs of PU-PEG-Lys surface or PU-PEG-Lys(P) control surface were immersed in anhydrous ethanol (10 ml) containing 4-nitrobenzaldehyde (40 mg) and acetic acid (0.008 ml) under nitrogen at 50 °C for 3 h. The surfaces were then washed and sonicated in absolute ethanol for 2 min. The discs were immersed in water (1 ml) containing acetic

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