Transactions of the Materials Research Society of Japan 29 [1] 177-180 (2004)

Segmented Polyurethane / 2-Methacryloyloxyethyl Phosphorylcholine Nano-Ordered Surface Morphology for Biomedical Applications

Ryo Ogawa, Junji Watanabe, and Kazuhiko Ishihara
Department of Materials Engineering, School of Engineering,
The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan
Fax: 81-3-5841-8647, e-mail: ryo@bmw.t.u-tokyo.ac.jp

The segmented polyurethane (SPU) is applied for biomedical devices. However, much plasma proteins were adsorbed on the SPU surface, and the clot formation occurred when the SPU is contacted with blood. We attempted the modification of SPU by photoinduced grafting with the 2-methacryloyloxyethyl phosphorylcholine (MPC) on the surface of SPU in order to reduce the protein adsorption. The photoinduced grafting was carried out using hydrogen peroxide and $Fe(II)^{2+}$ system. And the density of the poly(MPC) chains on the surface could be controlled by MPC in feed in the photoinduced grafting process. The surface can be almost covered with the MPC units, and showed the high hydrophilicity. The mechanical properties of the SPU introduced hydroperoxide groups did not change significantly. The adsorptions of immunoglobulin (IgG) were investigated. The SPU grafted with MPC inhibited the IgG adsorption on the surface effectively.
Key words: Phospholipid polymer, Photografting, Segmented polyurethane, Surface modification.

1. INTRODUCTION

The segmented polyurethane (SPU)s are widely used as an elastomer due to their good mechanical properties. However, their surfaces are poor functions in the view point of the biomaterials, the biocompatibility and chemical stability of the SPUs under biological conditions are not satisfactory for long-term implantation and contacting with blood.[1] Therefore, it is necessary to improve its surface properties, without having any adverse effects on the excellent properties of SPU. There have been many articles that reported to attempt to modify the surface of the SPU to obtain biocompatibility, such as the covalent bonding of alkyl groups for the selective adsorption of albumin,[2] poly(ethylene oxide), PEO, chains for the reduction of protein adsorption,[3] and heparin molecules for preventing thrombin activation on the surface.[4] However, these methods did not give enough biocompatibility. We have been investigating the preparation and evaluation of phospholipid polymers as novel biomaterials. The phospholipid polymers, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers (Fig.1), inhibit protein adsorption and cell adhesion, even when they contact human whole blood in the absence of an anticoagulant. The immobilization of the MPC polymer chains by the grafting polymerization of MPC on the surface of SPU is one of the good methods to obtain biocompatibility. And this method possibly maintains the performance of SPU. Also, the important advantage of the grafting is stability for long-term applications.

In this study, we attempted to introduce the poly(MPC) on the surface of SPU through photoinduced grafting of MPC. A number of different methods are used for surface grafting. The UV irradiation-induced polymerization was one of the surface grafting methods. It conducts conveniently and the cost is low. To modify the surface through the UV irradiation-induced polymerization, the photosensitive groups must be

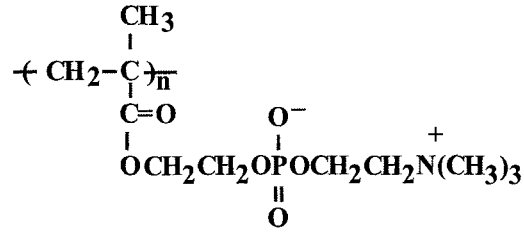

Fig. 1. Chemical structure of poly(MPC).

introduced on the SPU surface. As hydroperoxide (HPO) group is UV sensitive, thus we introduced the hydroperoxide groups onto the SPU surface in this study. The amount of the hydroperoxide group could be controlled by the time of UV irradiation, and the graft density of hydroperoxide groups enables us to control the grafting. We oxidized the SPU membrane in hydrogen peroxide solution under UV irradiation, and HPO groups were introduced mainly on the soft segments of SPU. When irradiated under UV light, the hydroperoxide groups on the surface decompose into macromolecular oxygen radicals and hydroxyl radicals. The oxygen radicals can initiate the grafting copolymerization. However, the hydroxyl radicals cannot initiate grafting but produce homopolymer. Thus, to minimize the production of homopolymers, ammonium iron (II) sulfate hexahydrate was added as a reductant.[5] The process is shown in Fig. 2.

The MPC has a methacrylate group, and it is easy to introduce the poly(MPC) chains onto the surface of SPU by the photoinduced grafting of MPC. With introducing MPC onto the surface of SPU through this method, this work will show the potential of MPC to applications for long-term implantations. The simplicity of the photoinduced grafting method will enable to modify various shapes of medical devices, such as

Step 1: Introduction of HPO Groups

$$H_2O_2 \xrightarrow{h?} 2HO^{\bullet} \quad (1)$$

$$RO^{\bullet} + SPU \longrightarrow ROH + SPU^{\bullet} \quad (2)$$

$$SPU^{\bullet} + O_2 \longrightarrow SPU\text{-}O\text{-}O^{\bullet} \quad (3)$$

Step 2: Grafting Copolymerization

$$SPU\text{-}OOH \xrightarrow{h?} SPU\text{-}O^{\bullet} + HO^{\bullet} \quad (4)$$

$$SPU\text{-}OOH + Fe^{2+} \xrightarrow{k1} SPU\text{-}O^{\bullet} + HO^{\bullet} + Fe^{3+} \quad (5)$$

$$SPU\text{-}O^{\bullet} + Fe^{2+} \xrightarrow{k2} SPU\text{-}O^{-} + Fe^{3+} \quad (6)$$

$$HO^{\bullet} + Fe^{2+} \longrightarrow HO^{-} + Fe^{3+} \quad (7)$$

$$SPU\text{-}O^{\bullet} + M \longrightarrow SPU\text{-}O\text{-}M^{\bullet} \quad (8)$$

Fig. 2. The process of photoinduced grafting.

catheter, pacing leads, and artificial hearts, and grant them excellent biocompatibility. And the grafting can control the density of poly(MPC) on the surface easily. The control of surface characteristics is important to dominate the interactions between material surface and living organism. In this study, taking the size of biomolecules(around dozens nanometers) into consideration, we attempted to control the surface structures in nano-scale in order to prevent the unfavorable interaction of biomolecules. Through this method, we can control the MPC layer in the scale of biomolecules. The nano-ordered surface will contribute to inhibiting the troubles between material surfaces and living organism.

2. MATERIALS AND METHODS

2.1. Membrane Preparation

A solution containing 5 wt% SPU (Tecoflex 60 / aliphatic polyether polyurethane, Fig. 3) was prepared. As a solvent, an EtOH / CH_2Cl_2 mixture (3 / 7 by volume) was used. The solution was stirred for 30 min and sonicated for another 30 min at room temperature. Twenty mL of the solution was cast on a 20-cm^2 glass dish. To evaporate the solvent, the dish was kept for 5 hours at room temperature, and the glass plate for covering the dish was used to control the evaporation rate of solvent. After this process, the dish was kept at 60 °C overnight. To evaporate the remaining solvent completely, the dish was then dried under vacuum condition at 60 °C overnight period. The SPU membrane formed in the dish was carefully peeled off. The thickness of the membrane was 200 _m. The SPU membrane was subsequently placed in 40 mL hydrogen peroxide solution (30%) and UV was irradiated with a high-pressure mercury lamp (250 W) for 8 hours under stirring at room temperature. The photooxidized membrane was rinsed with water to free excess hydrogen peroxide and dried at room temperature in vacuum. A given amounts of MPC, 15 mL aqueous solution of ammonium iron (II) sulfate hexahydrate (concentration of ammonium iron (II) sulfatehexahydrate is 1.5 x 10^{-3} mol/L) and photooxidized membrane, were charged into a glass tube closed with a rubber stopper. Graft copolymerization was carried out under UV irradiation at a distance of 10 cm for 1 hour at room temperature. The photoinduced grafting process is shown in Fig. 3. The membrane was rinsed with hot water (70 °C) for 2 days to remove the ungrafted polymers, and subsequently dried in vacuum at 50 °C for 24 h.

2.2. Characterizations

The amounts of HPO groups were determined by the iodometry method.

The mechanical strength of SPU membrane and that with HPO groups were evaluated the tensile strength measurement. The test was carried out using a STA-1150 (ORIENTEC, Tokyo, Japan). The samples were cut into a dog bone shape (the size was 12.5 mm x 2.5 mm). The crosshead speed was 10 mm/min. The number of specimens tested was three.

The surface compositions were gained by X-ray photoelectron spectroscopy (XPS), using an ESCA-200 (AXIS His 165, Shimadzu/Kratos, Kyoto, Japan). The measurement was carried out at room temperature. The take-off angle of the photoelectron was 90 degree.

Static contact angle measurements with water were carried out to gain the wettability of the grafted membranes.

To evaluate the amounts of protein adsorption on the surface, 15 mm diameter membranes were contacted with IgG solution with concentration of 1.6 g/dL in phosphate buffer solution (PBS, pH 7.4) at 37 °C for 60 min. After the membrane was rinsed with PBS, the remaining IgG adsorbed on the surfaces was removed with a 1 wt% aqueous solution of sodium dodecylsulfate (SDS). The amount of IgG in the SDS solution was then determined by the micro-BCA method using a clinical test kit (micro BCA protein assay reagent kit, #23235, Pierce, Rockford, IL, USA).

3. RESULTS AND DISCUSSION

On the SPU membranes, the HPO groups

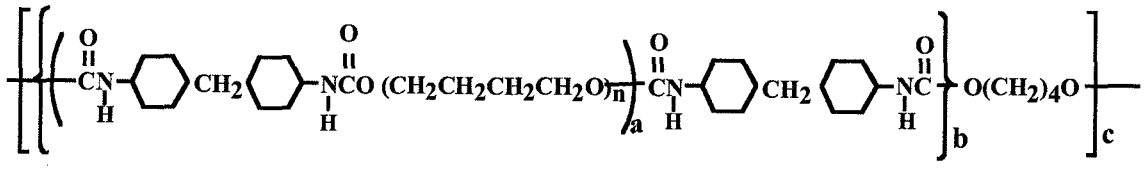

Fig. 3. Chemical structure of segmented polyurethane.

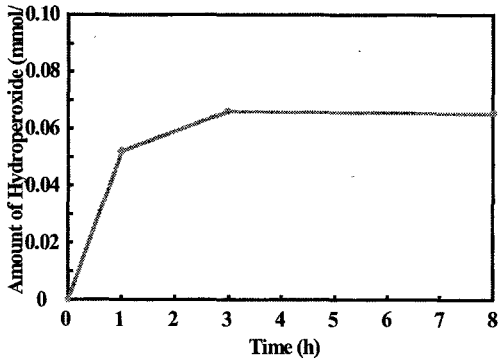

Fig. 4. The amount of HPO groups on the surface of SPU.

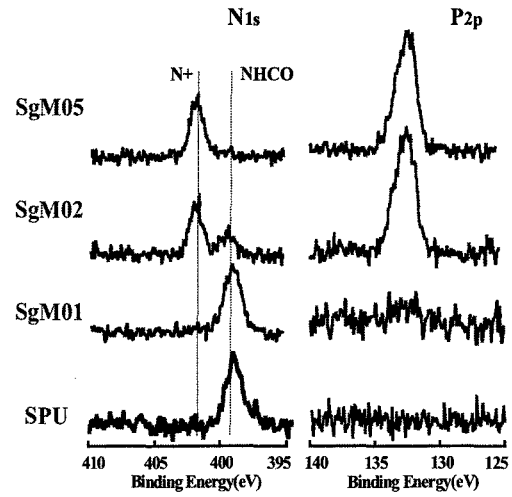

Fig. 5. XPS charts of SPU grafted with poly(MPC).

were introduced by UV irradiation. The amounts of HPO groups were determined by the iodometry method. As shown in Fig. 4, the amounts are increased with UV irradiation. In this research, we used the aromatic polyether polyurethanes as SPU. The amounts of HPO groups on the polyether polyurethane were less than that of polyester polyurethane.[5] This is because polyether chains are more durable against UV irradiation.

The effect of photooxidization on the mechanical properties was estimated by the tensile strength measurement. The Young's modulus of SPU was 49.2 MPa, and that of SPU with HPO groups (UV irradiation time was 8 hours) was 41.3 MPa. Maximum elongation of SPU was 724 %, and that of SPU with HPO groups was 578 %. It was revealed that UV irradiation reduced the mechanical properties of SPU.

The MPC units were introduced by photografting method. The samples were prepared in various monomer concentrations in feed. The monomer concentrations of 0.1, 0.2 and 0.5 were designated as SgM01, SgM02 and SgM05 respectively. The results of surface grafting were summarized in Table I.

To evaluate the poly(MPC) concentration on the surface using XPS, phosphorus atom concentration divided by carbon atom concentration (P/C) was calculated with the intensity of signal ratios. The result revealed that the MPC concentrations in feed dominated the density of MPC chains grafted on the surface of SPU. When the surface is covered by the MPC units completely, the P/C value will be 0.091 theoretically. Also, a nitrogen atom peak in urethane bonds of SPU completely disappeared in SgM05 (shown in Fig. 5). This XPS analyzed around 20 nm in depth from the surface. As the result, the poly(MPC) layer with thickness of about 20 nm covered the surface of SPU completely. The poly(MPC) is water-soluble polymer, and shows strong hydrophilicity.

In the static contact angle measurements, the contact angles of SgM01, SgM02, and SgM05 were around 40 degree, and that of SPU was about 70 degree (Fig. 6). The SPU grafted with poly(MPC) showed high hydrophilicity. There are significantly no distinctions among MPC feeds. The SgM01 was as hydrophilic as other grafted samples against P/C values. This result suggested that non-reacted HPO groups became hydroxyl groups, and then the contact angles decreased comparing with SPU.

The surface biocompatibility was estimated by protein (IgG) adsorption tests. The results are shown in Fig. 7. The result showed that SPU grafted with poly(MPC) reduced the IgG adsorption on the surface. Also, as the concentration of MPC units increased, the amounts of adsorbed IgG were reduced gradually. The static contacts angles showed that the very small amounts of MPC units gave strong hydrophilicity on the

Table I. The results of surface grafting.

Samples	MPC mole concentration in feed (mol/L)	Surface compositions (P/C)	Contact angles (deg)	The amounts of IGG adsorption (μ g/cm^2)
SPU	—	0.000	71.9 ± 2.3	2.93 ± 0.14
SgM01	0.1	0.003	44.1 ± 3.2	1.90 ± 0.38
SgM02	0.2	0.049	37.5 ± 7.0	1.37 ± 0.04
SgM05	0.5	0.079	38.6 ± 2.9	0.62 ± 0.02

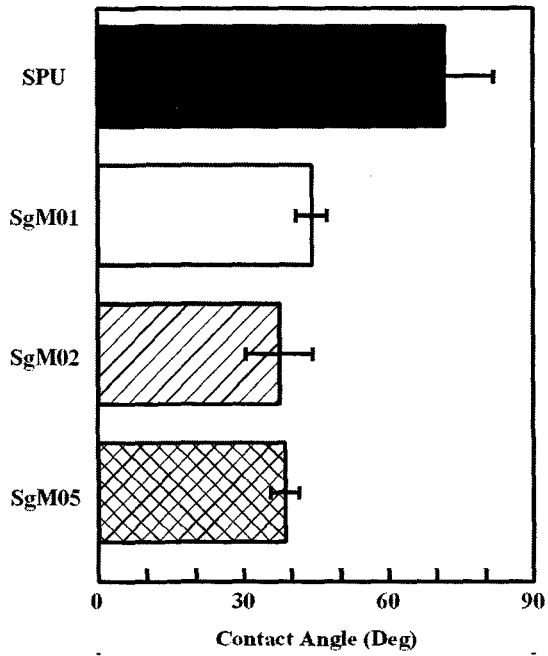

Fig. 6. Static wate contact angles of MPC grafted SPU.

surface of SPU, but protein adsorption test showed that the large amounts of MPC units proven more excellent biocompatibility. It is revealed that when the surface shows high hydrophilicity, it does not means that the surface can inhibit the protein adsorption. It is assumed that he poly(MPC) can maintain water structure, and proteins contacted with such water does not denature and adsorb on the surface. Thus, all SPU grafted with poly(MPC) showed hydophilicity at the same level, but the difference occurred for IgG adsorption. It was revealed that the surface of SPU should be covered with poly(MPC) completely to obtain the good biocompatibility.

It was concluded that photoinduced grafting methods could introduce MPC units on the surface of SPU. The desity of poly(MPC) on the surface of SPU was controlled, and nano-ordered layer of poly(MPC) was formed on the surface. The SPU grafted with poly(MPC) showed high hydrophilicity, but the amounts of IgG adsorption were different among SPU

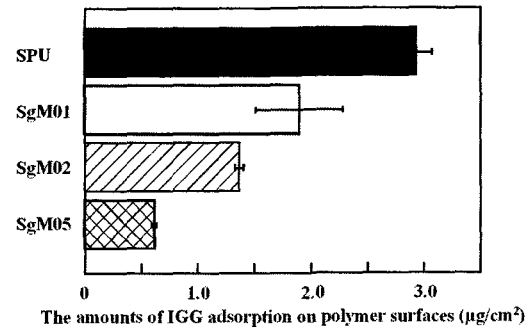

Fig. 7. The protein adsorption on the surface of MPC grafted SPU.

grafted with poly(MPC). Thus, the surface layer composed of poly(MPC) has significant effects on the surface properties. It is important to control the surface in nano-scale, and the surface that covered completely by MPC units is necessary to obtain excellent biocompatibility. The SPU grafted with poly(MPC) showed biocompatibity because of poly(MPC) and could applied for biomedical devices.

Acknowledgement

This research was supported by Grant for 21st Century COE Program "Human-Friendly Materials based on Chemistry" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by Grant-in-Aid for Scientific Research (B) (13480288) from JSPS.

References

[1] N. M. K. Lamba, K. A. Woodhouse, and S. L. Cooper, *Polyurethanes in Biomedical Applications*, CRC Press, Boca Raton, FL (1998).

[2] W. G. Pitt, T.G. Grasel, and S.L. Cooper, *Biomaterials* **9** , 36-46 (1998).

[3] E. Brinkman, L. van der Does Poot, and A. Bantjes, *Biomaterials* **11**, 200-05 (1990).

[4] K. D. Park, T. Okano, C. Nojiri, and S. W. Kim, *J Biomed Mater Res* **22** , 977-92 (1988).

[5] J. Guan, C. Gao, L. Feng and J. Shen *J Appl Polym Sci* **77**, 2505-12 (2000).

(Received October 13, 2003; Accepted November 10, 2003)