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MMA/MPEOMA copolymers as coating materials for improved blood compatibility: protein adsorption study

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Surface-induced thrombosis remains one of the main problems in the development of blood-contacting devices. When a foreign surface comes in contact with blood, the initial blood response is adsorption of blood proteins, followed by platelet adhesion and activation, leading to thrombus formation. A particularly effective polymer for the prevention of protein adsorption and platelet adhesion appears to be polyethylene oxide (PEO). In this study, water-insoluble copolymers of methyl methacrylate (MMA) and methoxy PEO monomethacrylates (MPEOMA) with different PEO molecular weights (200, 400, and 1000) and monomer composition were synthesized and characterized by gel permeation chromatography and ^{1}H-nuclear magnetic resonance spectroscopy. The synthesized copolymers were coated on glass slides by a spin coating method to prepare PEO-rich surfaces as blood-compatible surfaces. The surface properties of the copolymers and their interaction with blood proteins (albumin, γ-globulin, fibrinogen, and plasma proteins) were investigated by the measurement of water contact angles and by electron spectroscopy for chemical analysis, respectively. It was observed that the protein adsorption on the copolymer surfaces decreased with increasing PEO molecular weight and MPEOMA content in the copolymers. The copolymers with long PEO chains in MPEOMA (MMA/MPEO$_{1000}$MA copolymers) were effective in preventing protein adsorption, even though their MPEOMA content was less than the copolymers with shorter PEO chains.

1. Introduction

Knowledge of interfacial interaction of polymers with plasma protein and platelets is important in establishing polymer blood compatibility [1–3]. It is known that proteins are complex macromolecules with molecular weights ranging from thousands to millions and that they adsorb on all interfaces during the first few minutes of blood or biological fluid exposure [4–6]. Generally, the protein adsorption process results in platelet adhesion and activation of the coagulation pathways, leading to thrombus formation. Surface-induced thrombosis remains one of the main problems in the development of blood-contacting devices. There have been various efforts to minimize protein adsorption and platelet adhesion. A particularly effective polymer for the prevention of protein adsorption and platelet adhesion appears to be polyethylene oxide (PEO; or polyethylene glycol (PEG) when the molecular weight is less than about 10 000 [7]), probably owing to its minimum interfacial free energy with water, hydrophilicity, high surface mobility and steric stabilization effects, and unique solution properties and molecular conformation in water [8, 9].

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PEO surfaces have been prepared by many different methods including covalent coupling and graft copolymerization of PEO or PEO derivatives to substrates, which provide permanent PEO surfaces [10–19]. More simply, PEO surfaces have been prepared by physical adsorption of various water-soluble PEO-containing amphiphilic block or graft copolymers onto hydrophobic substrates [20–22]. Adsorption of PEO-containing block or graft copolymers would be more stable than that of PEO homopolymers, since the hydrophobic segments provide hydrophobic adsorption forces or anchor to the polymer substrate. This method seems applicable to many biomedical areas, owing to its simplicity and non-specificity. The main disadvantage is that the immobilized polymers may not remain on the surface permanently. Similar PEO surfaces with greater stability could be achieved by blending small amounts of PEO or PEO-containing block copolymers into the polymer matrix [23–27].

In this study, PEO surfaces were prepared by the coating of water-insoluble PEO-containing graft copolymers, methyl methacrylate (MMA) and methoxy PEO monomethacrylate (MPEOMA) copolymers, to glass slides. We investigated the adsorption behavior of blood

proteins (albumin, γ-globulin, fibrinogen, and plasma proteins) in terms of PEO molecular weight and monomer composition in the copolymers.

2. Materials and methods

2.1. Synthesis of MMA/MPEOMA copolymers

Monomers MMA and MPEOMA with different PEO molecular weights (200, 400, and 1000; designated $MPEO_{200}MA$, $MPEO_{400}MA$, and $MPEO_{1000}MA$, respectively), were purchased from Polysciences, USA. MMA was purified by washing with 10% NaOH and then vacuum distilled before use. MPEOMA was used as received. 2,2′-azo bisisobutyronitrile (AIBN; Aldrich, USA) was purified by recrystallization from methanol and used as an initiator for polymerization. The copolymers were synthesized by radical polymerization of monomers in toluene at 50 °C for 48 h with different MMA/MPEOMA feed ratio (Table I). A polymer mixture containing 14.0 wt % of monomers, 0.6 wt % of AIBN, and 85.4 wt % of toluene was bubbled with nitrogen for 15 min then sealed in an ampoule. After the polymerization was finished, the polymer was precipitated into cooled diethyl ether, washed, and then dried.

The average molecular weights of the prepared copolymers were estimated by gel permeation chromatography (GPC; Waters 2690, USA) using polymethyl methacrylate (PMMA) calibration standards. MMA/MPEOMA composition in the copolymers was determined by ^{1}H-nuclear magnetic resonance spectroscopy (NMR; Bruker DRX-300, Germany) using $CDCl_3$ as a solvent.

2.2. Preparation of the copolymer surfaces

To prepare copolymer surfaces, 5 wt % of MMA/MPEOMA copolymers were dissolved in tetrahydrofuran and coated on glass slides five times using a spin coater (Solitec 5100, USA; each coating with 1500 r.p.m. for 30 s and then vacuum dried).

The copolymer surfaces were characterized by measuring water contact angles. The water contact angle, an indicator of the wettability or hydrophilicity of surfaces, was measured by a sessile drop method at room temperature using an optical bench-type contact angle goniometer (Model 100-0, Rame-Hart, USA). Drops of purified water, 4 μl, were deposited onto the copolymer surface using a micro-syringe attached on the goniometer. After 30 min equilibrium, the microscopic measurement of the contact angles was performed with the goniometer.

2.3. Blood protein adsorption

Human blood proteins, albumin, γ-globulin, fibrinogen, and plasma, were used to study the adsorption behavior of proteins on the copolymer surfaces. Albumin, γ-globulin, and fibrinogen were obtained from Sigma as crystallized and lyophilized powders. The protein powders were dissolved in phosphate-buffered saline (PBS, pH 7.3 ∼ 7.4) to make solutions of 1 mg ml^{-1}. Fresh human blood containing citrate/phosphate/adenine-1 mixture solution (CPDA-1) as an anticoagulant (CPDA-1 to blood ratio, 1 : 7) was supplied by the Blood Center of the Korean Red Cross. Blood was centrifuged at 2000 ***g*** for 20 min to obtain platelet-poor plasma. The copolymer-coated glasses were placed on 24-well polystyrene plates and equilibrated with PBS for 30 min. After removing the PBS from the wells by pippetting, each protein solution was added to the wells. After 1 h incubation at 37 °C, the copolymer-coated glasses were washed with PBS, followed by washing with purified water to remove unadsorbed proteins and then vacuum dried.

The protein-adsorbed surfaces were analyzed by electron spectroscopy for chemical analysis (ESCA). The ESCA (ESCALAB MK II, V. G. Scientific Co., UK) was equipped with AlK_α radiation source at 1487 eV and 300 W at the anode. The nitrogen 1s peaks from the

TABLE I Composition and molecular weights of copolymers synthesized

PEO (Mol. wt)	MMA/MPEOMA composition[a]		Mol. wt[c]	
	Monomer	Copolymer[b]	M_w	M_w/M_n
200	95/5	93/7	50 000	2.1
	90/10	90/10	53 000	2.2
	85/15	84/16	53 000	2.1
	75/25	78/22	58 000	2.2
400	97/3	97/3	38 000	1.8
	93/7	94/6	34 000	1.7
	90/10	90/10	36 000	1.8
	85/15	86/14	32 000	1.6
1000	98.5/1.5	98.5/1.5	32 000	1.6
	97/3	97/3	27 000	1.4
	95/5	96/4	24 000	1.4
	93/7	94/6	21 000	1.4

[a]Composition, mol%.
[b]By ^{1}H-NMR measurement.
[c]By GPC measurement using PMMA calibration standards.

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

3. Results and discussion

3.1. Characterization of MMA/MPEOMA copolymers synthesized

Fig. 1 shows the structure of MMA/MPEOMA copolymers synthesized. The monomer composition and PEO molecular weight in MPEOMA used are listed in Table I. All of the copolymers listed were water-insoluble and thus can be used as coating materials, whereas the copolymers with the MPEOMA compositions more than those listed in Table I were water-soluble. The weight-average molecular weights (M_w) of the copolymers determined by GPC were in the range of about 21 000 to 58 000. The molecular weight distribution of the copolymers was relatively narrow (polydispersity index (M_w/M_n) $\leq$ 2.2). MMA/MPEOMA composition in the copolymers determined by ^{1}H-NMR was not so different from the monomer feed composition as seen in Table I, indicating that the reactivities of MMA monomer and MPEOMA macromonomer with PEO side chain are not so different; the length of macromonomer side chains used in this study do not have a pronounced effect on the macromonomer's reactivity [28].

3.2. Characterization of the copolymer surfaces

The copolymer surfaces were characterized by measuring water contact angles. The surface coated with the polymer having the monomer composition of 100/0 (i.e. polymethyl methacrylate homopolymer (PMMA) used as a control) showed a water contact angle of about 60°. The contact angles of the copolymer surfaces decreased with increasing MPEOMA content and also increasing PEO molecular weight in MPEOMA, as seen in Fig. 2. The decrease in the contact angles (and thus the increase in wettability or hydrophilicity) on the surfaces may be due to the hydrophilic property of PEO chains extended into the water phase. The surface wettability or hydrophilicity is one of the main parameters affecting the interaction of biological species like proteins or cells with polymeric materials [29].

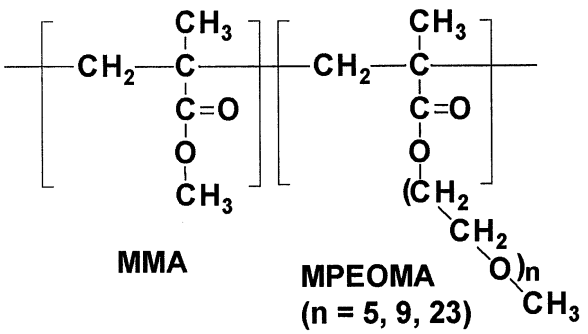

Figure 1 Structure of copolymers synthesized.

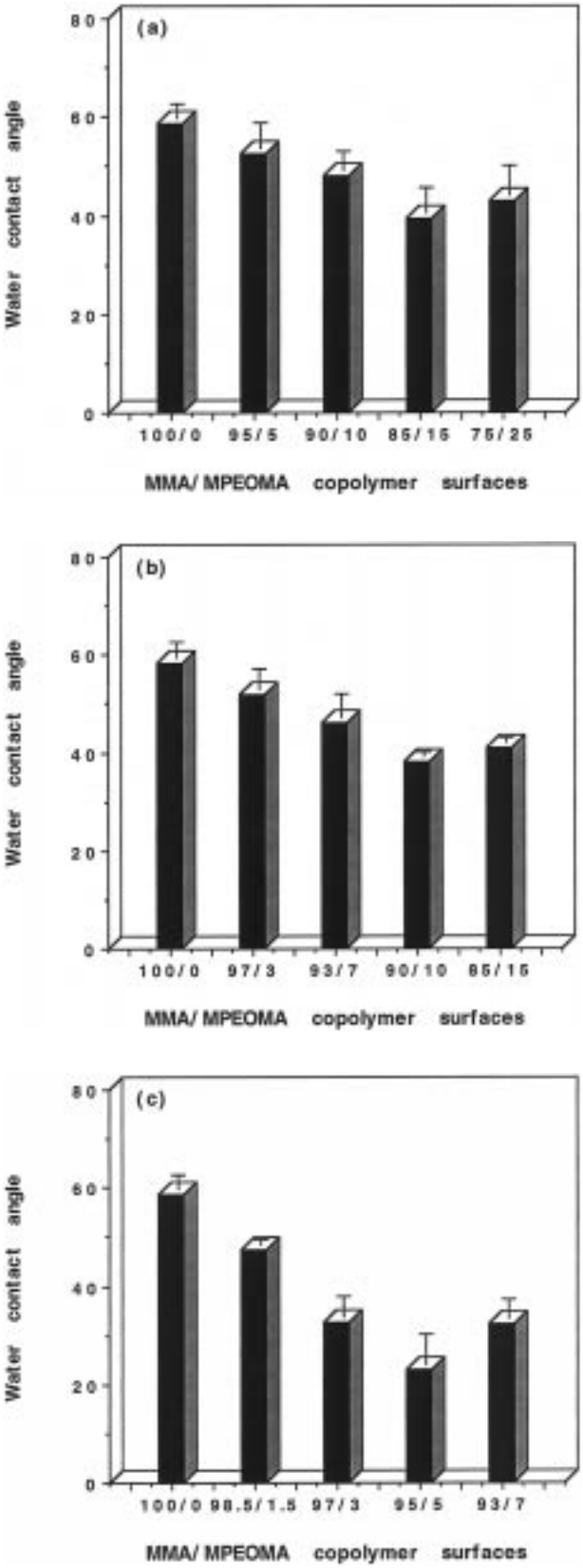

Figure 2 Water contact angles of copolymer surfaces; (a) MMA/$MPEO_{200}$MA, (b) MMA/$MPEO_{400}$MA, and (c) MMA/$MPEO_{1000}$MA copolymer surfaces. Sample numbers, $n = 3$.

3.3. Interaction of blood proteins with the surfaces

Human blood proteins, albumin, γ-globulin, and fibrinogen, were used as model systems to study the adsorption behavior of proteins on the copolymer surfaces. Human plasma, which contains more than 200

TABLE II Three-dimensional structures and elemental composition of model proteins used

Protein	Mol. wt.[a,b]	Major axis[a] (nm)	Minor axis[a] (nm)	Atomic% (omitting H)[c]			
				C	N	O	S
Albumin	66 000 ~ 69 000	15	3.8	66.6	15.1	17.4	0.90
γ-globulin	153 000 ~ 156 000	23.5	4.4	63.5	13.9	22.6	trace
Fibrinogen	340 000 ~ 400 000	70	3.8	67.1	11.6	20.3	1.10

[a]From [31].
[b]From [32].
[c]By ESCA analysis of protein powder.

kinds of different proteins [30], was also used for the comparison study. Protein adsorption onto polymer surfaces is important because of its possible involvement in the initial stage of blood coagulation. It is recognized that preadsorbed albumin surfaces appear to be passive, while preadsorbed γ-globulin or fibrinogen surfaces show active platelet deposition [3]. Table II shows three-dimensional structures of model proteins used. The albumin is the major constituent of blood plasma (about 60% of the total plasma proteins) and is one of the smallest proteins in the plasma [31, 32]. The shape of this protein is a prolate ellipsoid with a size of about $15 \times 3.8 \times 3.8$ nm. It contains a comparatively large number of polar and charged residues and thus, it is highly soluble in water and negatively charged at pH 7.4 (isoelectric point (IP), 4.7 ~ 5.5). It also has big hydrophobic patches on its surface. The fibrinogen is an exceptionally elongated molecule with an axial ratio (major axis : minor axis) of about 18 : 1. It contains about 10% charged residues and is negatively charged at pH 7.4 (IP, about 5.8). The γ-globulin has intermediate size and shape among the model proteins used. Its IP value (5.8 ~ 7.3) is nearer the neutral point than other plasma proteins. Table II also compares the elemental composition of the model proteins. The nitrogen contents of the proteins are about 11 ~ 15% depending on the proteins, as determined by ESCA. They are mainly derived from peptide bonds in the structure of the proteins.

The blood proteins were adsorbed onto the copolymer surfaces, and the relative adsorbed amount of proteins was evaluated by ESCA. Although ESCA is not a very good method for the study of protein adsorption, it is a simple and easy method to obtain semiquantitative information on protein adsorption. As we investigated protein adsorption on polymer surfaces by ESCA and by using ^{125}I-labeled proteins in our previous studies [20, 21], we observed that the protein adsorption on the surfaces analyzed by both methods show the same trend. For ESCA analysis, the nitrogen peak (binding energy, ~ 400 eV) from the survey scan spectrum was used as an indicator of the protein adsorption on the surface.

Fig. 3 shows an ESCA survey scan spectra of MMA/$MPEO_{1000}$MA copolymer surfaces after fibrinogen adsorption. The nitrogen peak from the control PMMA homopolymer surface (monomer composition 100/0; Fig. 3a) was higher than MMA/$MPEO_{1000}$MA copolymer surfaces (Fig. 3b,c), indicating the larger amount of protein adsorption on the PMMA surface. This is probably due to the hydrophobic and/or polar interactions of the protein molecules with the PMMA surface. Fig. 4 compares the relative adsorbed amount of blood proteins on the MMA/$MPEO_{1000}$MA copolymer surfaces, which was determined as follows: relative adsorbed amount of protein = (N% of MMA/MPEOMA

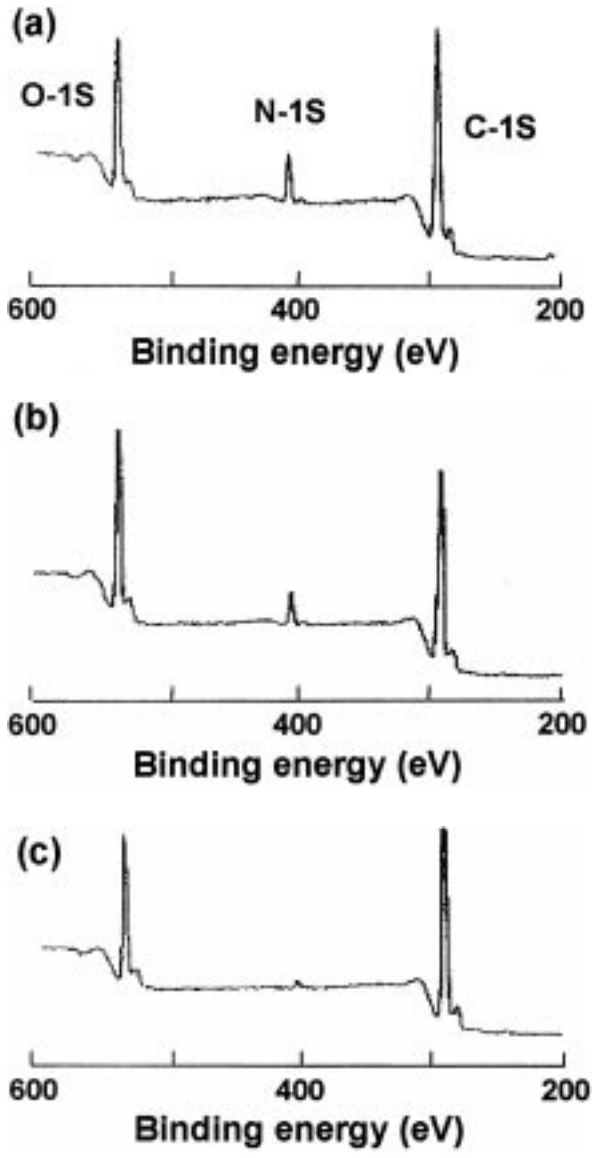

Figure 3 ESCA survey scan spectra of MMA/$MPEO_{1000}$MA copolymer surfaces after fibrinogen adsorption (1 h adsorption in 1 mg ml^{-1} fibrinogen solution). MMA/$MPEO_{1000}$MA monomer composition; (a) 100/0, (b) 98.5/1.5, and (c) 93/7.

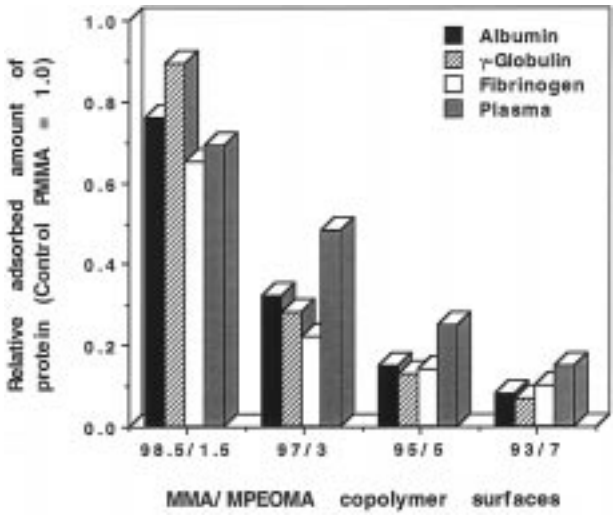

Figure 4 Relative adsorbed amount of proteins on MMA/$MPEO_{1000}MA$ copolymer surfaces. $n = 3$ (standard deviations (SD) $\leq \pm 5\%$).

copolymer surface)/(*N*% of control PMMA surface). As seen in Fig. 4, the protein adsorption on the MMA/$MPEO_{1000}MA$ copolymer surfaces decreased with increasing $MPEO_{1000}MA$ content, regardless of protein types used. Fig. 5 compares the relative adsorbed amount of three model proteins (albumin, γ-globulin, and fibrinogen) on the MMA/MPEOMA copolymer surfaces with different PEO molecular weights. The protein adsorption on the copolymer surfaces decreased with increasing PEO molecular weight and MPEOMA content in the copolymers. The copolymers with long PEO chains in MPEOMA (MMA/$MPEO_{1000}MA$ copolymers) were effective in preventing protein adsorption, even though their MPEOMA contents were less than the copolymers with shorter PEO chains; the MMA/$MPEO_{1000}MA$ copolymer with the monomer composition of 93/7 showed a similar effect on protein adsorption with the MMA/$MPEO_{400}MA$ copolymer with the composition of 85/15 or the MMA/$MPEO_{200}MA$ copolymer with the composition of 75/25.

Possible explanations for the reduced protein adsorption of MMA/MPEOMA copolymer surfaces particularly with large MPEOMA content or long PEO chains include PEO's 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 solution properties of PEO produce surfaces that are in a liquid-like state, with the polymer chains exhibiting considerable flexibility or mobility [7, 33, 34]. Among common water-soluble polymers, PEO is the most flexible in water because it has flexible ether linkages in its backbone and does not have bulky side groups; thus it will not be hindered sterically in water. It appears that the PEO molecule has a large excluded volume in water [9]. PEO surfaces in water with rapidly moving hydrated PEO chains and a large excluded volume tend to repel protein molecules that approach the surface.

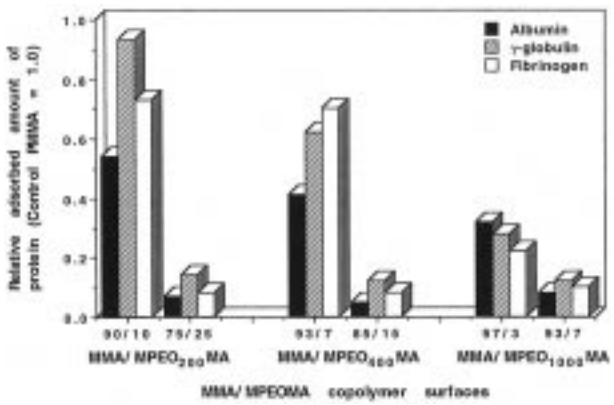

Figure 5 Relative adsorbed amount of proteins on MMA/MPEOMA copolymer surfaces. $n = 3$ (SD $\leq \pm 5\%$).

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