Improvement of blood compatibility on cellulose dialysis membrane. III. Synthesis and performance of water-soluble cellulose grafted with phospholipid polymer as coating material on cellulose dialysis membrane

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To improve the surface blood compatibility on a cellulose hemodialysis membrane, a blood compatible polymer with a phospholipid polar group, poly[2-methacryloyloxyethyl phosphorylcholine(MPC)], was immobilized on the surface through the coating of a water-soluble cellulose grafted with poly(MPC) (MPC-grafted cellulose, MGC). The MGC was synthesized by graft copolymerization of MPC on a water-soluble cellulose using cerium ion as an initiator. The coating process on the cellulose membrane with an aqueous solution of the MGC was convenient, and the MGC on the surface was not significantly detached even after immersion in water. The permeability and mechanical strength of the membrane coated with the MGC did not decrease compared with the original membranes. The MGC-coated cellulose membrane was blood compatible, as determined by the prevention of platelet adhesion and aggregation after contact with platelet-rich plasma. From these results, it is concluded that the MGC may be a useful material for improving the blood compatibility of the cellulose hemodialysis membrane. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Hemodialysis is one of the most important methods for blood purification.[1] The properties required for a hemodialysis membrane are an excellent ultrafiltration rate, permeability by solutes, mechanical strength, and blood compatibility. Many synthetic polymer membranes have been investigated to raise the efficiency of dialysis; however, cellulose membranes are still used worldwide for 85% of hemodialysis. Although the cellulose membrane has both good permeability and mechanical strength, its blood compatibility must be further improved for better hemodialysis.[2] Thus, it is necessary to infuse an anticoagulant such as heparin during hemodialysis to prevent coagulation. Moreover, the cellulose membrane induces significant activation of the complement system because of strong interactions between the membrane surface and complement proteins.[3]

Some investigations have been carried out to solve these problems associated with the cellulose hemodialysis membrane—that is, the surface of the membrane has been modified with low-molecular-weight compounds and hydrophilic polymers.[4–6] Although these modifications did reduce the activation of complement, platelet adhesion and concomitant clot formation were not inhibited. Therefore, a more effectiveness of modification of the cellulose membrane surface is necessary.

Recently, we found that polymers with a phospholipid polar group, 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymerized with hydrophobic monomers such as *n*-butyl methacrylate (BMA) showed improved blood compatibility.[7–12] The poly(MPC-*co*-BMA) with a 0.32 MPC mole fraction suppressed blood cell adhesion and activation when the copolymer contacted with human platelet-rich plasma and whole blood even in the absence of an anticoagulant.[8,11,12] The total blood coagulation time determined by the Lee–White method on glass was extended from 8 to 30 min by coating poly(MPC-*co*-BMA) on the glass surface.[11] The amount of plasma proteins adsorbed on the MPC copolymer from human plasma was drastically reduced with an increase in the MPC mole fraction in the copolymer.[9,11] These

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results clearly indicated that the MPC moieties in the copolymer play an important role in improved blood compatibility.

The MPC can polymerize in water and graft on a cellulose membrane surface even in a heterogeneous system. Therefore, we synthesized cellulose membranes grafted with poly(MPC) and investigated their blood compatibility *in vitro* and *ex vivo*.[13–15] The results of these studies showed that the introduction of poly(MPC) chains on the membrane surface was effective in preventing platelet adhesion and activation. Moreover, complement activation induced by contact with the cellulose membrane was reduced with an increase in the MPC mole fraction on the surface. However, the efficiency of the graft polymerization of MPC on the membrane surface was not satisfactory, and the grafting process was not readily applicable to dialyzer processing.

We considered that a water-soluble polymer having both affinity to the cellulose base membrane and excellent blood compatibility could improve the surface blood compatibility of the cellulose membrane by a convenient technique, such as coating, using its aqueous solution. Therefore, we chose the cellulose unit as the backbone and poly(MPC) as a side chain for the coating polymer. This communication describes the synthesis of a water-soluble cellulose grafted with poly(MPC)(MGC) and its performance as a coating material for improved blood compatibility on a cellulose hemodialysis membrane.

EXPERIMENTAL

Materials

We synthesized MPC by a previously described method and recrystallized it from acetonitrile.[8] The chemical formula of the MPC is shown in Figure 1. Cellulose powder was obtained from Merck Co., Ltd., and used without further purification. Acetic acid and acetic acid anhydride were purified by distillation, and the fractions bp 139.0°C and bp 118.0°C, respectively, were used. The regenerated cellulose hemodialysis membrane, Cuprophan™, was obtained from Enka, A.G. (Wappertal-Barmen, Germany). The thickness of the membrane was 20 μm. Other reagents were commercially available and used without further purification.

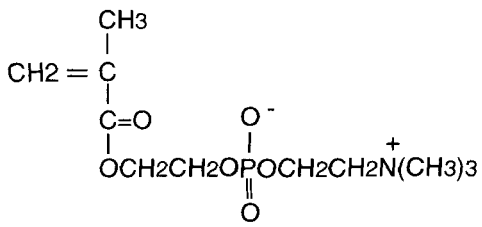

Figure 1. Chemical formula of MPC.

Synthesis of water-soluble cellulose grafted with MPC

The synthetic route of the water soluble-cellulose grafted with poly(MPC) (MPC-grafted cellulose, MGC) is shown in Figure 2. It consisted of three reactions, acetylation of cellulose, hydrolysis of the acetyl cellulose with degradation of the main chain of the cellulose to form a water-soluble cellulose, and graft polymerization of MPC initiating from the water-soluble cellulose. In a flask, 10 g of cellulose powder, 38 ml of acetic acid, 38 ml of acetic acid anhydride, and 4 ml of sulfonic acid were placed. The mixture was heated at 50°C for 60 min with stirring. The reaction mixture was then poured into distilled water, and the resulting precipitate was collected by filtration and then dried. The obtained cellulose acetate was identified by Fourier transform-infrared (IR) spectroscopy (DX-20, Nicolet, WI).

The obtained cellulose acetate (0.25 g) was suspended in a 50-ml aqueous solution of 1 N sodium hydrogen carbonate, then 100 ml aqueous solution of 3 N sodium hydroxide was added to the solution with stirring. After the solution became transparent, hydrochloric acid was slowly added to neutralize it. A part of the solution was removed and sufficiently dried to confirm the structure of the product by IR.

To an aqueous solution containing the water-soluble cellulose, aqueous solutions containing MPC and 0.1 mmol/ml cerium ammonium nitrate (pH 1.0) were added. After argon gas was bubbled through the mixture for 10 min to eliminate any oxygen in the solution, the mixture was heated at 40°C for 1 h under an argon atmosphere. The solution was transparent during the reaction. The reaction mixture was dialyzed against distilled water for 3 days to remove any low-molecular-weight impurities such as unreacted MPC, cerium ions, or other inorganic ions. The obtained MGC was stored as an aqueous solution (0.5 wt%) in a refrigerator to prevent spontaneous gelation. A portion of the solution was removed and dried to determine the chemical structure of the MGC and the mole fraction of the MPC in the MGC using IR spectroscopy and phosphorus analysis, respectively. A gel-permeation chromatograph (GPC; Tosoh's system with a refractive index detector and a size-exclusion type column, TSK-gel G3000PWXL; Tosoh, Tokyo, Japan) was used to estimate the molecular size [number average molecular weight (Mn)] of the MGC in the water system. The calibration was carried out by a comparison between the elution time of the sample polymer and that of poly(oxyethylene)

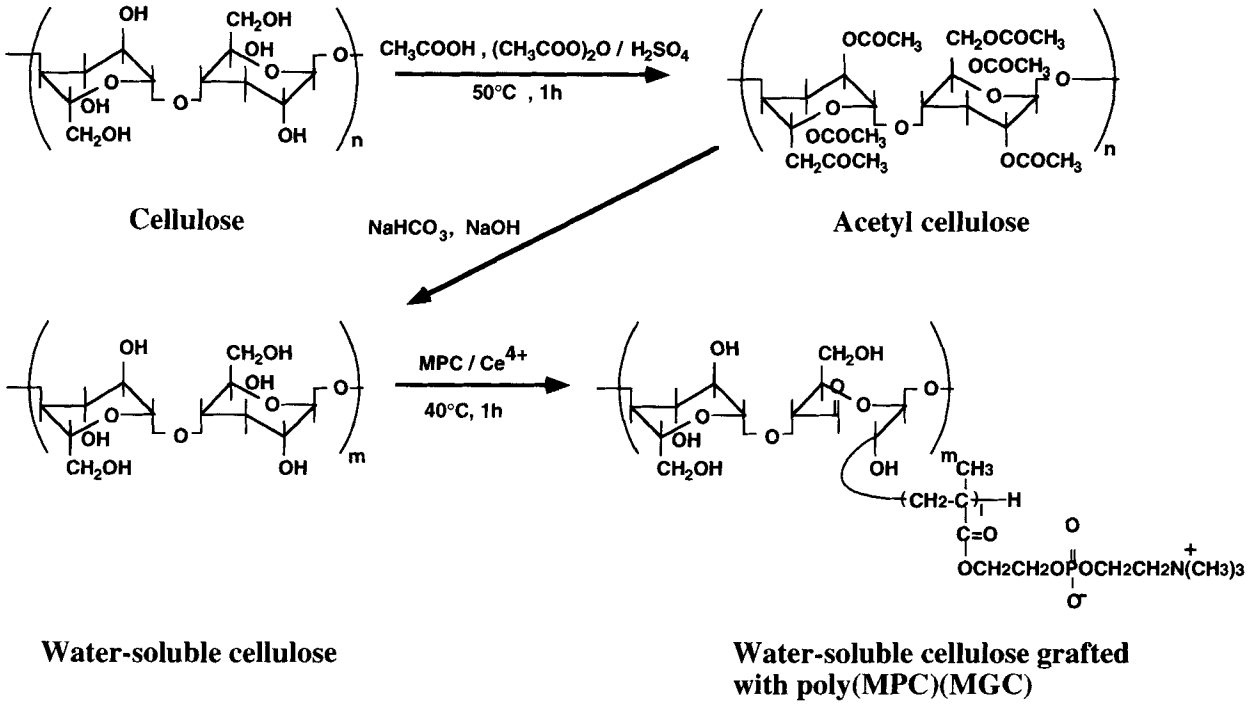

Figure 2. Synthetic route of the water-soluble cellulose grafted with poly(MPC) (MPC-grafted cellulose, MGC).

(POE) standards (Tosoh). The synthetic results of the MGC are summarized in Table I.

Coating of the MGC on cellulose membrane

The coating of the MGC on the cellulose membrane was carried out using a 0.5 wt% aqueous solution of the MGC. The cellulose membrane was immersed in the solution for 3 min, and the membrane was removed and dried under atmospheric conditions for 2 h, then dried *in vacuo* for 15 h. The structure of the grafted MPC on the cellulose membrane was confirmed using X-ray photoelectron spectroscopy (XPS, ESCA-750; Shimadzu, Kyoto, Japan) and IR spectroscopy. The ratio of phosphorus atom (P) in the MPC unit versus carbon atom (C) was determined from the XPS elemental analysis. The mole fraction of MPC on the membrane surface defined as [number of MPC unit(mol)]/[number of MPC unit (mol) + number of cellulose unit(mol)] was calculated from the value of P/C.[13] The amount of MGC on the cellulose membrane was determined by phosphorus analysis of the membrane coated with the MGC. The results are summarized in Table II.

Elution test of the MGC from the membrane

Cellulose membranes coated with the MGC (1.0 × 1.0 cm) were immersed into various solvents for 180 min to elute the MGC. The solvents used in this study were distilled water, 40 vol% ethanol aqueous solution, and ethanol. Moreover, boiling water was also used as an elution solvent—that is, the mem-

TABLE I
Synthetic Result of Water-Soluble Cellulose Grafted with Poly(MPC)(MGC)

Code	MPC Concentration in Feed Solution (mmol/L)	MPC Unit Mole Fraction[a] in the MGC	Mn[b] (×10⁴)
MGC-1	60.0	0.062	2.0
MGC-2	80.0	0.13	2.3
MGC-3	100	0.34	3.1

[a]Determined by phosphorus analysis.
[b]Estimated by GPC measurement with poly(oxyethylene) standard.

TABLE II
Characterization of Cellulose Membrane Coated with the MGC

Code	Amount of MGC on the Membrane (μg/cm²)[a]	MPC Unit Mole Fraction[b] at the Membrane Surface
MGC-1	46	0.033
MGC-2	53	0.11
MGC-3	48	0.25

[a]Determined by phosphorus analysis.
[b]Determined by XPS analysis.

brane coated with the MGC was boiled for 30 min, then cooled to room temperature. A specific amount of these solvents was removed, and the amount of the MPC unit in the solvent was determined from phosphorus analysis. After the membrane was immersed in water for 180 min at 37°C, the membrane was dried under reduced pressure for 1 day and the surface analyzed by XPS to determine the surface mole fraction of MPC.

Measurement of membrane tensile strength

Tensile strength of the membrane in the wet state was measured with an autograph (DSS-500; Shimadzu) to estimate its mechanical property. The membrane sample was 40 × 5 mm, and the crosshead speed was 10 mm/min. Five samples of each membrane were used for this measurement, the mean value calculated and a comparative analysis was made using analysis of variance and Student *t* test.

Permeability measurement

Permeation of urea and creatinine was evaluated by the following experimental method using a U-shaped glass cell at 37°C.[13] The original cellulose membrane and that coated with the MGC-3 saturated with water were interposed between two parts of the glass cell. A 50-ml aliquot of distilled water containing the desired amount of solute was placed on one side of the cell, and a 50-ml aliquot of distilled water was placed on the other side. Both solutions were continuously stirred with a magnetic stirrer during the experiment with samples of solution (0.5 ml) removed after specific periods of time, and the amount of solute in each solution determined. The initial concentrations of urea and creatinine were 200 and 20 mg/dl, respectively. The concentration of urea was determined using a clinical test kit (B-Test Wako; Wako Pure Chemical, Ltd. Osaka, Japan) based on the urease-indophenol method. The amount of creatinine was determined by the following procedure. In a test tube containing 0.5 ml of sample solution, 4.5 ml of distilled water was added, then the test tube was kept at 25°C. Two milliliters of 0.04 N picric acid aqueous solution and 2 ml of 0.75 N sodium hydroxide aqueous solution were added to the creatinine solution. After 15 min, the solution turned orange and absorbance at 515 nm was recorded. The calibration curve was made using creatinine solutions with specific concentrations by the procedure described earlier.

In vitro blood contacting test

Whole blood was prepared from Japanese white rabbits weighing approximately 3.0 kg using the following method: the carotid artery was cannulated using polyethylene tubing, and 90 ml of fresh blood per rabbit was collected in a disposable syringe containing 10 ml of a 3.8 wt% aqueous sodium citrate solution. The citrated whole blood was immediately centrifuged for 15 min at 750 rpm to obtain citrated platelet-rich plasma (PRP). The number of platelets in the PRP determined using a Coulter counter (ZB-I, Coulter Electronics, Hialeah, FL) was 7.0×10^7 cells/ml.

The cellulose membrane and that coated with the MGC were interposed between two parts of a separable acrylic well 1.5 cm in diameter for cell culture. A phosphate-buffered solution (PBS, pH 7.4, ionic strength; 0.15) was poured into the well to equilibrate the membrane surface. After 15 h, the PBS was removed and 1 ml of PRP was poured into the well and kept for 30 or 180 min at room temperature. After removal of the PRP, the membrane was rinsed twice with 1 ml of PBS, and immersed into PBS containing 2 wt% glutaraldehyde to fix the adhered platelets. The membrane was freeze-dried and coated with gold to observe the surface using a scanning electron microscope (SEM; JEOL JSM-5400, Tokyo, Japan).

RESULTS

Characterization of MGC

Hydrolysis of cellulose acetate to form a water-soluble cellulose proceeded in a homogeneous solution. The chemical structure of the water-soluble cellulose was identified by IR spectroscopy. The IR absorptions on the water-soluble cellulose were the same as those of native cellulose. The number of repeating units of water-soluble cellulose was below 10, which was estimated by GPC with POE standards.

Graft polymerization of MPC on the water-soluble cellulose in water using cerium ions as an initiator also proceeded homogeneously. The chemical structure of MGC was identified by IR spectroscopy, elemental analysis, and GPC. When the IR spectrum of the MGC was compared with that of the original cellulose, absorptions at 1720, 1250, 970, and 800 cm^{-1} appeared after grafting. These IR absorptions were assigned to C=O, P=O, $N^+(CH_3)_3$, and C−O−P in the MPC moiety, respectively. As shown in Table I, the mole fraction of MPC in the MGC could be regulated by changing the concentration of MPC in the feed. Moreover, the Mn was more than 2×10^4.

Properties of cellulose membrane coated with the MGC

Coating of cellulose membrane with the MGC was carried out using its aqueous solution—that is, the membrane was immersed in a solution and dried *in vacuo*. The amount of MGC immobilized on the membrane was determined by phosphorus analysis; the results are indicated in Table II. Surface characterization of the membrane was performed with XPS. Figure 3 shows the XPS chart of both the original cellulose membrane and that coated with the MGC-3. The peaks attributed to nitrogen (404 eV) and phosphorus (134 eV) were observed on the surface of the membrane coated with MGC-3. The ratio of nitrogen and phosphorus was almost 1.0. Table III summarizes elution of the graft copolymer when the cellulose membranes were immersed in various solutions. These values were low compared with the total amount of MGC on the membrane—every value was <10% of these initial values. The MPC mole fraction at the membrane surface in the dry state was slightly smaller than that in the bulk polymer. However, after immersion in water, the MPC mole fraction at the surface increased.

The tensile strength of the original cellulose membrane in the wet state was 1.4 ± 0.2 kgf/mm^2, and that coated with the MGC was 1.3 ± 0.1 kgf/mm^2. There was no significant difference between them ($P > .01$).

Figure 4 shows the time dependence of the amount of solute that permeated through the original cellulose membrane, and that coated with the MGC. The permeation amount of both urea and creatinine through the original membrane linearly increased with time. The permeation rate of urea was larger than that of creatinine. The same tendency was found in the case of the cellulose membrane coated with MGC. The permeation rate of solutes through the MGC-treated membrane corresponded to that of the original membrane within an experimental error.

Platelet adhesion on cellulose membrane coated with the MGC

Figures 5 and 6 show SEM pictures of cellulose membranes coated with MGC after contact with PRP for 30 and 180 min, respectively. When PRP came in contact with the original cellulose membrane for 30 min, numerous platelets were adherent and deformed on the surface. This tendency was clearer with longer contact times. By contacting PRP on the membrane for 180 min, deformation and aggregation of platelets adhered on the surface proceeded, and a large fibrin net was formed. On the other hand, platelet adhesion was effectively reduced by coating with the MGC. Although some platelet adhesion was observed on the surface of the membrane coated with MGC-1 and MGC-2 by contacting PRP for 180 min, it was not found on the surface of the membrane coated with MGC-3.

DISCUSSION

Fundamental characteristics of the MPC polymer, such as poly(MPC-*co*-BMA), suggest the development of novel blood compatible dialysis membranes.[7–12] Poly(MPC-*co*-BMA) could suppress both adhesion of platelets and adsorption of the complement protein, C5. However, it is difficult to use poly(MPC-*co*-BMA) as a coating material on a cellulose dialysis membrane, because regenerated cellulose membranes used for hemodialysis are too hydrophilic to immobilize the poly(MPC-*co*-BMA) in a stable state. If elution of the polymer occurred during hemodialysis, thrombus formation would be induced on the bare cellulose membrane surface. Moreover, detachment of the copolymer results in infusion into the patient and may have adverse effects. Therefore, covalent grafting of the MPC polymer on the cellulose membrane was designed to prevent elution of the MPC polymer.[13–15]

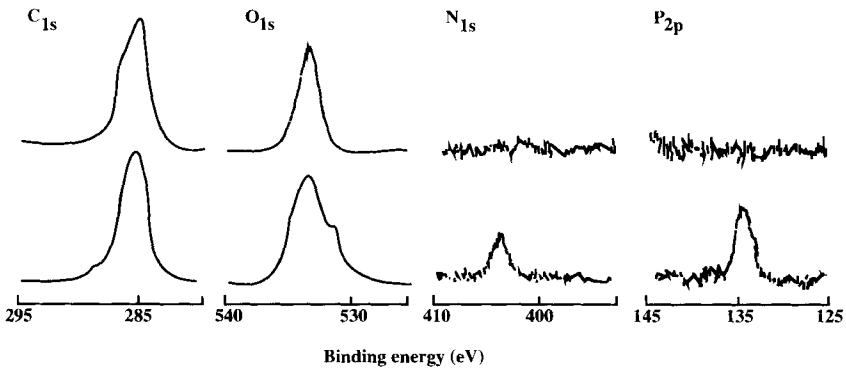

Figure 3. XPS spectra of C_{1s}, O_{1s}, N_{1s}, and P_{2p} observed on the original cellulose membrane and that coated with MGC-3.

TABLE III
Elution of the MGC from the MGC-Treated Cellulose Membrane

Code	Amount of MGC on the Membrane ($\mu g/cm^2$)	Amount of MGC Eluted ($\mu g/cm^2$)			MPC Unit Mole Fraction[c] at the Membrane Surface after Immersion in Water
		In Water		In 40% Aqueous Ethanol Solution at 37°C[a]	
		at 37°C[a]	at 100°C[b]		
MGC-1	46	0.0	2.4	2.8	0.08
MGC-2	53	2.4	2.5	4.5	0.18
MGC-3	48	1.5	4.5	0.0	0.39

[a]Immersion for 180 min.
[b]Immersion for 30 min.
[c]Determined by XPS analysis after the membrane was immersed in water at 37°C for 180 min.

Preparation of the cellulose membrane grafted with poly(MPC) directly and *in vitro* blood compatibility on the membrane surface were reported in earlier studies.[13,14] Grafting of MPC initiated with cerium ions did not affect the permeability and mechanical strength of the membrane. Complement activation, which was strongly induced on the original cellulose dialysis membrane, was suppressed by grafting of poly(MPC) on the surface.[14] We also found that the grafting of poly(MPC) on the cellulose hollow fiber membranes could improve the *ex vivo* blood compatibility even in the absence of an anticoagulant.[15]

The direct grafting of poly(MPC) on a cellulose membrane was effective for improving blood compatibility on the surface; however, it was not an easy process because the grafting reaction proceeded in a heterogeneous system. Therefore, we tried to prepare water-soluble graft copolymer (MGC)s composed of a poly(MPC) chain and a cellulose backbone and applied them as coating materials on the cellulose membranes.

As shown in Figure 7, when MGC was immobilized on the surface of the cellulose membrane from an aqueous solution by drying, the cellulose backbone in the MGC could act as a fixation site for the poly(MPC) chains on the surface because of the strong affinity resulting from hydrogen bonding between the base cellulose and the cellulose backbone. That is, the cellulose backbone could orient to the base cellulose membrane selectively, and the poly(MPC) chains could spread out when blood contacts membrane. Because the treatment process includes only coating and drying, it is an easy process to improve blood compatibility on the cellulose membrane. Moreover, the synthetic reaction of the MGC can proceed homogeneously, and the yield of the MGC is high.

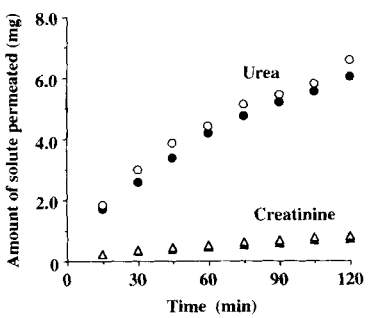

Figure 4. Permeation curve of urea and creatinine through the original cellulose membrane (open plots) and that coated with MGC-3 (closed plots) at 37°C.

Coating with the MGC on cellulose membrane surface was confirmed by XPS and phosphorus analysis based on the MPC moiety in the MGC. The MPC mole fraction at the membrane surface corresponded to that in the bulk MGC, and the amount of MGC on the surface was about 50 $\mu g/cm^2$. Only a slight amount of MGC coated on the surface was eluted when the membrane was immersed in distilled water and a 40 vol% aqueous solution of ethanol. As shown in Figure 4, permeability of creatinine and urea through the cellulose membrane modified with the MGC did not change compared with the original cellulose membrane. The tensile strength of the cellulose membrane also did not change after coating with the MGC. Therefore, the coating of MGC on the cellulose membrane did not show an adverse effect on membrane performance.

From Figures 5 and 6, it can be seen that the number of blood cells adherent on the cellulose membrane decreased dramatically by coating with MGC. In earlier studies, we showed that the MPC moiety on the polymer membrane was important in suppressing protein adsorption and platelet adhesion.[13–16] In this study, increase in the surface MPC mole fraction induced reduction of platelet adhesion even after 180 min contact with PRP.

The mechanism of blood compatibility observed on the poly(MPC-*co*-BMA) is the construction of a self-assembled biomimetic membrane by adsorbing phospholipids from plasma.[11] We considered that the mechanism could not apply in the case of the cellulose membrane coated with MGC because the cellulose backbone as a base showed a hydrophilic nature compared with the poly(BMA). However, the MPC moiety on the membrane surface gave the cellulose membrane excellent blood compatibility.

Protein adsorption of membranes for medical use

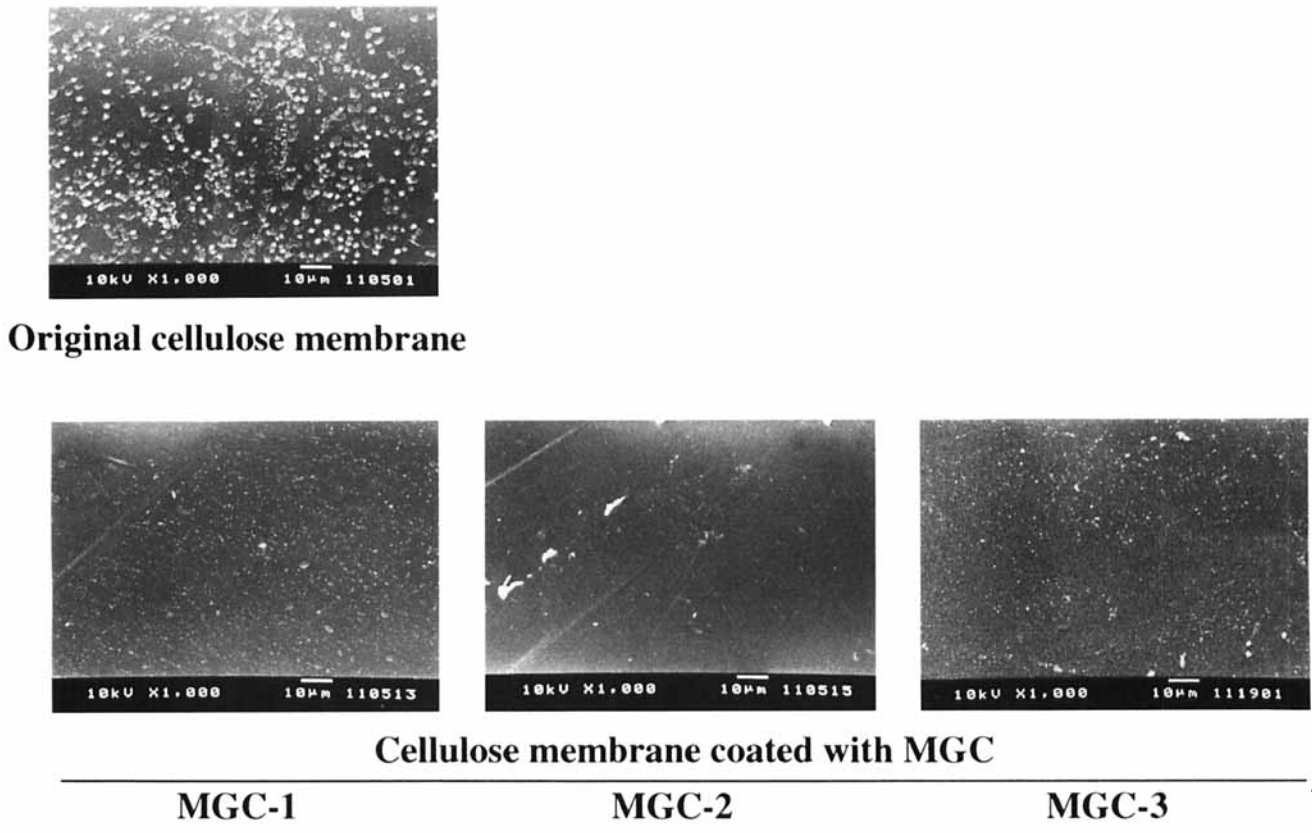

Figure 5. SEM pictures of the original cellulose membrane and that coated with MGC after contact with PRP for 60 min.

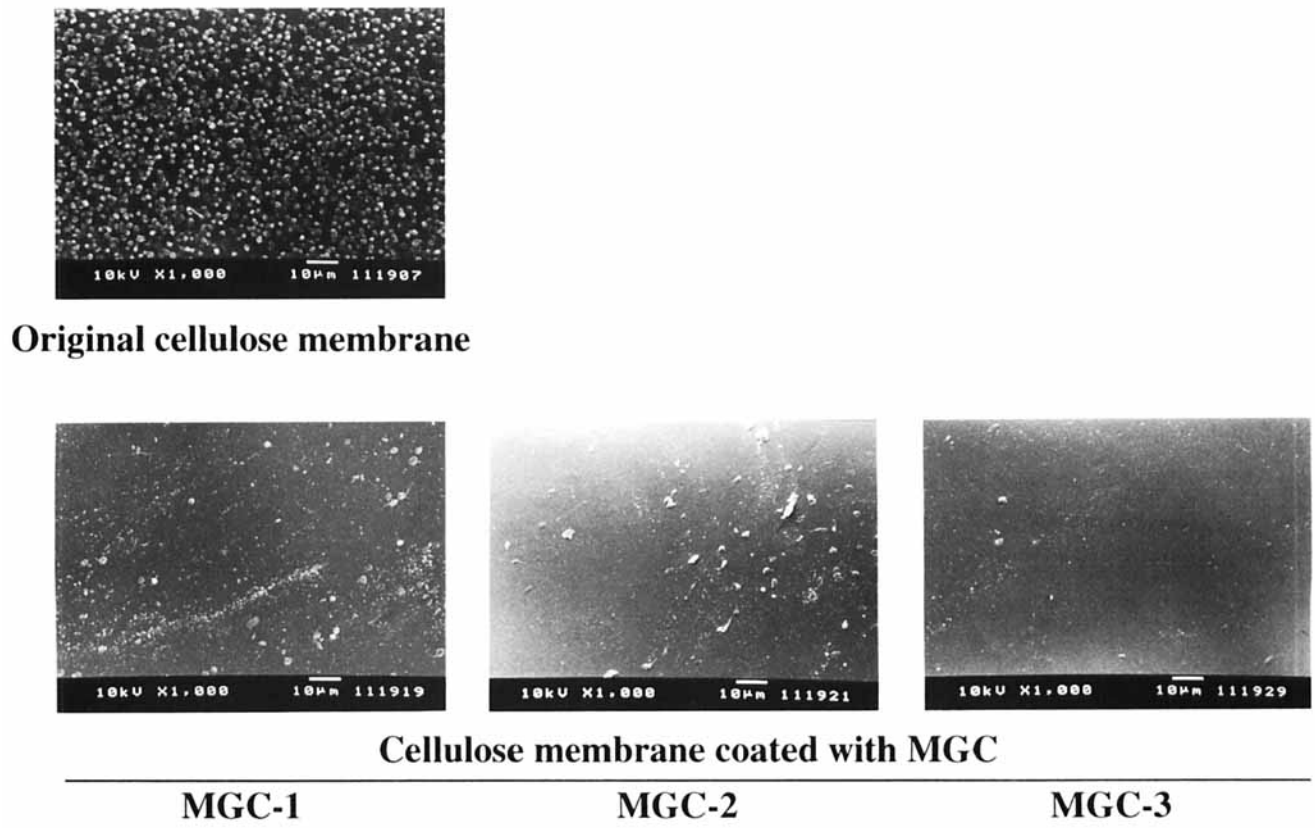

Figure 6. SEM pictures of the original cellulose membrane and that coated with MGC after contact with PRP for 180 min.

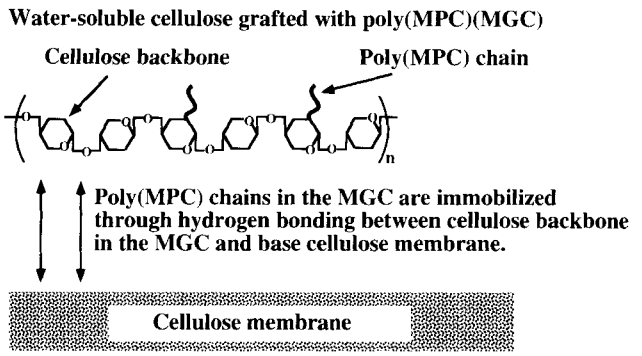

Figure 7. Concept of immobilization of poly(MPC) chain on cellulose membrane by coating with MGC.

influences the performance of membranes with regard to permeability and selectivity.[16] Although protein adsorption on the MGC-treated membrane has not been evaluated, we have found that the amount of proteins adsorbed on the cellulose membrane grafted with poly(MPC) reduced significantly compared with that on the original cellulose membrane.[15] Therefore, we consider that the cellulose membrane covered with the MGC may maintain permeability of solute when the membrane is exposed in the bloodstream. From these results, we conclude that the MGC is a suitable material for modification of the surface of the cellulose hemodialysis membrane that requires blood compatibility, biocompatibility, and permeability of solute.

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References

1. T. Nishimura, "Polymer materials for blood purification," in *Biomedical Applications of Polymeric Materials*, T. Tsuruta, T. Hayashi, K. Kataoka, K. Ishihara, and Y. Kimura (eds.), CRC Press, Boca Raton, 1993, pp. 191–218.
2. C. Woffindin and N. A. Hoenich, "Blood-membrane interactions during haemodialysis with cellulose and synthetic membranes," *Biomaterials*, **9**, 53–57 (1988).
3. P. R. Craddock, J. Fehr, A. P. Dalmasso, and H. S. Jacob, "Hemodialysis leukopenia: Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellulose membranes," *J. Clin. Invest.*, **59**, 879–888 (1977).
4. T. Akizawa, T. Kitaoka, S. Koshikawa, T. Watanabe, K. Imamura, T. Tsurumi, Y. Suma, and S. Eiga, "Development of a regenerated cellulose noncomplement activating membrane for hemodialysis," *Trans. Am. Soc. Artif. Int. Organs*, **32**, 76–80 (1986).
5. T. Akizawa, K. Kino, S. Koshikawa, Y. Ikada, A. Kishida, M. Yamashita, and K. Imamura, "Efficiency and biocompatibility of a polyethylene glycol grafted cellulosic membrane during hemodialysis," *Trans. Am. Soc. Artif. Int. Organs*, **35**, 333–335 (1989).
6. A. Kishida and Y. Ikada, "Chemical modification of cellulose membrane and the subsequent decrease of the complement activation," *Jpn. J. Artif. Organs*, **17**, 47–50 (1988).
7. K. Ishihara, T. Ueda, and N. Nakabayashi, "Preparation of phospholipid polymers and their properties as polymer hydrogel membranes," *Polym. J.*, **22**, 355–360 (1990).
8. K. Ishihara, R. Aragaki, T. Ueda, A. Watanabe, and N. Nakabayashi, "Reduced thrombogenicity of polymers having phospholipid polar groups," *J. Biomed. Mater. Res.*, **24**, 1069–1077 (1990).
9. K. Ishihara, N. P. Ziats, B. P. Tierney, N. Nakabayashi, and J. M. Anderson, "Protein adsorption from human plasma is reduced on phospholipid polymer," *J. Biomed. Mater. Res.*, **25**, 1397–1407 (1991).
10. T. Ueda, H. Oshida, K. Kurita, K. Ishihara, and N. Nakabayashi, "Preparation of 2-methacryloyloxyethyl phosphorylcholine copolymers with alkyl methacrylates and their blood compatibility," *Polym. J.*, **24**, 1259–1269 (1992).
11. K. Ishihara, H. Oshida, T. Ueda, Y. Endo, A. Watanabe, and N. Nakabayashi, "Hemocompatibility of human whole blood on polymers with a phospholipid polar group and its mechanism," *J. Biomed. Mater. Res.*, **26**, 1543–1552 (1992).
12. K. Ishihara, T. Tsuji, T. Kurosaki, and N. Nakabayashi, "Hemocompatibility on graft copolymers composed of poly(2-methacryloyloxyethyl phosphorylcholine) side chain and poly(*n*-butyl methacrylate) backbone," *J. Biomed. Mater. Res.*, **28**, 225–232 (1994).
13. K. Ishihara, K. Fukumoto, J. Aoki, and N. Nakabayashi, "Improvement of blood compatibility on cellulose dialysis membrane. 1. Grafting of 2-methacryloyloxyethyl phosphorylcholine onto a cellulose membrane surface," *Biomaterials*, **13**, 145–149 (1992).
14. K. Fukumoto, K. Ishihara, R. Takayama, J. Aoki, and N. Nakabayashi, "Improvement of blood compatibility on cellulose dialysis membrane. 2. Blood compatibility of phospholipid polymer grafted cellulose membrane," *Biomaterials*, **13**, 235–239 (1992).
15. K. Ishihara, K. Fukumoto, H. Miyazaki, and N. Nakabayashi, "Improvement of hemocompatibility on a cellulose dialysis membrane with a novel biomedical polymer having a phospholipid polar group," *Artif. Organs*, **18**, 559–564 (1994).
16. K. Ishihara, S. Ohta, T. Yoshikawa, and N. Nakabayashi, "Protein adsorption resistible membrane for biosensor composed of polymer with phospholipid polar group," *J. Polym. Sci.: Polym. Chem.*, **30**, 929–932 (1992).

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