룸부로키나제가 공유결합으로 고정화된 폴리우레탄 표면분석

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LK-immobilization on polyurethane through the covalent bond and its surface characterization

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INTRODUCTION

Polyurethane has been used in many blood contacting devices such as vascular prostheses, blood filters, catheters, heart valves, pacemaker lead insulation, ventricular assist devices and total artificial hearts.[1] These applications arise from the superior physical and mechanical properties of polyurethane elastomers and their relatively good blood and tissue compatibility. When compared to other commercial plastics, polyurethanes are outstanding in strength, flexibility and fatigue resistance.

The interaction between tissue and a foreign material is influenced by the nature of the tissue-material interface, and thus, by the surface properties of the material. This is particularly the case in blood-material contact, where thrombogenesis at the interface is influenced in part by the nature of the prosthetic surface. It thus becomes crucial to be able to characterize the surface of a biomaterial before mechanics studies of biological interactions with its surface.

A number of procedures have been investigated to improve blood compatibility of biomaterials by various surface modification methods, especially by immobilizing bioactive compounds.[2,3] Lumbrokinase, a potent and novel fibronolytic enzyme from the earthworm, was extracted by Mihara et.al. in 1983. They reported that lumbrokinase is heat stable and stable within a broad pH range and shows both a plasminogen activator activity and a direct digestive action on fibrin.[4,5] In a previous study, GH Ryu et. al. immobilized lumbrokinase on a polyurethane surface via maleic anhydride methylvinyl ether copolymer(MAMEC) to improve the biocompatibility of polyurethane against thrombosis.[6] There was the possibility that Lumbrokinase and MAMEC might be released from the polyurethane surface in this method because MAMEC was not covalently bonded but just coated.

In this study, we immobilized lumbrokinase on a polyurethane surface through the covalent bond, not coating, by using 1,6-Diisocyanatohexane and maleic acid methyl vinyl ether copolymer(MAcMEC). We evaluated the surface characteristics of the modified polyurethane.

MATERIALS AND METHODS

Lumbrokinase Purification

Lumbrokinase (LK) was purified from the earthworm (*L. rubellus*) powder using a modified method of Mihara et al.. LK fractions were obtained by ammonium sulfate precipitation and diethylaminoethyl (DEAE) anion exchange column chromatography. The protein concentration of purified LK was determined by lowry protein assay

Lumbrokinase immobilization

Polyurethane (PU) sheets (1*2 cm, Pelethane®2363-80AE; Dow Chemical Co.) were extracted with methanol for 3days to remove the low-molecular-weight components. After extraction, the sheets were dried under vacuum at 50°C for 48 h to remove the residual solvents.

After extraction with methanol, PU sheets were reacted with 1,6-Diisocyanatohexane(HMDI) in dry toluene for 1h at 40°C and washed twice with dry toluene. HMDI-treated PU were reacted with maleic acetic acid methylvinyl ether copolymer(MAcMEC) as linker in dry formamide for 4 h or 20 h at 60°C and then reacted in LK solution of 1mg/ml in 0.05M KH_2PO_4 (pH 4.5) for 20 h at room temperature. Continuously HMDI, MAcMEC and LK treated PU were washed in 0.05M KH_2PO_4 (pH 4.5) for 1 h, followed by 24 h drying under vacuum at room temperature.

The concentration of LK bound on PU surface by above two methods determined by the modified Bradford assay (dye-binding method).

Surface characterization

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Lumbrokinase grafted via MAcMEC on the polyurethane surface was confirmed using a FTIR spectrophotometer, coupled with an ATR accessory. The sample sheets(1*2*1 cm) were pressed against the crystal, and the spectra were collected at 4 cm^{-1} resolution under nitrogen.

Dynamic Contact Angle Measurement

The advancing and receding contact angles were determined by the Wilhelmy plate method.[7] In this method, distilled water was used as a liquid of known surface tension(72.6 dynes/cm). To measure the contact angle, clean sample sheets were immersed into and drawn out of the water at speed of 79.64 microns/sec.

RESULTS

The ATR-FTIR spectra show the characteristic peaks of untreated PU, HMDI-treated PU, and MAcMEC treated PU. On the HMDI treated PU surface, a characteristic peak of isocyanate(-NCO) group was absorved at 2266 cm^{-1}. This indicated that HMDI was well bonded covalently on the PU surface as a linker. After reaction with MAcMEC, the sharp peak of isocyanate group on the HMDI treated PU surface was disappeared.

The surface concentration of lumbrokinase was determined by modified dye-binding method. This assay involves the formation of a complex between lumbrokinase and dye (coomassie Brilliant Blue G).[6] The surface concentration of lumbrokinase on the modified polyurethane surface(PU-HMDI-MAcMEC-LK) was 1.52 $\mu g/cm^2$.

The covalent angle data of the samples were listed in table 1. The polyurethane surface is typically hydrophobic and the surface of the PU-MAcMEC is more hydrophilic. This is mainly caused from the carboxyl group of MAcMEC after reaction with 1,6-Diisocyanatohexane.

DISCUSSION

The LK-immobilization scheme on the polyurethane surface involves the covalent bond formation reaction. LK immobilization was performed by a coupling of 1,6-Diisocyanatohexane to polyurethane through a allophanate reaction. The free isocyanate groups remaining on the surface were then coupled to the carboxyl functional groups of MAcMEC. So, MAcMEC could be bonded to the polyurethane surface tightly and coupled through covalent bond formation reacted with functional groups such as amino groups on LK. When the MAcMEC react with the amino group from LK, the pH of reaction solution needs to be lower than 5 because of reaction efficiency.

LK immobilization was confirmed by ATR-FTIR and quantification of the immobilized enzyme. Contact angle measurement shows that the hydrophilicity of the LK-immobilized surface is increased.

In conclusion, a new surface modification method for lumbrokinase on polyurethane surface was developed and its surface characterization was evaluated by the chemical analysis.

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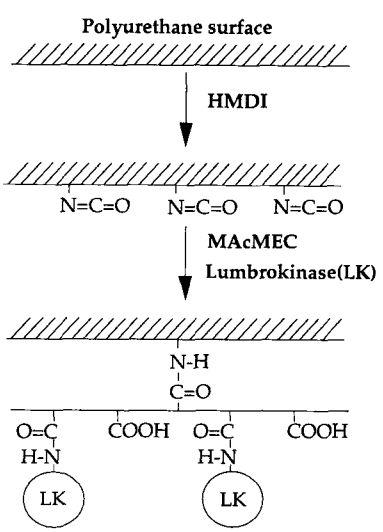

Figure 1. Sturcture of PU-HMDI-MAcMEC-LK

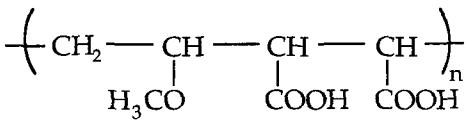

Figure 2. The structure of MAcMEC

TABLE 1.
Wilhelmy Plate Contact Angle Data in Water

Material	θ_{adv}	θ_{rec}
PU control (MeOH extracted)	89.40	51.02
PU-MAcMEC	53.64	24.19
PU-MAcMEC-LK	53.77	28.55