醫工學會誌：第 13 卷 第 4 號, 1992
J. of KOSOMBE : Vol. 13, No. 4

研 究 論 文
92 - 13 - 4 - 01

# Enhancement of Blood Compatibility of Albumin-Immobilized Polyurethane

**Gyu Ha Ryu, Dong Keun Han*, Young Ha Kim*, and Byounggoo Min**

**=Abstract=**

Surface treatment with albumin on a blood contacting material inhibits platelet adhesion, activation and subsequent thrombus formation. Though adsorbed albumin improves blood compatibility, rapid desorption occurs when this surface is exposed under circulating blood. In this study, we immobilized human serum albumin on the polyurethane (PU, Pellathane® 2363−80AE) surface to investigate its blood compatibility and extended effects on blood-material interface. PU surface was treated with hexamethylene diisocyanate(HMDI) and PU-HMDI was further grafted with albumin to produce albumin-immobilized PU surface (PU-Albumin). Surface of PU-Albumin was characterized by ATR-FTIR, ESCA, SEM and dynamic contact angle. Blood compatibility was evaluated by *in vitro* protein adsorption, platelet adhesion, and occlusion time in *ex vivo* rabbit A-A shunt. Immobilization of albumin was confirmed from the disappearance of the-NCO peak observed at 2250 $cm^{-1}$ on the PU-HMDI surface by IR and the existence of S atomic % by ESCA. The concentration of albumin immobilized on PU surface was in the order of 5.8 ug/㎠. Albumin grafted PU also showed slight increase of the hydrophilicity in the Wilhelmy plate method and less fibrinogen adsorption than PU control. PU-Albumin surface showed less platelet adhesion, activation and reduced thrombogenicity. The *ex vivo* occlusion time of untreated PU was 50 min, that of albumin immobilized PU was extended to 150 min, which indicates that PU-Albumin surface has better blood compatibility than PU.

## I. INTRODUCTION

The initial events following exposure of polymer surfaces to blood lead to cellular adhesion and coagulation[1,2]. Many efforts have been made with the aim of passivating the foreign surface by appropriate surface modifications. Serum albumin contains no peptide sequences to interact with cell membrane or enzyme receptors in the coagulation cascade. It also reduces subsequent platelet adhesion and surface activation of the coagulation pathway[3-5]. Many investigators have examined the effects of albumin adsorption and immobilization on the blood compatibility of polymer surface[6,7]. However, despite the enhanced blood compatibility of adsorbed albumin, it is readily desorbed from the surface when exposed to circulating blood. We grafted the human serum albumin on the

〈접수 : 1992년 11월 24일〉

Department of Biomedical Engineering, College of Medicine, Seoul National University, 28 Yeongun-dong, Jongno-gu, Seoul 110−744, Korea
* Polymer Chemistry Laboratory, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul, Korea

polyurethane (PU) surface by stable covalent bonds and investigated its surface characteristics and blood compatibility.

## MATERIALS AND METHODS

### Albumin Immobilization Onto PU Surface

PU sheet (2×5 cm, Pellathane® 2363−80AE, Dow chemical Co., Midland, MI) and PU tubing (30 cm in length, Royalthene® R-380 PNAT, ID 1.5mm×OD 2.0 mm, Uniroyal chemical Co.) were extracted with methanol. The methanol-extracted PU sheet and tubing were immersed in 150 ml of toluene followed by the addition of 0.5 ml of stannous octoate and 8 ml of hexamethylene diisocyanate(HMDI). After being reacted for 1 hr at 40℃ under nitrogen, the sheet and tubing were washed in toluene and then anhydrous ether to yield PU-HMDI[8]. PU-HMDI was further grafted with 150 ml of 0.2% (w/v) human serum albumin (HSA, Sigma chemical Co., St. Louis, MO) in phosphate-buffered saline(PBS, PH 7.4) containing 0.02% sodium azide for 12 hrs at 4℃. The sheet and tubing were washed with PBS(PH 7.4) and dried *in vacuo* at 20℃ to produce PU-Albumin.

### Concentration and Stability of Immobilized Albumin

The assay described by Bradford[9] was modified and used to estimate the surfaces concentration of immobilized albumin. A standard curve with samples of known albumin concentration was prepared in parallel with PU-Albumin sheets. Following albumin-dye precipitation, the samples were centrifuged at 1,500g for 10 min and the absorbances of the dye-depleted solution were read at 595 nm with spectrophotometer (Shimadzu UV-240). To measure the leakage and the stability of immobilized albumin, PU-Albumin sheets were stirred in PBS (pH 7.4) for a week. The test solution was added to the protein reagent and its dye-depleted solution was analyzed as above.

### Characterization of Polymer Surface

ATR-IR

Attenuated total reflection infrared (ATR-IR) data were obtained from the surfaces of PU and modified PU sheets using a Jasco A-102 IR spectrophotometer, coupled with an ATR-6 accessory and 45℃ KRS-5 crystal.

ESCA

The elemental compositions of modified PU surfaces were determined using a Physical Electronic PHI 558 electron spectroscopy for chemical analysis (ESCA) spectrometer. The source was a 10kV, 30mA monochromatized x-ray beam from a magnesium anode. Surface elemental compositions relative to carbon were calculated from peak heights with a correction for atomic sensitivity. Survey spectra were obtained at 100 eV pass energy giving good sensitivity, while higher-resolution data were carried out at 50 eV pass energy. Surface charge build-up was corrected by considering the shift of $C_{1s}$ peak at 285 eV.

Dynamic Contact Angle

The advancing and receding contact angles were determined by the Wilhelmy plate method[10]. The contact angle data of modified PU sheets were evaluated with a Wilhelmy plate contact angle apparatus (WET-TEK, Biomaterials Int.) in double-distilled water at constant temperature (20℃) and humidity (30% r.h.).

### *In Vitro* Evaluations

$^{14}C$-fibrinogen Adsorption

Purified human fibrinogen(Calbiochem Co.) was radiolabelled by reductive alkylation with $^{14}C$-formaldehyde (Du Pont) and sodium cyano-

borohydride using the method of Dottavio-Martin and Ravel[11]. Each of PU and PU-Albumin beads (0.8g) filled in disposable syringes was first contacted with bovine plasma containing $^{14}C$-labelled fibrinogen as a function of adsorption time(1 min-2 hrs) or plasma concentration (0.2%-10% normal). Then samples were rinsed with PBS (pH 7.4) solution twice and placed in a 2% SDS/PBS(pH 7.4) solution for 2 days. After this, the surface concentration of fibrinogen desorbed from samples was determined by counting the radioactivity with a scintillation counter (Beckman DP 5500).

Platelet Adhesion to Polymer Surfaces

Nine volumes of human whole blood were drawn into plastic syringes containing one volume of 3.8% sodium citrate solution and centrifuged at 300g for 10 min to obtain platelet-rich plasma (PRP). Platelet concentration of PRP was $4 \times 10^8$/ml. PU and PU-Albumin beads (0.8g, 14㎠) were carefully weighed into plastic syringes and equilibrated with 2 ml of PBS (pH 7.4). Then the buffer was removed and 2 ml of PRP was introduced. The syringes were sealed with parafilm(American Can Co., Greenwich, CT) and then rotated for 15, 30, 45, and 60 min, respectively at room temperature. At each time point, platelets were counted using a Coulter counter (Coulter Electronics. Inc., Hialeah, FL). Control sampes without beads were also incubated with PRP and used as a reference for each time point.

### *Ex Vivo* A-A Shunt Model[12]

An arterio-aterial (A-A) shunt was performed under the conditions of low flow rate and low shear rate. Male rabbits (New Zealand White, 2-3 kg) were anesthetized with ketamine /urethane, and right carotid arteries were exposed surgically. PU and modified PU tubings (ID 1.5㎜×OD 2.0 ㎜, 30 ㎝ in length), equilibrated overnight with PBS (pH 7.4), were rinsed and carefully inserted into the clamped carotid artery of rabbit. The flow rate was measured with ultrasonic flow meter (ES-4100Z, ARS Electronic) and controlled to 2.5 ml/min continuously using a suture tourniquet. The occlusion time was defined as the time the blood flow decreases to zero.

## RESULTS AND DISCUSSION

### Immobilization of Albumin

To introduce free isocyanate groups (-NCO) to the PU sheets, allophanate reaction of HMDI with the -NH groups of PU surface was performed. The optimal HMDI treatment condition for PU was described elsewhere[8]. The -NCO groups on PU surface could react with albumin. ATR-IR spectra of PU-HMDI surfaces showed a sharp characteristic band of -NCO at 2250 $cm^{-1}$. The free -NCO groups on the PU-HMDI surfaces undergo nucleophilic reactions with $-NH_2$ and /or -OH groups of albumin. The immobili-zation of albumin was confirmed by the disappearance of the -NCO peak observed at 2250 $cm^{-1}$on the ATR-IR spectra of PU-HMDI surfaces. Albumin was immobilized on PU-HMDI surfaces at various time and temperature, and the optimal condition was found to be the reaction time of 12 hrs at 4℃. The -NCO group is sensitive to moisture and can be decomposed into amine and $CO_2$ via an unstable carbamic acid, therefore water is not a good solvent. It was, however, possible to immobilize albumin in water. This might be due to the higher reactivity of the -NCO group with $-NH_2$ than with $H_2O$. Serum proteins are stable in PBS (pH 7.4) at 4℃. This is the main reason that the reaction must be performed in PBS (pH 7.4) at 4℃ for the stability of albumin, and this is also the reason that

the reactivity of -NCO group with $H_2O$ is reduced at 4℃. The concentration of the immobilized albumin on PU-Albumin sheets was determined by modified Bradford assay and was in the order of 5.8 ug/㎠.

To confirm the stability of immobilized albumin, the changes of remaining immobilized albumin concentration were measured as a function of time. During the first 10 hrs, there was a 5% loss from bound albumin, and there was no further loss thereafter. The initial decrease in albumin concentration may be due to the desorption of physically adsorbed albumin on PU-Albumin surface.

All these observations suggest that albumin was tightly bound to PU surface by covalent bonds.

ESCA

As shown in Table Ⅰ, an analysis of the atomic composition for modified PU surfaces was carried out by ESCA. In the case of PU-HMDI, the introduction of -NCO groups was confirmed from the drastic increase of N atomic % compared with PU control. Also, the existence of S atom in PU-Albumin surface means that albumin, which contains cysteine, was grafted onto the surface of PU-HMDI.

Surface Wettability

Table Ⅰ lists the contact angle data of modified PU surfaces in water. PU control displayed a typical hydrophobic surface with a advancing angle of 86.3° and a receding angle of 40.6°. However, PU-HMDI surface showed that the receding angle was wetting due to the instability of -NCO groups, which readily reacts with water in the PU-HMDI/water interface[8].

Especially PU-Albumin surface exhibited small advancing angle compared to PU and PU-HMDI, indicating that the surface is somewhat hydrophilic by grafting of albumin.

Meanwhile, the receding angle of PU-Albumin surface showed complete wetting like that of PU-HMDI, which represents the increase of hydrophilicity. But this result does not imply that such a wetting behavior of PU-Albumin is only due to the effect of immobilized albumin. It may also contain the effect of $NH_2$ groups produced by the reaction of -NCO groups and water during albumin immobilization.

**Table 1.** Surface Properties of PU-Albumin

| Material | ESCA (atomic %) | | | | Contact angle | |
|---|---|---|---|---|---|---|
| | C | O | N | S | $\theta$ adv | $\theta$ rec |
| PU, MeOH ext. | 76.9 | 21.5 | 1.5 | — | 86 | 41 |
| PU-HMDI | 73.5 | 14.4 | 11.0 | — | 85 | wet |
| PU-Alb | 72.9 | 15.2 | 9.7 | 2.2 | 61 | wet |

### *In Vitro* ¹⁴C-fibrinogen Adsorption

Figure 1 shows the fibrinogen binding kinetics for diluted plasma. The adsorption isotherms of fibrinogen for 1 and 20 min are also indicated in Figure 2. PU-Albumin surface showed less fibrinogen adsorption than PU control.

### *In Vitro* Platelet Adhesion

The platelet adhesion results on PU and PU-Albumin are summarized in Table Ⅱ. Table Ⅱ indicates that substantially fewer platelets adheres to the modified PU when the surface is immobilized with albumin. Many investigators[13-15] have reported that surface pretreatment with albumin reduces subsequent platelet adhesion.

**Table 2.** *In vitro* and *Ex vivo* Blood Compatibility of PU and PU-Albumin

| Materials | ¹Platelt retension (%) (n=3) | ²Occlusion time(min) (n=2~4) |
|---|---|---|
| PU | 62.1±1.23 | 50± 5 |
| PU-Albumin | 84.6±2.38 | 150±10 |

1 : After 1 hr incubation with PRP at R.T.

1, 2 : Mean±S.D.

### *Ex Vivo* Occlusion Time

As listed in Table Ⅱ, albumin-immobilized PU shows a considerable prolongation of occlusion time compared with the untreated PU. The occlusion time of PU was 50 min, but that of albumin-immobilized PU was extended to 150 min. These data are in good agreement with the results of *in vitro* tests, fibrinogen adsorption and platelet adhesion.

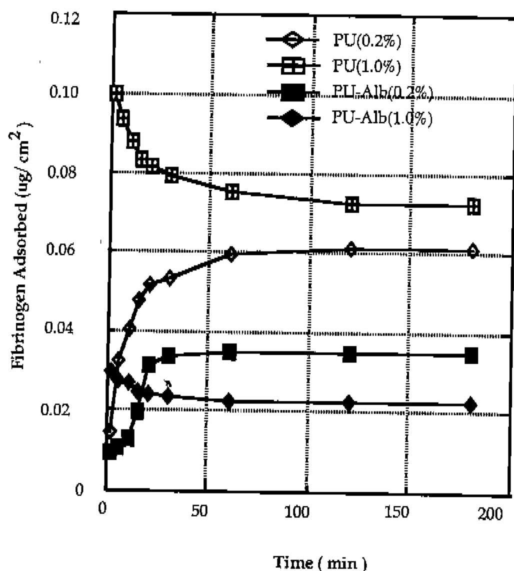


**Fig. 1.** Fibrinogen adsorption on polymer surfaces of 0.2% and 1.0% normal plasma concentration.

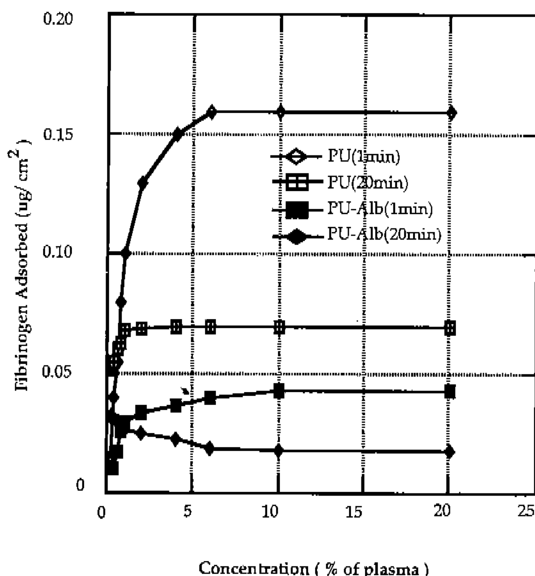


**Fig. 2.** Fibrinogen adsorption on polymer surfaces of diluted plasma after 1 and 20 minutes incubation time.

## CONCLUSION

Albumin was immobilized on PU and its amount was about 5.8 ug/㎠, which corresponded to a few tens of albumin layers based on albumin molecular dimensions of 38 Å×150 Å suggested by Peters[16]. Albumin-immobilized PU was proved to reduce fibrinogen adsorption, platelet adhesion, and surface activation of the coagulation pathway. Improved blood compatibility of PU-Albumin surface might be explained from the "multilayered protein passivation mechanism" proposed by Matsuda et al[17]. Albumin was tightly-bound by stable covalent bonds on PU surface, as confirmed from the *in vitro* stability test. But the upper protein layers may be more loosely-bound. These loosely-bound layers can be exchanged with other proteins and delaminated when adhered or aggregated platelets are formed on these layers. This organized structure may play an important role in the inhibition of platelet adhesion and activation.

## REFERENCES

1) Baier RE and Dutton RC : Initial events in interactions of blood with a foreign surface, J. Biomed. Mater. Res., 3:191, 1969.
2) Vroman L, Adams AL, and Klings M : Interactions among human blood proteins at interfaces, Fed. Proc., 30:1494, 1971.
3) Mason RG : The interaction of blood hemostatic elements with artificial surfaces, Prog. Hemost. Thromb., 1:141, 1972.
4) Lee RG and Kim SW : The role of carbohy-

drates in the platelet adhesion to foreign surfaces, J. Biomed. Mater. Res., 8:393, 1974.

5) Kim SW, Lee RG, Oster H, Coleman D, Andrade JD, Lentz DL, and Olsen D : Platelet adhesion to polymer surfaces, Trans. Am. Soc. Artif. Intern. Org., 20:449, 1974.

6) Mohammad SF and Olsen DB : Immobilized albumin-immunoglobulin G for improved hemocompatibility of biopolymers, Trans. Am. Soc. Artif. Intern. Organs, 35:384-387, 1989.

7) Riccitelli SD, Schlatterer RG, Hendrix JA, Williams GB, and Eberhart RC : Albumin coatings resistant to shear induced desorption, Trans. Am. Soc. Artif. Intern. Organs, 31:250-256, 1985.

8) Han DK, Park KD, Jeong SY, and Kim YH : Preparation and surface characterization of PEO-grafted and heparin-immobilized polyurethanes, J. Biomed. Mater. Res., 23:87-104, 1989.

9) Bradford MM : A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72:248-254, 1976.

10) Smith L, Doyle C , Gregonis DE, and Andrade JD : Surface oxidation of cistrans polybutadiene, J. Appl. Polym. Sci. 26:1269-565, 1978.

11) Dottavio-Martin D and Ravel JM : Radiolabeling of protein by reductive alkylation with [$^{14}$C] formaldehyde and sodium cyanoborohydride, Anal. Biochem., 87:562-565, 1978.

12) Nojiri C, Okano T, Grainger D, Park KD, Nakahama S, Suzuki K, and Kim SW : Evaluation of nonthrombogenic polymers in a new rabbit A-A shunt model, Am. Soc. Artif. Intern. Org. J., 10:596-601, 1987.

13) Roohk HV, Pick J, Hill R, Hung E, and Bartlett RH : Kinetics of fibrinogen and platelet adherence to biomaterials, Trans. Am. Soc. Artif. Intern. Organ, 22:1, 1976.

14) Salzman EW : Surface effects in hemostasis and thrombosis, Chapter 10 in Chemistry of Biosurfaces (Ed. M.L.Hair), Marcel Dekker, New York, Vol. 2, 1971, pp. 489-516.

15) Packham MA, EvansG, Glynn MF, and Mustard JF : The effect of plasma proteins on the interaction of platelets with glass surfaces, J. Lab. Clin. Med., 73:686, 1969.

16) Peters T, Jr : Serum albumin, Adv. Protein Chem., 37:161-245, 1985.

17) Matsuda T, Takano H, Hayashi K, Taenaka Y, Takaichi S, Umezu M, Nakamura T, Iwata H, Nakatani T, Tanaka T, Takatani S, and Akutsu T : The blood interface with segmented polyurethanes : "Multilayered protein passivation mechanism", Trans. Am. Soc. Artif. Intern. Organs, 30:353-358, 1984.