의공학회지 : 제15권, 제1호, 1994
J. of KOSOMBE : Vol. 15, No. 1

연 구 논 문
94-15-1-03

좌심실보조장치의 혈액주머니용 코팅재료로서 PU-PEO-SO_3의 *in vivo* 혈액적합성에 관한 연구

한 동 근 · 김 종 원* · 유 규 하* · 최 진 욱* · 이 규 백* · 김 영 하 · 민 병 구*

=Abstract=

In Vivo Blood Compatibility of PU-PEO-SO_3 as Coating Material for Blood Sac of Left Ventricular Assist Device (LVAD)

Dong Keun Han, Jong won Kim*, Gyu Ha Ryu*, Jin Wook Choi*, Kyu Back Lee*, Young Ha Kim, and Byoung Goo Min*

Sulfonated poly(ethyleneoxide)-grafted polyurethane (PU-PEO-SO_3) prepared by bulk modification was coated on a blood sac for electrohydraulic left ventricular assist device (ELVAD) implanted in dogs and its *in vivo* blood compatibility on shear stress was studied as compared with untreated PU. The effect of the wall shear stress on the protein adsorption unlike platelet adhesion is dependent on the surface characteristics of the material, although less proteins seem to be adsorbed in the region of the high shear stress. The thickness of total proteins adsorbed on PU-PEO-SO_3 (400 Å) by transmission electron microscopy (TEM) was considerably lower than that of untreated PU (1,000~1,600 Å), but PU-PEO-SO_3 showed high albumin adsorption, low fibrinogen and IgG adsorption, and low platelet adhesion as compared with untreated PU, suggesting that PU-PEO-SO_3 is more *in vivo* blood compatible. Therefore, it appears that such a blood compatible PU-PEO-SO_3 is useful for blood contacting biomaterials including artificial organs.

Key words : Polyurethane, PU-PEO-SO_3, Blood compatibility, LVAD, Biomaterial

INTRODUCTION

Plasma protein adsorption on the artificial surface is strongly influenced by not only the surface characteristics of materials but also the fluid dynamics inside the blood pump, and it would affect subsequent platelet adhesion and activation, which plays a major role in the initiation of thrombus formation at the blood-material interface *in vivo*.

There exist a few papers which were reported on the quantitative description of the adsorbed protein distribution inside the artificial heart[1, 2]. Mabuchi *et al.*[2] tried to find the distribution of adsorbed plasma proteins onto the surface of the blood pump by ^{125}I conjugated antibody method. Though many studies on the protein adsorption

한국과학기술연구원 고분자화학팀
Polymer Chemistry Laboratory, Korea Institute of Science and Technology
* 서울대학교 의과대학 의공학교실
* Department of Biomedical Engineering, College of Medicine, Seoul National University, Seoul 110-744, Korea
통신저자: 한동근, P.O. Box 131, Cheongryang, Seoul 130-650, Korea, Tel. (02) 962-8801 (ext, 3469), Fax. (02) 965-3852

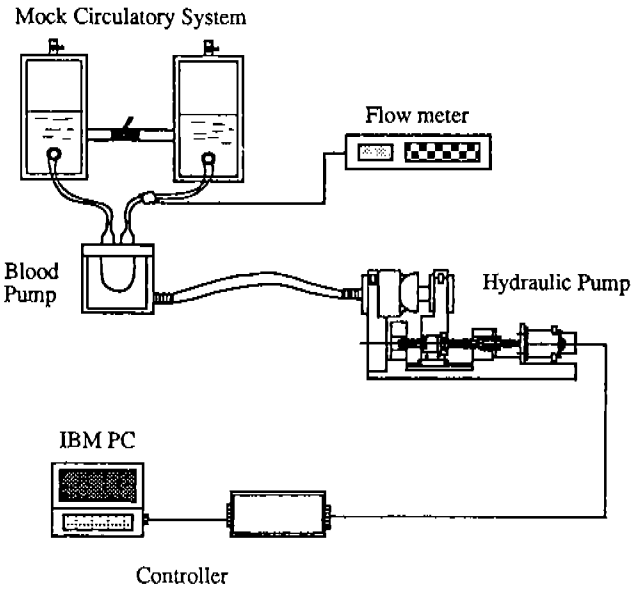

Fig. 1. Schematic diagram of the electrohydraulic left ventricular assist device(ELVAD)

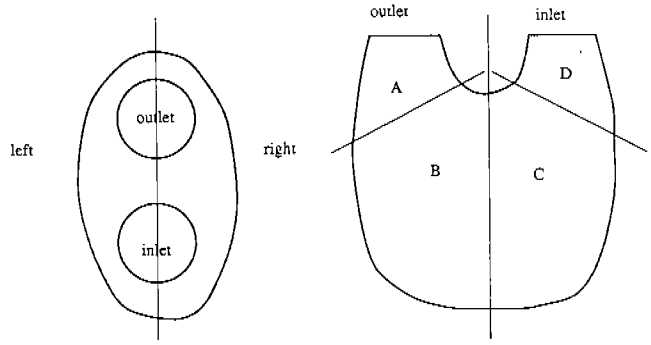

Fig. 2. Schematic diagram of the sectionized ventricle for ELVAD

in the artificial heart were performed[3~5], its relationship to the mechanism of platelet adhesion and activation is not well known yet at the blood-material interface.

In this study, *in vivo* blood compatibility of sulfonated poly(ethyleneoxide)-grafted polyurethane(PU-PEO-SO_3) coated on a blood sac for left ventricular assist device (LVAD) was investigated relative to untreated PU by canine implantation. Also to study protein adsorption and platelet adhesion on the wall shear stress in blood-material interactions, the three major plasma proteins such as fibrinogen, albumin, and gamma globulin(IgG) and platelets adsorbed on ventricular surfaces were examined by transmission electron microscopy(TEM), enzyme linked immunosorbent assay(ELISA), and scanning electron microscopy (SEM) analyses.

MATERIALS AND METHODS

1. Coating of PU-PEO-SO_3 to blood sac for LVAD

Sulfonated poly(ethyleneoxide)-grafted polyurethane (PU-PEO-SO_3) was prepared by bulk solution reaction as described in detail previously[6, 7]. In brief, first, propane sultone in tetrahydrofuran(THF) was added dropwise to amino-terminated PEO(molecular weight: 1000; NOF Corp., Japan) solution in THF, and reacted at 50℃ for 5 hrs to get sulfonated PEO(H_2N-PEO-SO_3). Consecutively, H_2N-PEO-SO_3 in N, N-dimethyl acetamide(DMAc) was reacted with excess amount of hexamethylene diisocyanate (HMDI) in DMAc at 50℃ for 3 hrs in the presence of triethylamine(TEA) to yield isocyanated PEO-SO_3(OCN-PEO-SO_3). Finally, OCN-PEO-SO_3 in DMAc was grafted to PU bead(Pellethane® 2363-80AE; Dow Chemical Co.) dissolved in DMAc for 3 days at 50℃ in the presence of TEA to produce PU-PEO-SO_3. The obtained PU-PEO-SO_3 was dissolved in DMAc(2.5%, w/v) and coated on blood sac inside LVAD. Untreated PU was used as a control.

2. Electrohydraulic left ventricular assist device (ELVAD)

The ELVAD is composed of three main parts[8]; a hydraulic pump, a blood pump, and a controller as shown in Fig. 1. The prong type of sac(Fig. 2), which is solution-cast by PU(Pellethane® 2363-80AE, Dow Chemical Co.), is located inside the transparent acrylic resin chamber where the pulsatile pressure is transferred from the hydraulic pump through a tube. Motor's torque is converted by the ball-screw mechanism to produce hydraulic pressure. The ELVAD can operate in two control modes; synchronous and asynchronous modes with the signal of the electrocardiogram.

3. Flow visualization

To obtain the wall shear stress inside the ventricle of the ELVAD, the flow characteristics were analyzed by flow vis-

Table 1. Summary of animal LVAD experiments

Case	Animal	Weight (kg)	Ventricular surface	Implantation time (hrs)
I	dog	17	PU	6
II	dog	18	PU-PEO-SO_3	6

ualization. A planar He-Ne laser (5 mW) light source illuminated the acrylic resin chamber through a cylindrical lens. Polystyrene particles (IRA 904, Amberite ion exchange resin; Rohm & Hass Co.) were suspended in the testing fluid as scatterers. Glycerin mixed water (36.7% (v/v) glycerin) was used as the blood analogue fluid. Flow patterns formed by these scatterers inside the ventricle were captured by a video camera (NTSC type, CCD; Hitachi Co.) and a video recorder (Hitachi Co.). The captured images were digitized using an image board (PCVISON plus, Imaging Tech.) and the IBM PC 386. Digital images were transferred in off-line to the Macintosh FXII computer (Apple Computer Co.) and processed using the software package, Adobe photoshop (Version 1.0, Adobe Systems Inc.).

4. Animal experiments and sample segmentation

Two acute implantations of ELVAD were performed with two mongrel dogs. Experimental conditions were summarized in Table 1. The inlet cannula (wire reinforced type, 32 Fr, Stockert Shiley Co.) was sutured to the left atrial auricle after incision of the left myocardium. The outlet cannula (bent type, 28 Fr, Stockert Shiley Co.) was sutured to the aorta.

Two Sinkhole bileaflet heart valves solution-cast with PU (Pellethane® 2363-80AE, Dow Chemical Co.) were used[7]. In the second animal experiment, the inner surface of the ventricle where the blood is contacted was coated with PU-PEO-SO_3.

After animal death, the blood contacted surfaces were rinsed mildly with phosphate buffered saline (PBS, pH 7.4, 0.15M). The ventricle was sectionized into 8 segments as shown in Fig. 2. The levels of the wall shear stress were determined for each segment by considering the flow characteristics inside the ventricle resulted from flow visualization. The minimal size of each segment was limited for the reliable quantification of adsorbed proteins.

5. TEM observation

Samples from each specimen were sliced into 0.5 mm thick strips under a stereomicroscope. The strips were stained with 1% (w/v) osmium tetroxide (OsO_4) solution for 30 min to measure the total thickness of the adsorbed protein layers.

Monospecific rabbit antisera (primary antisera, IgG fraction, Sigma Chemical Co.) against bovine serum albumin (BSA) and gamma globulin (IgG) were used. The IgG fraction of rabbit antisera against bovine fibrinogen was obtained from immunized rabbits and isolated using protein A Sepharose CL-4B (Pharmacia Co.). The samples were incubated in rabbit primary antisera against each protein for 24 hrs at 40℃, then rinsed with PBS-BSA containing 0.05% Tween-20 (TPBS). In the reaction of samples with primary antiserum against BSA, PBS gelation was used for its specificity instead of PBS-BSA. The samples were incubated in gold conjugated second antibody (mean particle size = 10 nm; Sigma Chemical Co.) for 1 hr at room temperature. After rinsing with TPBS, the samples were immersed in a 1% (w/v) OsO_4 solution for 10 min, followed by fixation in Karnovsky's solution for 5 min, and then rinsed with PBS buffer (pH 7.4).

Then samples were freeze-dried overnight, coated with a 6% polyvinylpyrrolidone (Sigma Chemical Co.) solution, and cut with an ultramicrotome. A cross-sectional view was observed with a Hitachi transmission electron microscopy (TEM, model H-600 EM).

6. ELISA

Adsorbed plasma proteins (fibrinogen, albumin, and IgG) of each segment were quantified by an enzyme linked immunosorbent assay (ELISA). The plasma proteins on each specimen of the ELVAD were eluted with 2% (w/v) sodium dodecyl sulfate (SDS)/PBS (pH 7.4) for 2 days and dialyzed against PBS (pH 7.4). The protein samples (0.1 ml each) were added into each well of the microelisa plate (Dynatech Co.) and incubated overnight at 40℃. After washing the plate with PBS-Tween 20 solution (TPBS, 0.05 % (v/v) Tween 20/PBS), the samples were incubated in 0.1 ml aliquots of rabbit primary antisera to canine fibrinogen, albumin, and IgG at room temperature. Then the samples were washed with TPBS and incubated in 0.1 ml aliquots

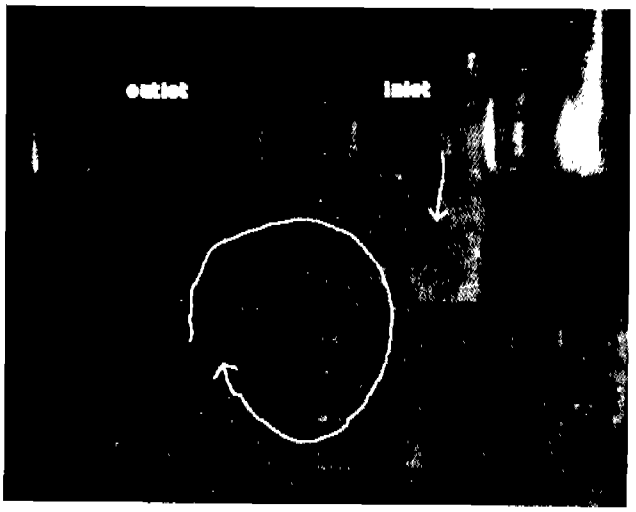

Fig. 3. Image of the typical flow pattern inside the ventricle at diastolic phase

of peroxidase-conjugated anti-rabbit second antibody (Sigma Chemical Co.) at a dilution of 1 : 1,500 for 2 hrs at room temperature. After rinsing the plate with TPBS, 0.2 ml aliquot of ELISA substrate solution (0.1% 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 0.005% H_2O_2, 0.09M NaH_2PO_4, and 0.05M citric acid, pH 4.6) was added to each well and mixed. The absorbance of each well was read at 405 nm in the microelisa reader (Dynatech Co.).

7. SEM observation

Samples (1 × 1 mm) were cut from sectionized segments, rinsed with PBS, fixed with 2% glutaraldehyde solution in PBS buffer for 24 hrs at 37℃, dehydrated with several dilutions of ethanol and water, and then lyophilized. The sample was coated with an evaporated gold layer and the morphology of adhered platelets was observed with a scanning electron microscopy (SEM, Hitachi S-510) at 15 kV. The degree of platelet adhesion was evaluated by counting the numbers of platelets adhered on the overall surface.

RESULTS AND DISCUSSION

A large vortex was developed at diastolic phase inside the ventricle as shown in Fig. 3. The small picture in the south-eastern part is the original image of the flow pattern formed at diastolic phase. The white curve in the picture indicates the direction of the vortex. A small region of flow separation could be observed near the outlet valve. The degrees of the wall shear stress can be categorized into 2 groups as listed in Table 2.

Fig. 2 shows schematic diagram of the sectionized ventricle for ELVAD. The blood-contacted ventricles according to the level of the shear rate after animal death were sectionized. Because analysis of adsorbed protein might be influenced by the size of the ventricle segment, the number of segments was limited to 8 for a ventricle. The wall shear stress of segment A is considered to be the lowest among four parts since there exists a small region of flow separation at diastolic phase. Less proteins seem to be adsorbed in the region of the high shear stress, but the statistical significance of this fact was not obtained. It is interesting, however, to note that in the case of PU-PEO-SO_3, the significant change on protein adsorption was observed between high and low shear stress. Namely, more albumin adsorption and much less fibrinogen and IgG adsorption were found in low shear stress (segment A) than in high one (segment B, C, and D).

Table 2. Protein adsorption[a] and platelet adhesion[b] on shear level of ventricular surfaces

Segment	Shear level	PU				PU-PEO-SO_3			
		Fibrinogen	Albumin	IgG	Platelet	Fibrinogen	Albumin	IgG	Platelet
A	low	94.8	0.8	1.2	2	54.4	1.1	NA[c]	1
B	high	32.5	0.5	0.8	2	30.1	0.5	0.5	2
C	high	34.1	0.4	0.9	2	28.7	0.5	0.5	1
D	high	57.7	0.8	0.7	1	45.9	1.6	NA	1

[a] By ELISA, unit : ng/cm^2
[b] By SEM, relatively adhesion level : 2 > 1
[c] NA : not available

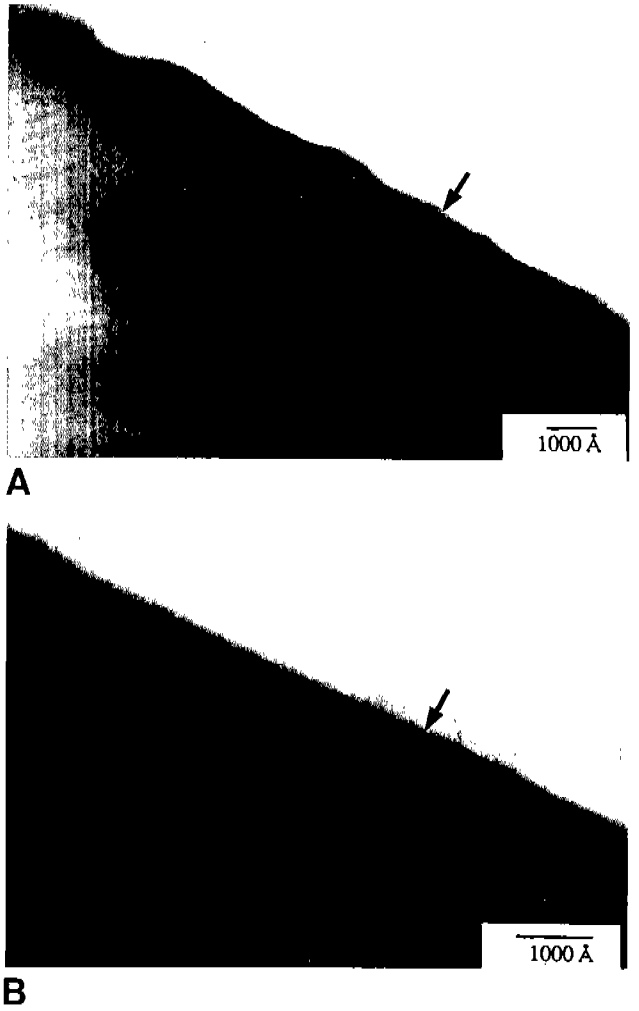

Fig. 4. Cross-sectional TEM views of adsorbed proteins on **(a)** PU and **(b)** PU-PEO-SO_3 surfaces

Fig. 4 shows cross-sectional TEM views of adsorbed proteins on (a) PU and (b) PU-PEO-SO_3 surfaces. The thickness of the adsorbed protein layer on PU surface was 1,000-1,600 Å, while that of PU-PEO-SO_3 surface was only about 400 Å. This means that the surface of PU-PEO-SO_3 may passivate protein adsorption to a great extent due to synergistic effect of the grafted PEO and sulfonate groups. Fig. 5 shows typical cross-sectional TEM view of adsorbed fibrinogen on PU surface. The dots in the adsorbed protein layer indicate the stained fibrinogens. The adsorption behaviors of other proteins, such as albumin and IgG, on the surfaces were similar to the adsorption of fibrinogen.

The amount of adsorbed proteins on each segment of PU and PU-PEO-SO_3 ventricles by ELISA is also shown

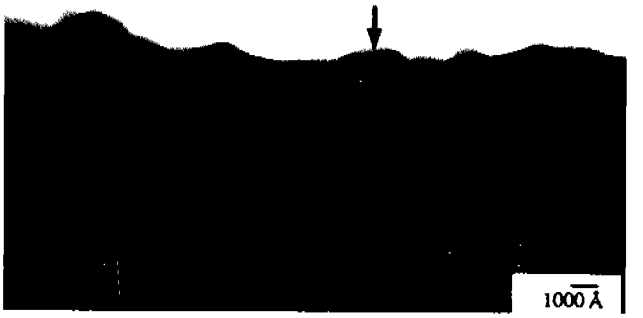

Fig. 5. Typical cross-sectional TEM view of adsorbed fibrinogen on PU surface

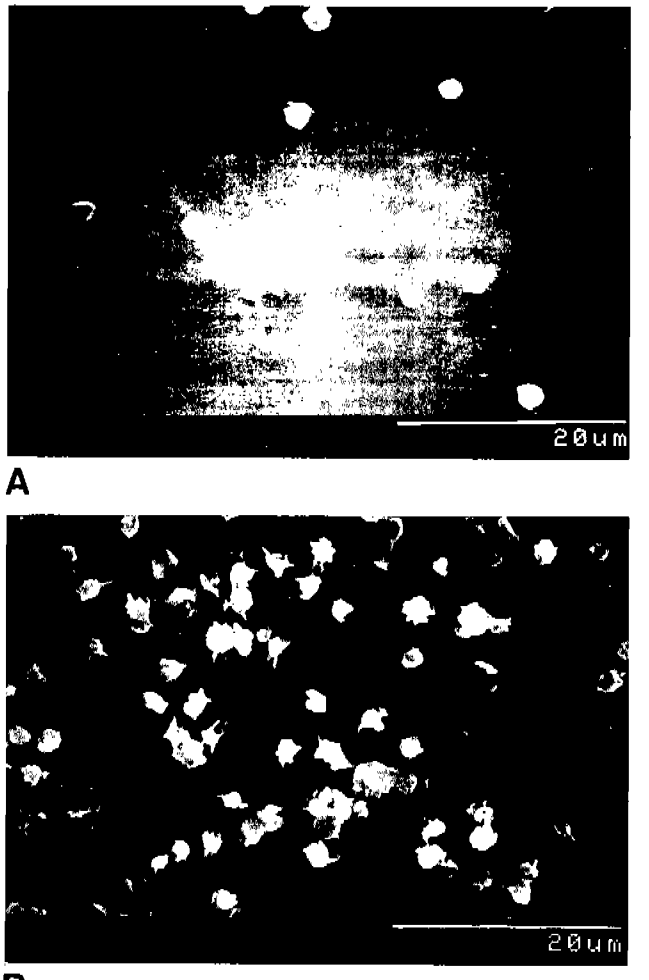

Fig. 6. SEM picture of adhered platelets on ventricular surfaces: **(a)** low level, **(b)** high level

in Table 2. The degree of total protein adsorption on PU-PEO-SO_3 was relatively lower than untreated PU, but only albumin adsorption on PU-PEO-SO_3 was high as compared with PU, thus indicating that PU-PEO-SO_3 has specific high affinity for albumin. In addition, less adsorption of fibrinogen and IgG was revealed on PU-PEO-SO_3 surface than PU one. These results are well consistent with *in vitro* protein study as described elsewhere[9, 10].

The degree of platelet adhesion by SEM observation on overall surface of each sample was determined and categorized them into 2 groups as shown in Fig. 6. Results of platelet adhesion for each segment are also shown in Table 2. Although the degree of platelet adhesion was little correlated with the shear level in the ventricle of two experiments, overall platelet adhesion on PU-PEO-SO_3 was considerably low as compared with PU.

Although PU-PEO-SO_3 surface is more inert than PU surface to the plasma proteins, protein adsorption to the surface is dependent on the wall shear stress. Therefore, the effect of the wall shear stress on the protein adsorption unlike platelet adhesion is dependent on the surface characteristics of the material, as mentioned by Pitt and Cooper[11]. Because of the short implantation time, the relationship between the protein adsorption and platelet adhesion on materials was not observed in present study. It must be needed to measure the level of the wall shear stress more absolutely and accurately in order to compare these results to others and to find the dependency of the surface characteristics on the shear level.

From these results of animal ELVAD experiments, PU-PEO-SO_3 coated on a ventricle showed high albumin adsorption, low fibrinogen and IgG adsorption, and low platelet adhesion as compared with untreated PU, suggesting that PU-PEO-SO_3 is more *in vivo* blood compatible. Such an improved blood compatibility of PU-PEO-SO_3 is due to the synergistic effect of the hydrophilicity and dynamic mobility of PEO chains and the electrical repulsion of negatively charged sulfonate(SO_3) groups, as explained by "negative cilia" model[12, 13].

CONCLUSIONS

PU-PEO-SO_3 was coated on a blood sac for electrohydraulic left ventricular assist device(ELVAD) implanted in dogs and its *in vivo* blood compatibility on shear stress was studied as compared with untreated PU.

A well-developed large vortex was observed at the center of the artificial ventricle by *in vitro* flow visualization and the blood-contacted ventricle was sectionized according to the level of the shear rate from results of flow visualization after animal death.

Less proteins seem to be adsorbed in the region of the high shear stress, but the statistical significance of this fact was not obtained. The effect of the wall shear stress on the protein adsorption unlike platelet adhesion is dependent on the surface characteristics of the material. Because of the short implantation time, the relationship between the protein adsorption and platelet adhesion on materials was not observed.

The thickness of total proteins adsorbed on PU-PEO-SO_3(400 Å) by TEM was considerably lower than that of untreated PU(1,000-1,600 Å), but PU-PEO-SO_3 coated on a ventricle showed high albumin adsorption, low fibrinogen and IgG adsorption, and low platelet adhesion as compared with untreated PU, suggesting that PU-PEO-SO_3 is more *in vivo* blood compatible. Therefore, such a blood compatible PU-PEO-SO_3 is expected to be useful for blood contacting biomaterials including artificial organs.

ACKNOWLEDGEMENT

This work was supported by the Korean Ministry of Science and Technology(MOST) Grant No. 829-4480-1.

REFERENCES

1. J. Kim, G.H. Ryu, I. Shin, K.B. Lee, D.K. Han, Y.H. Kim, and B.G. Min. *Effect of shear rates on protein adsorption in the total artificial heart*. Am. Soc. Artif. Intern. Organs J., 38, 532-535, 1992
2. K. Mabuchi, H. Nakamura, K. Imachi, K. Ijiri, T. Chinzei, Y. Abe, T. Yonezawa, I. Sugawara, H. Nozawa, K. Imanishi, and I. Fujimasa. *Changes with respect to time in the* in vivo *adsorption of plasma proteins onto artificial heart blood pumps.* Am. Soc. Artif. Intern. Organs J., 38, 536-542, 1992
3. J.W. Boretos, W.S. Pierce, R.E. Baier, A.F. Leroy, and H.J. Donarchy. *Surface and bulk charateristics of a polyether urethane for artificial hearts.* J. Biomed Mater Res, 9, 327-340,

1975

4. T. Matsuda, H.Takano, K.Hayashi, Y. Taenaka, S. Takaichi, M. Umezu, T. Nakamura, H. Iwata, T. Nakatani, T. Tanaka, S. Takatani, and T. Akutsu. *The blood interface with segmented polyurethanes: multilayered protein passivation mechanism.* Trans Am. Soc. Artif. Intern. Organs, 30, 353-358, 1984
5. C. Nojiri, S.W. Kim, H.A. Jacobs, G.M. Pantalos, and D.B. Olsen. *Scanning and transmision electron microscopic evaluation of the U100 total artificial heart blood contacting surface.* Trans Am. Soc. Artif. Intern. Organs, 35, 679-683, 1989
6. D.K. Han. *Characteristics and biocompatibility of sulfonated poly(ethyleneoxide)-grafted polyurethanes.* Ph.D. Dissertation, Dept. of Biomed. Eng., Seoul National Univ., 1993
7. D.K. Han, K.B. Lee, K.D. Park, C.S. Kim, S.Y. Jeong, Y.H. Kim, H.M. Kim, and B.G. Min. In vivo *canine studies of Sinkhole valve and vascular graft coated with biocompatible PU-PEO-SO_3.* Am. Soc. Artif. Intern. Organs J., 39, 537-541, 1993
8. J.W. Choi. *A study on the development of an electrohydraulic left ventricular assist device.* Ph.D. Dissertation, Dept. of Biomed. Eng., Seoul National Univ., 1993
9. D.K. Han, G.H. Ryu, K.D. Park, S.Y. Jeong, Y.H. Kim, and B.G. Min. *Adsorption behavior of fibrinogen to sulfonated polyethyleneoxide-grafted polyurethane surfaces.* J. Biomater. Sci. Polym. Edn, 4, 401-413, 1993
10. D.K. Han, G.H. Ryu, K.D. Park, S.Y. Jeong, B.G. Min, and Y.H. Kim. *Adsorption behaviors of plasma proteins to various modified polyurethane surfaces.* J. Biomed. Mater. Res., submitted, 1994
11. W.G. Pitt and S.L. Cooper. *FTIR-ATR studies of the effect of shear rate upon albumin adsorption onto polyurethaneurea.* Biomaterials, 7, 340-347, 1986
12. D.K. Han, S.Y. Jeong, Y.H. Kim, B.G. Min, and H.I. Cho. *Negative cillia concept for thromboresistance: Synergistic effect of PEO and sulfonate groups grafted onto polyurethanes.* J. Biomed. Mater. Res., 25, 561-575, 1991
13. Y.H. Kim, D.K. Han, S.Y. Jeong, and B.G. Min. *Negative cilia concept for enhanced blood compatibility: Grafting of PEO-sulfonate onto polyurethane.* Progress in Pacific Polymer Science, Y. Imanishi (Ed.), Springer-Verlag, Berlin, 1992, pp. 235-244