Polymer(Korea) Vol. 21, No. 2, pp 332-341(1997)

UV 처리에 의해 PEO가 고정화된 PMMA 필름 표면에서의 단백질 흡착 및 혈소판 점착 거동

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(1997년 1월 4일 접수)

Plasma Protein Adsorption and Platelet Adhesion onto PEO-entrapped PMMA Film Surfaces Prepared by Photo-induced Polymerization

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(Received January 4, 1997)

요 약: PEO가 1～20 wt% 함유된 MMA를 UV 처리(100 W, 365 nm)에 의해 중합하여 PEO가 고정화된 PMMA 필름을 제조하였다. 이때 사용한 PEO의 분자량은 400, 10000, 100000이었다. 제조된 필름 표면들은 물접촉각 측정과 ESCA에 의해 분석되었다. 필름 내에 고정화된 PEO의 안정성을 평가하기 위해 필름을 일주일까지 물 속에 담가 계속 흔들어 준 후 꺼내어 무게 변화를 측정하였다. PEO가 고정화된 필름 표면에서의 혈장 단백질 흡착 및 혈소판 점착 거동을 조사한 결과, 필름 내에 고정화된 PEO의 분자량 증가에 따라 또한 PEO의 표면 밀도 증가에 따라 단백질 흡착 및 혈소판 점착이 감소하는 경향을 보였다. 특히 분자량 100000의 PEO가 고정화된 필름 표면에서 PEO의 표면 밀도가 높을 때 단백질 흡착 및 혈소판 점착 감소가 상당히 낮게 나타나, 혈액 적합성 재료로서의 사용 가능성을 높게 해 주고 있다.

ABSTRACT: Polyethylene oxide (PEO)-entrapped polymethyl methacrylate (PMMA) films were prepared by photo-induced polymerization of methyl methacrylate (MMA) containing 1～20 wt% of PEO with different molecular weight (400, 10000, and 100000). The photopolymerization was carried out using a 100 W ultraviolet light source (wavelength, 365 nm). The prepared PEO-entrapped PMMA film surfaces were characterized by the measurement of water contact angle and electron spectroscopy for chemical analysis (ESCA). The stability of PEO entrapped in PMMA films was also examined by immersing the films in water for up to 7 days with continuous shaking and measuring the weight changes. The behavior of plasma protein adsorption and platelet adhesion on the PEO-entrapped PMMA film surfaces was investigated. It was observed that the plasma protein adsorption and platelet adhesion on the film surfaces decreased with increasing PEO molecular weight and its surface density. The PEO 100,000-entrapped surfaces with high PEO content were very effective for the prevention of protein adsorption and platelet adhesion.

Keywords: *PEO surfaces, polymethyl methacrylate (PMMA), photo-induced polymerization, protein adsorption, platelet adhesion.*

INTRODUCTION

Polyethylene oxide (PEO or polyethylene glycol (PEG) when the molecular weight is less than about 10000) is well recognized as an effective polymer for low protein adsorption and low cell adhesion.[1-3] It seems that many factors are involved in PEO's passivity in aqueous solution such as its minimum interfacial free energy with water, hydrophilicity, high surface mobility and steric stabilization effects, and unique solution properties and molecular conformation in water.[3-10]

Immobilization of PEO or its derivatives onto a surface of polymeric substrate has been attempted by various methods to generate a PEO surface. Those methods include physical adsorption, covalent coupling, and graft copolymerization. PEO surfaces have been prepared by physical adsorption of various PEO-containing amphiphilic block copolymers onto existing hydrophobic substrates.[4,5,11,12] The hydrophobic block segments of the PEO-containing block copolymers provide hydrophobic adsorption forces or anchor to the polymer substrate. This method seems applicable to many biomedical areas, due to its simplicity and nonspecificity. But the main disadvantage is that the immobilized polymers may not remain on the surface permanently.

Covalent coupling of PEO or PEO derivatives to substrate is the most effective way of creating a permanent PEO surface. Commonly used technique include direct coupling, which leads to surfaces containing pendant PEO chains.[13-18] The direct coupling methods use PEO molecules which have first been derivatized using a reactive coupling agent. The activated PEO reacts with a functional group on the surface; hence covalent coupling method is possible only if the surface has chemically active functional groups which can react with PEO derivatives. This limits the application of the techniques only to certain materials, and the procedures are usually very complicated and time-consuming.

For inert surfaces without any functional groups, such as polyethylene (PE), polypropylene (PP), and polytetrafluoro ethylene (PTFE), PEO coupling is possible only when the surface is premodified with reactive functional groups. Reactive functional groups utilize initiating species like free radicals or peroxides which are generated on polymer surfaces by ultra-violet (UV) irradiation,[19,20] high energy gamma irradiation,[21,22]
and plasma or corona discharge.[23-25] Methoxy PEG monomethacrylate macromers are commonly used to prepare this type of PEO surfaces. The graft copolymerization method seems widely applicable, because the initiating species for graft copolymerization of monomer can be generated on any polymer substrates by the pre-irradiation technique.

In this study, PEO surfaces were prepared by photo-induced polymerization of methyl methacrylate (MMA) containing PEO. The PEOs with different molecular weight (400, 10000, and 100000) were melted and dissolved in MMA monomer solution without using any solvents. The prepared PEO-entrapped PMMA film surfaces were characterized by the measurement of water contact angle and electron spectroscopy for chemical analysis (ESCA). The stability of PEO entrapped in PMMA films was also examined. The behavior of plasma protein adsorption and platelet adhesion on the PEO-entrapped PMMA film surfaces was investigated in terms of PEO molecular weight and its surface density.

EXPERIMENTAL

Materials. MMA monomer (Aldrich, U.S.A)

was purified by washing with 10% NaOH and then vacuum distillation before use. PEO with different molecular weight (Aldrich; 400, 10000, and 100,000) and 2,2-dimethoxy-2-phenyl-acetophenone (benzil dimethyl ketal (BDMK; Aldrich)) as an initiator were of chemical grade and used as received.

Preparation of PEO-entrapped PMMA Films. PEO (0~20 wt%; MMA base) was added to 1 g MMA and heated to about 100 ℃ to melt the PEO. 4 μL of BDMK initiator (from a stock solution of 100 mg BDMK/mL MMA) was added and mixed well to homogeneity. Dry nitrogen gas was purged into the solution for 1 min to remove oxygen in the solution. The solution was injected to form a film between two clean cover glasses (No. 1, Corning, U. S. A.) with a 0.33 mm-thick PTFE gasket and clamped with binder clips, similarly to the method described by Drumheller et al.[26] It was placed in a pre-warmed (about 100 ℃) chamber and a 100 W high pressure mercury vapor lamp light (365 nm, Blak-Ray UVP, U. S. A.) was irradiated onto the film for up to 80 min. After UV irradiation, the cover glasses were gently peeled away from the photopolymerized PMMA film. The films prepared were stored in a vacuum oven until use.

Characterization of PEO-entrapped PMMA Films. The prepared PEO-entrapped PMMA film surfaces were characterized by measuring water contact angles. The water contact angle, an indicator of the surface hydrophilicity, was measured by a sessile drop method at room temperature using an optical bench-type contact angle goniometer (Model 100-0, Rame-Hart, U. S. A.). Drops of purified water, 3 μL, were deposited onto the film surface to form a sessile drops using a micro-syringe attached on the goniometer. The direct microscopic measurement of the contact angles was done with the goniometer. More than 5 different positions were measured for each film surface and averaged. The changes in chemical structure of PMMA film surfaces by the entrapped PEO with different molecular weight and content were investigated by ESCA. The ESCA (ESCALAB MKII, V. G. Scientific Co., U. K.) was equipped with Al Kα radiation source at 1487 eV and 300 W at the anode. Survey scan and carbon 1S core level scan spectra were taken to analyze the film surfaces.

To examine the stability of PEO entrapped in PMMA films, the films (1.5 cm × 4.0 cm × 0.33 mm) were weighed after thorough drying and immersed in water for 1 and 7 days with continuous shaking using orbital shaker (Model 160, Aros, U. S. A.). After that, the films were taken out from the water, rinsed with fresh water, dried to constant mass in vacuum oven, and then weighed again. The PEO extraction was determined as follows:

$$\text{PEO extraction}(\%) = (W_{dry} - W_{dry,\,final})/W_{dry}$$

where W_{dry} denotes the weight of the dry film before immersion in water and $W_{dry,\,final}$ the weight of the dry film after water immersion.

Plasma Protein Adsorption. Human plasma (Sigma, U. S. A.) was diluted with phosphate buffered saline (PBS, pH 7.4) to make 10 % solution. The control and PEO-entrapped PMMA films were placed on 24 well polystyrene plates (Corning, U. S. A.) and equilibrated with PBS for 30 min. After removing the PBS solution from the wells by pipetting, the plasma protein solution was added to the wells. After 1 hr incubation at 37 ℃, the films were washed with PBS, followed by washing with water to remove unadsorbed proteins. After vacuum drying, the protein-adsorbed film surfaces were analyzed by ESCA. The nitrogen 1S peaks from

the survey scan spectra were used for the analysis of proteins adsorbed on the surfaces.

Platelet Adhesion. Blood from healthy canines (adult mongrel dogs not on medication) was collected with a PP syringe containing 3.8% sodium citrate solution (final dilution, 1:9). Blood was centrifuged at 300 g for 15 min at 4 ℃ to obtain platelet-rich plasma (PRP) and portions further centrifuged at 2000 g for 15 min at 4 ℃ to obtain platelet-poor plasma (PPP). The platelet concentration of PRP was adjusted to 5×10^4 platelets/μL by adding PPP to PRP. The number of platelets was counted by a haemocytometer. The control and PEO-entrapped PMMA films were placed on the 24 well plates and equilibrated with PBS for 30 min. PRP was pre-warmed to 37 ℃ and added to the 24 well plates after removing the PBS solution. After 2 hr incubation at 37 ℃, the films were washed carefully with PBS to remove weakly adhered platelets. The platelets adhered on the surfaces were examined by a scanning electron microscope (SEM, JSM-840A, Jeol, Japan). Adhered platelets were fixed with 2.5% glutaraldehyde (Gibco Laboratories, U. S. A.) for 15 min at room temperature. After thorough washing with PBS, the platelets adhered on the film surfaces were dehydrated in an ethanol-graded series (50, 60, 70, 80, 90, and 100%) for 10 min each and allowed to dry in a clean bench at room temperature. The platelet-attached surfaces were gold deposited in vacuum and examined by SEM with a tilt angle of 45°. The platelet density on the surfaces was also estimated by SEM. Different fields were randomly counted and the results were expressed as the average number of platelets adhered per mm^2 of surface.

RESULTS AND DISCUSSION

Characterization of PEO-entrapped PMMA Films.

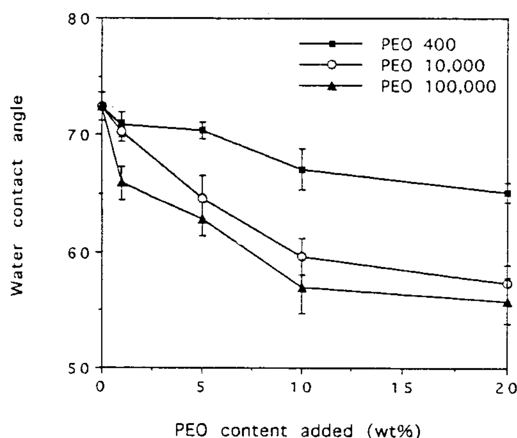

Figure 1. Water contact angles of PEO-entrapped PMMA film surfaces as a function of PEO content added. Sample numbers, n=5.

PEO did not dissolve in MMA at temperature below T_m of PEO, about 60 ℃. However, melts of PEO in MMA were homogeneous and transparent at temperature above 60 ℃. Photopolymerized PMMA film surfaces showed water contact angles of about 72°. The PEO-entrapped PMMA films were also transparent and a little flexible compared to the PMMA homopolymer films. The entrapped PEO seems to act as a kind of plasticizer. The water contact angles of the PEO-entrapped PMMA film surfaces decreased with increasing PEO molecular weight and its content as shown in Fig. 1. The decrease in the contact angles (and thus the increase in surface hydrophilicity) is due to the entrapped PEO projecting from the surfaces (Fig. 2). It is well known that surface modifiers or additives tend to migrate to the surface region, resulting in the higher concentration on the surface than the bulk.[27]

The changes in chemical structure of PMMA film surfaces by the entrapped PEO with different molecular weight and content were analyzed by ESCA carbon 1S spectra. The PMMA homopolymer film surface had alkyl carbon

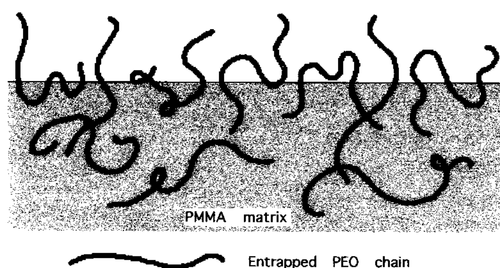

Figure 2. Schematic diagram showing a PEO-entrapped PMMA film.

(-C-C-, binding energy~285.0 eV), ether carbon (-C-O-,~286.6 eV), and carboxylic carbon (O=C-O-,~289.1 eV) peaks, as labeled in Fig. 3. The PEO-entrapped PMMA film surfaces showed the significantly increased ether carbon peaks. The increase in the ether carbon peaks is derived from the PEO molecules entrapped on the surfaces since all carbons in PEO are ether carbons. Table 1 lists up the results of ESCA

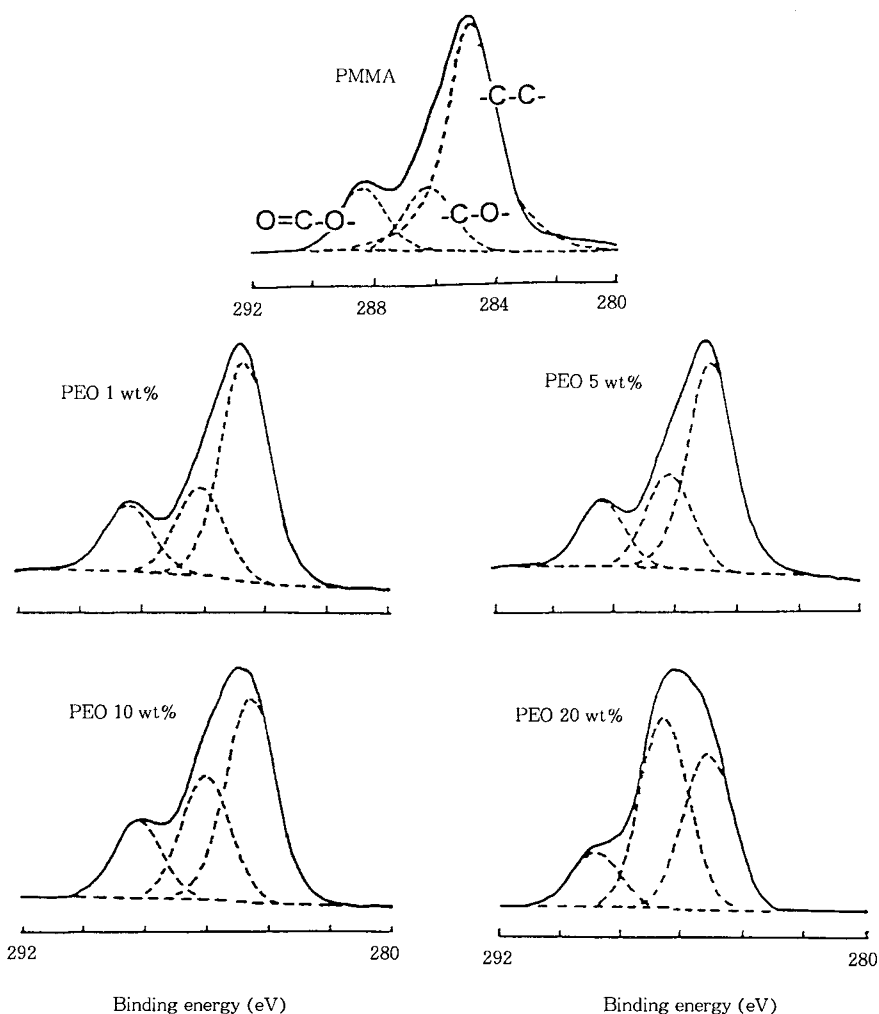

Figure 3. ESCA carbon 1S core level spectra of PMMA homopolymer and PEO 100000-entrapped PMMA film surfaces.

Table 1. ESCA Analysis of PMMA Homopolymer and PEO 100000-entrapped PMMA Film Surfaces

surface	wt%	atomic %			
	PEO	-C-C-	-C-O-	O=C-O-	-C-O-/-C-C-
PMMA	0	68	17	16	0.25
PEO 400	1	53	28	19	0.53
-entrapped	5	54	28	18	0.52
PMMA	10	53	29	18	0.55
	20	53	32	15	0.60
PEO 10000	1	65	21	14	0.32
-entrapped	5	62	22	16	0.35
PMMA	10	51	31	19	0.61
	20	46	40	15	0.87
PEO 100000	1	59	23	18	0.39
-entrapped	5	57	25	18	0.44
PMMA	10	50	31	19	0.62
	20	39	47	14	1.21

analysis of the PEO-entrapped PMMA film surfaces. From the Table, we can see that the atomic % of ether carbon on the surfaces increased as the PEO content added in the MMA to be entrapped increased, indicating the increase in PEO surface density on the surfaces.

To examine the stability of PEO entrapped in PMMA films, the films were immersed in water for 1 and 7 days. As seen in Fig. 4, all PEO-entrapped PMMA films demonstrated some extraction of PEO, the extraction decreasing with the increase in PEO molecular weight. The low molecular weight PEO (400) was extracted to a considerable extent after 1 day immersion in water, while the high molecular weight PEO (100000) showed no significant extraction even after 7 day immersion, except for the case of 20 % addition of PEO in MMA monomer. It seems that longer PEO chains were more tightly entrapped into the PMMA substrates than shorter ones, probably due to the more entanglement between PEO chains and PMMA chains.

Interactions of Plasma Proteins and Platelets with the Surfaces. Human plasma, which contains more than 200 kinds of different proteins,[28] was adsorbed onto the control PMMA homopolymer and PEO-entrapped PMMA film surfaces and the relative adsorbed amount of proteins on the surfaces was evaluated by ESCA. The nitrogen signal from peptide bonds was used as an indicator of surface protein adsorption. Fig. 5 shows ESCA survey scan spectra of the control and PEO-entrapped PMMA film surfaces after plasma protein adsorption. The nitrogen peak (binding energy, ~ 399.0 eV) from the control PMMA surface was much higher (about 5.1 atomic %) than the

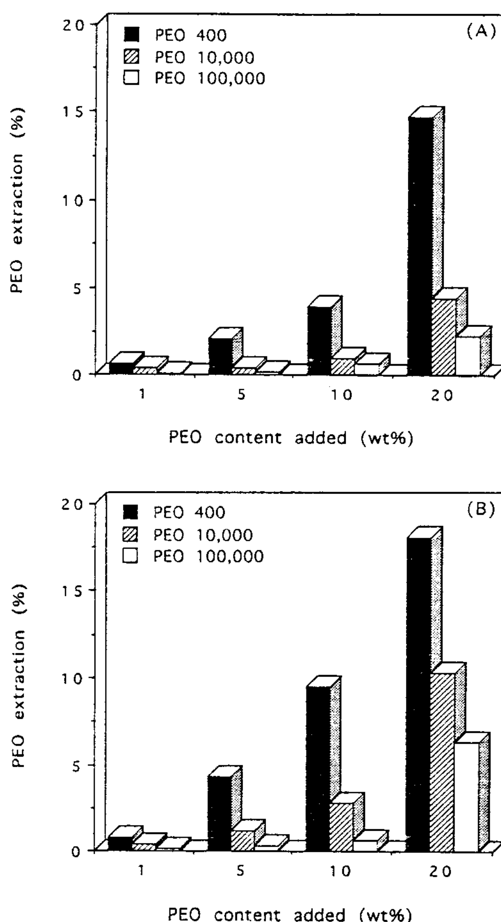

Figure 4. Extraction of entrapped PEO from PMMA films by immersion in water for (A) 1 day and (B) 7 days.

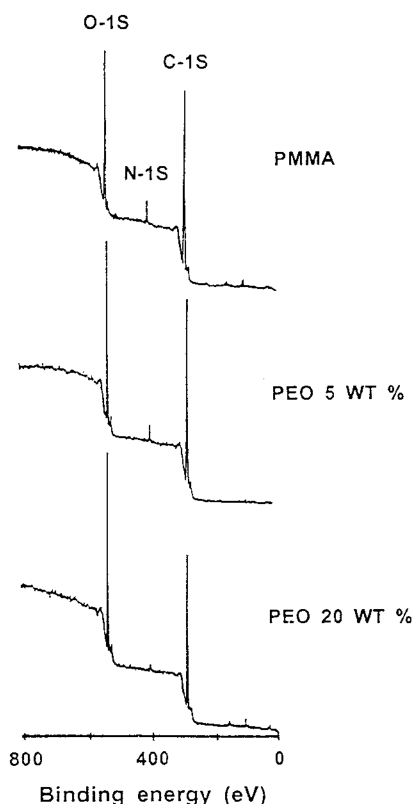

Figure 5. ESCA survey scan spectra of control and PEO 100000-entrapped PMMA film surfaces (1 hr adsorption in 10% plasma protein solution).

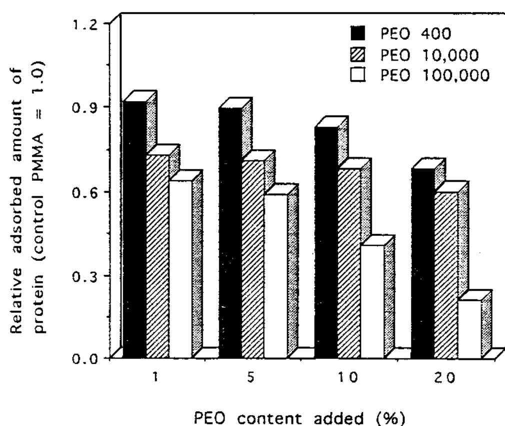

Figure 6. Relative adsorbed amount of proteins on PEO-entrapped PMMA film surfaces.

PEO-entrapped PMMA surfaces, indicating the larger amount of protein adsorption on the control surface. This is probably due to the hydrophobic interaction of the protein molecules with the hydrophobic PMMA surface. For the PEO-entrapped PMMA surfaces, the nitrogen peaks decreased with the increasing PEO content added. This indicates that the protein adsorption was reduced with the increasing PEO surface density. Fig. 6 compares the relative adsorbed amount of proteins on the PEO-entrapped PMMA film surfaces. It was determined as follows:

$$\text{Relative adsorbed amount of proteins} = \frac{(\text{N \% of PEO-entrapped surface})}{(\text{N \% of control surface})}$$

As seen in Fig. 6, the adsorption of plasma protein decreased with increasing PEO molecular weight and its content in the PEO-entrapped PMMA films. In the case of the surfaces entrapped with PEO 100000 with 10 and 20% addition, the amount of adsorbed proteins was reduced about 60 and 80%, respectively, as compared to the control surface, while the PEO 400 and PEO 10000-entrapped surfaces did not show effective reduction of protein adsorption.

For platelet adhesion study, PRP separated from fresh canine blood was incubated on the PEO-entrapped PMMA film surfaces and the platelets adhered on the surfaces were observed by SEM. Fig. 7 shows the SEM pictures of platelets attached to the PEO 10000 and PEO 100000-entrapped PMMA surfaces with different PEO content added. The platelet adhesion on the surfaces decreased with increasing PEO molecular weight and its surface density. The PEO-entrapped surfaces with high PEO content were effective for the prevention of platelet adhesion (Fig. 8).

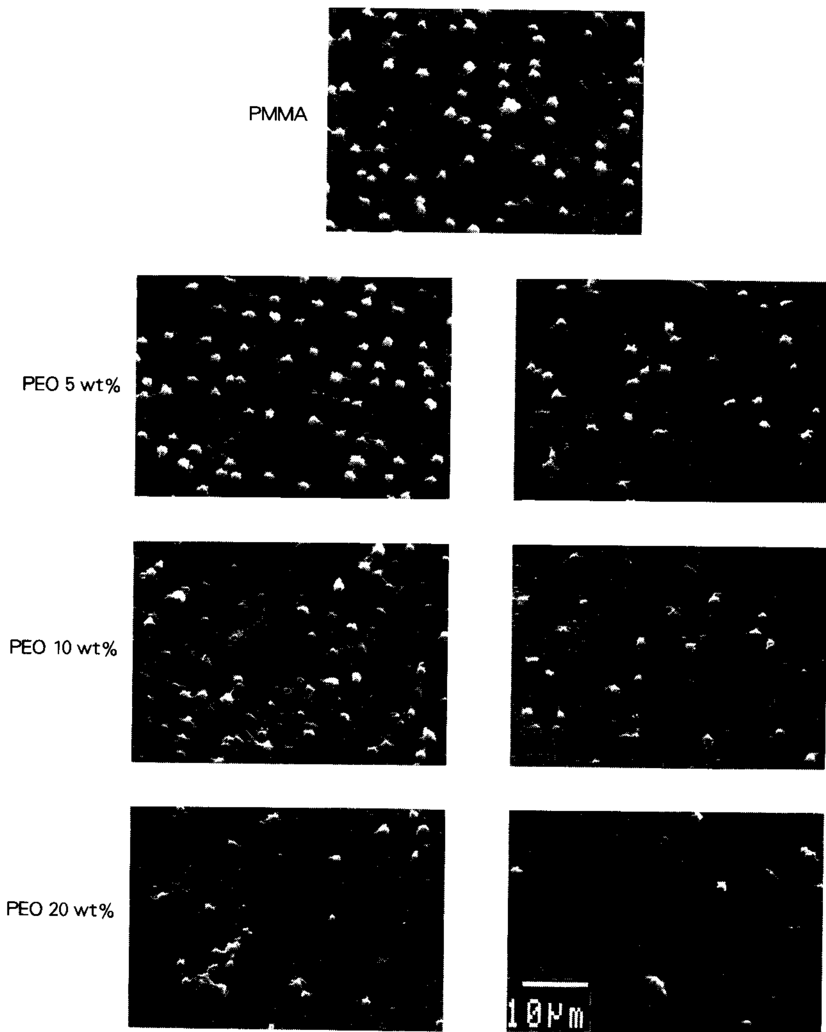

Figure 7. SEM pictures of platelets adhered on control and PEO-entrapped PMMA film surfaces (2 hr adhesion in PRP).

Possible explanations for PEO's passivity of the PEO-entrapped surfaces include its minimum interfacial free energy with water, hydrophilicity, high surface mobility and steric stabilization effects, and unique solution properties and molecular conformation in water, as discussed earlier. The hydrophilicity and unique solubility properties of PEO produce surfaces that are in a liquid-like state with the polymer chains exhibiting considerable flexibility or mobility.[2,10] PEO is the most flexible in water among common water-soluble polymers because it has flexible ether linkages in its backbone and does not have bulky side groups and thus will not be hindered sterically in water. It appears that the PEO molecule has a large excluded volume in water.[3] PEO surfaces in water with rapidly moving hydrated PEO chains and a large excluded volume tend to repel protein or platelet molecules which approach the surface.

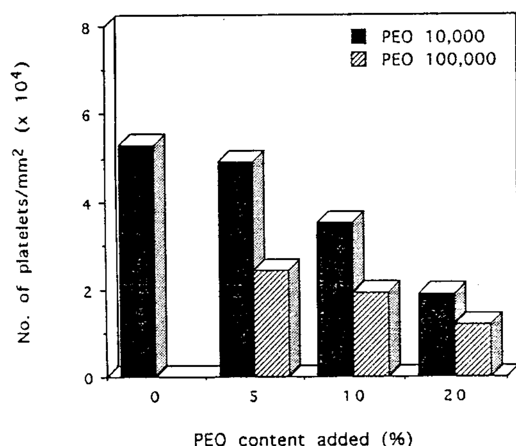

Figure 8. Number of platelets adhered on control and PEO-entrapped PMMA film surfaces.

CONCLUSIONS

PEO surfaces were prepared by photo-induced polymerization of MMA containing PEO with different molecular weight (400, 10000, and 100000). The photopolymerized PEO-entrapped PMMA films were transparent and a little flexible compared to the PMMA homopolymer films. It seems that the entrapped PEO acts as a kind of plasticizer. The water contact angles of the PEO-entrapped PMMA film surfaces decreased with increasing PEO molecular weight and its content. The decrease in the contact angles was due to the entrapped PEO projecting from the surfaces. The surface density of PEO increased as the PEO content added in the MMA increased, as verified by ESCA. All PEO-entrapped PMMA films demonstrated some extraction of PEO, the extraction decreasing with the increase in PEO molecular weight. However, the high molecular weight PEO (100000) showed no significant extraction even after 7 day immersion in water. The plasma protein adsorption and platelet adhesion on the PEO-entrapped PMMA film surfaces decreased with increasing PEO molecular weight and its surface density. The PEO 100000-entrapped surfaces with high PEO content were effective for the prevention of protein adsorption and platelet adhesion.

This study demonstrated that photo-induced polymerization of MMA containing PEO without using any solvents can be a feasible method to prepare PEO surfaces as blood-compatible surfaces.

Acknowledgment: This paper was supported by 1995 Research Fund of Hannam University.

REFERENCES

1. J. D. Andrade, S. Nagaoka, S. Cooper, T. Okano, and S. W. Kim, *ASAIO J.*, **10**, 75 (1987).
2. E. W. Merrill and E. W. Salzman, *ASAIO J.*, **6**, 60 (1983).
3. J. H. Lee, H. B. Lee, and J. D. Andrade, *Prog. Polym. Sci.*, **20**, 1043 (1995).
4. J. H. Lee, J. Kopecek, and J. D. Andrade, *J. Biomed. Mater. Res.*, **23**, 351 (1989).
5. J. H. Lee, P. Kopeckova, J. Kopecek, and J. D. Andrade, *Biomaterials*, **11**, 455 (1990).
6. S. I. Jeon, J. H. Lee, J. D. Andrade, and P. G. de Gennes, *J. Colloid Interface Sci.*, **142**, 149 (1991).
7. M. Amiji and K. Park, *J. Biomat. Sci., Polym. Edn.*, **4**, 217 (1993).
8. J. D. Andrade, *Medical Instrum.*, **7**, 110 (1973).
9. D. L. Coleman, D. E. Gregonis, and J. D. Andrade, *J. Biomed. Mater. Res.*, **16**, 381 (1982).
10. R. Kjellander and E. Florin, *J. Chem. Soc., Faraday Trans. I*, **77**, 2053 (1981).
11. J. H. Lee and J. D. Andrade, "Polymer Surface Dynamics", ed. by J. D. Andrade, p. 119, Plenum Press, New York, 1988.
12. M. Amiji and K. Park, *Biomaterials*, **13**, 682 (1992).
13. A. Kishida, K. Mishima, E. Corretge, H. Konishi, and Y. Ikada, *Biomaterials*, **13**, 113 (1992).
14. N. P. Desai and J. A. Hubbell, *J. Biomed. Mater. Res.*, **25**, 829 (1991).
15. G. R. Llanos and M. V. Sefton, *Macromol.*, **24**, 6065 (1991).

16. E. Kiss, C. G. Golander, and J. C. Eriksson, *Prog. Colloid Polym. Sci.*, **74**, 113 (1987).
17. C. Nojiri, T. Okano, H. A. Jacobs, K. D. park, S. F. Mohammad, D. B. Olsen, and S. W. Kim, *J. Biomed. Mater. Res.*, **24**, 1151 (1990).
18. D. K. Han, S. Y. Jeong, and Y. H. Kim, *J. Biomed. Mater. Res., Appl. Biomat.*, **23**, 211 (1989).
19. Y. Mori, S. Nagaoka, H. Takiuchi, T. Kikuchi, N. Noguchi, H. Tanzawa, and Y. Noishiki, *Trans. ASAIO*, **28**, 459 (1982).
20. E. Brinkman, A. Poot, L. van der Does, and A. Bantjes, *Biomaterials*, **11**, 200 (1990).
21. Y. H. Sun, W. R. Gombotz, and A. S. Hoffman, *J. Bioactive Compat. Polym.*, **1**, 316 (1986).
22. B. Jansen and G. Ellinghorst, *J. Biomed. Mater. Res.*, **19**, 1085 (1985).
23. K. Fujimoto, H. Inoue, and Y. Ikada, *J. Biomed. Mater. Res.*, **27**, 1559 (1993).
24. B. J. Jeong, J. H. Lee, and H. B. Lee, *J. Colloid Interface Sci.*, **178**, 757 (1996).
25. J. H. Lee, B. J. Jeong, and H. B. Lee, *J. Biomed. Mater. Res.*, **34**, 105 (1997).
26. P. D. Drumheller and J. A. Hubbell, *J. Biomed. Mater. Res.*, **29**, 207 (1995).
27. B. E. Rabinow, Y. S. Ding, C. Qin, M. L. McHalsky, J. H. Schneider, K. A. Ashline, T. L. Shelbourn, and R. M. Albrecht, *J. Biomat. Sci., Polym. Edn.*, **6**, 91 (1994).
28. H. G. Schwick and K. Heide, *Trends Biochem. Sci.*, **2**, 125 (1977).