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Sulfonated Poly(ethylene glycol) Containing Methacrylate Copolymer Surfaces; Preparation, Characterization and *In Vitro* Biocompatibility

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Abstract: Poly(ethylene glycol) (PEG1K) and sulfonated PEG (PEG1K-SO_3) methacrylate (MA) copolymers have been prepared and characterized. The structures of the synthesized copolymers were confirmed by ^1H and ^{13}C NMR spectroscopy and elemental analysis. The bulk characteristics of the copolymers were evaluated by viscosity and thermal analysis. The surface properties of the copolymers were investigated using dynamic contact angle measurements and electron spectroscopy for chemical analysis. The hydrophilicity of the surfaces modified with PEG1KMA or PEG1K-SO_3MA increased, possibly as a result of the orientation of the hydrophilic PEG1KMA/ PEG1K-SO_3MA chains into the water phase. Platelets adhered less to the surfaces of the copolymers than they did to a polyurethane control. In addition, adhesion of platelets to the copolymer surfaces decreased upon increasing the chain density of PEG1KMA and sulfonated PEG1KMA in the copolymers. Both bacterial adhesion and protein adsorption were significantly reduced on the copolymer surfaces and their levels differ depending on the kind of surface or media.

Keywords: sulfonated-PEG methacrylate copolymers, platelet adhesion, protein adsorption, bacterial adhesion.

Introduction

Polymeric material is essential for the development and improvement of medical devices and system in artificial organs. Surface-induced thrombus formation is a still serious problem in surgical therapy and application of medical devices and artificial organs.[1]

Although a substantial amount of work in the improvement of the blood compatibility of polymeric materials has been carried out, the results are still inconclusive. Therefore, the need for the generation of highly blood compatible materials has been increasing. A variety of approaches have been taken to improve blood compatibility of polymeric materials. One approach involves surface modification by (1) chemical modification by the grafting of hydrophilic components, such as poly(ethylene glycol) (PEG), (2) surface modification by incorporating bioactive agents such as fibrolytic enzymes (*t*-plasminogen activator and urokinase), various prostaglandins (PGE_1), and potent anticoagulant (heparin and hirudin), through either physical or chemical coupling, and (3) biological modification using protein or cell seeding.[2]

In our previous studies, a new approach to improve blood compatibility, biostability, and anticalcification of polymers has been developed. This approach is based on following concepts: First, the hydrophilic environment of the blood-material interface can reduce the protein adsorption and platelet adhesion and activation. And this might be achieved by the grafting of hydrophilic polymers such as PEG. In addition, the advantages of PEG use include low interfacial free energy,[3-6] its nonadhesive property, highly dynamic motions and extended chain conformation at the blood-material interface.[5-7] Second, sulfonated polymers have been shown anticoagulant activity like heparin.[7-12] In addition, the negatively charged pendent sulfonate group expels blood component further by electrical repulsion. Based upon these

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concepts, we have grafted sulfonated PEG (Mw 1,000 g/mol) onto polyurethane (PU-PEG-SO_3) to induce a synergistic effect of PEG and sulfonate groups.

The proposed hypothesis to improve blood compatibility has been proven both *in vitro*, *ex vivo* and *in vivo*.[7-17] In addition, PEG-SO_3 surface demonstrated a reduction in surface crack, calcium deposition and bacterial adhesion. The enhanced blood compatibility correlates with the improved biostability and anticalcification. This is mainly due to the synergistic effect of the hydrophilicity and the dynamic motion of PEG and negative sulfonate group.

Based upon the previous studies, sulfonated PEG concept has been extended to the copolymer system, which contains hydrophilic part of PEG/PEG-SO_3 methacrylate and hydrophobic part of octadecylmethacrylate (OMA). This copolymer can be applied as coatings or processing additives for medical devices and implants. Hydrophobic (HPB) part may be anchored into a suitable existing HPB substrate and hydrophilic PEG/PEG-SO_3 can be reoriented into aqueous phase when it comes into contact with blood. Previously, we have reported the effects of PEG/PEG-SO_3 acrylate with different PEG chain length on platelet and bacterial repellence elsewhere.[18]

In this study, methacrylate copolymers having the various chain densities of PEG/PEG-SO_3 (same PEG length, PEG Mw 1,000 g/mol, PEG1K) have been prepared and characterized. The chain density effect of PEG1K and PEG1K-SO_3 in copolymers on biological responses including platelet and protein interactions, and bacterial adhesion to copolymer (coated or blended) surfaces have been evaluated.

Experimental

Materials. PEG 1K mono-methacrylate (PEG1KMA, Monomer-Polymer & Dajac Lab., Inc., Feasterville, PA, USA) were dissolved in chloroform, precipitated in diethyl ether, and dried under vacuum at room temperature. OMA (Aldrich Co. St Louis MO, USA) was purified by vacuum-distillation and α,α-azobis(isobutyronitrile) (AIBN) was purified with recrystallization using methanol. Unless otherwise specified, all chemicals were purchased from Aldrich Co. and Sigma Chemical Co.

Polyurethanes (PU, Pellethane®, Dow Chemical Co., Midland, MI, USA) were dissolved with *N, N*-dimethylacetamide (DMAc), then precipitated with methanol to remove low molecular weight components. After purification, the PU were dried under vacuum. Purified PU were dissolved in DMAc for solvent casting of PU films.

Glass beads (mean diameter 150~212 μm, Sigma Co.) were cleaned by soaking in chromic acid for overnight, washing with flowing water for overnight, then alternatively with ethanol and distilled water for 1h and drying under vacuum at 60 °C. Purified glass beads were coated with PU and then copolymer solutions (0.1% w/v in DMAc). The beads were suction filtered and dried at 25 °C under vacuum for 24 h. Finally, the beads were mechanically sieved (U.S. standard #70) to remove aggregation.

Polyethylene films (PE, thickness 0.5 mm, Han-Wha Chemical Co., Korea) were used after purification with methanol extraction.

Synthesis of (sulfonated) PEG Methacrylate Copolymers. Sulfonation of PEG1KMA monomer was allowed to react with Na metal and then propane sultone to obtain sulfonated PEG1KMA (PEG1K-SO_3MA). Briefly, PEG1KMA was reacted with Na metal at 50 °C for overnight and unreacted Na splices were eliminated by filtering process. Then, propane sultone was added to the reaction solution and subsequently stirred at 50 °C for 24 h. PEG1K-SO_3MA solution was precipitated in excess diethyl ether. After filtering, the precipitate was dried at room temperature in a vacuum oven.

Copolymers were synthesized through free radical polymerization of PEG1KMA/OMA and PEG1K-SO_3MA/OMA monomers in a various molar ratio at 60 °C for 48 h in ethanol using AIBN as an initiator, respectively. The stoichiometry of monomers in the feed was manipulated to prepare a variety of copolymer compositions, i.e., PEG1K and PEG1K-SO_3 content in the copolymers. Molar ratio of hydrophilic monomer (PEG1KMA or PEG1K-SO_3MA) to hydrophobic monomer (OMA) was 1:9, 3:7, 5:5, 7:3, and 9:1, respectively. The polymerization mixture of 20 wt% monomer mixtures, 0.15 wt% AIBN and 80 wt% ethanol, was bubbled with nitrogen for 30 min and reacted in a three-necked flask equipped with a condensor, thermocontroller, and magnetic stirrer. After the polymerization, the reaction volume was reduced to about 60% by solvent evaporation under reduced pressure and precipitated in a *n*-hexane or diethyl ether and finally dried in vacuum at room temperature for 2 days. The copolymer structures were shown in Figure 1.

The copolymers were coated onto PU or PE film, or blended with PU film. Copolymer coating solution was prepared with 1wt% DMAc. Coating was done by the method similar to that of the glass bead coating. Blended PU film was prepared by adding copolymer (approximate 10 wt% of PU weight) in the PU/DMAc solution. The mixed solution was cast on cleaned glass plates. The solvent was slowly evaporated at 50 °C for 1 week in drying oven following by vacuum drying for 2 days at room temperature to eliminate the residual solvent.

Characterization of (sulfonated) PEG Methacrylate Copolymers.

Bulk Characterization. The structures of copolymers were characterized by Fourier transform infrared spectrometer (FTIR, Mattson Alpha Centauri, Bucks, England), nuclear magnetic resonance spectrometer (^{1}H-NMR, ^{13}C-NMR, Jeol JNM-PMX 60NMR; Tokyo, Japan), and elemental analyzer (EA, Fisons EA 1108, Italy). The composition of copolymers was estimated by ^{1}H-NMR spectra.

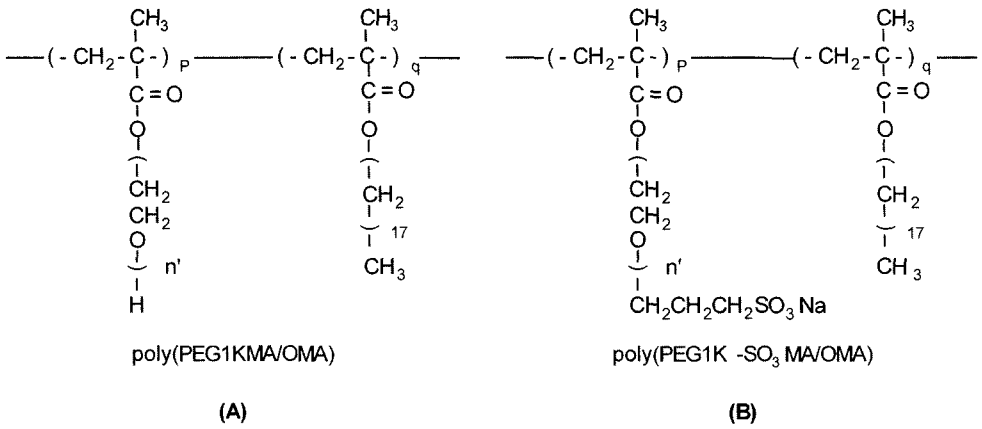

Figure 1. Structures of the synthesized copolymer.

In addition, the bulk characteristics of copolymers were evaluated by viscometry and differential scanning calorimetry (DSC, Dupont TGA 9900, USA). DSC was performed at a heating rate of 10 °C/min. Inherent viscosity in $CHCl_3$ was measured using an Ubbelohde Viscometer (Schott Gerate AVS 400, Kapillare Nr. 531 03/0o, Germany) at 25 °C. Solubility tests were conducted with polymer concentrations of approximately 1% (w/v) at room temperature.

Surface Characterization.

Dynamic Contact Angle Measurement (DCA): Dynamic contact angle analysis was performed at room temperature with the Wilhelmy plate technique using an equipment manufactured by Cahn Instruments, Inc. (DCA-322, USA). Modified surfaces were analyzed for dynamic advancing (θa) and receding (θr) contact angles, an indicator of surface wettability, using a Wilhelmy balance. The films (1×2 cm^2) were immersed into and taken out from triple-distilled water with rates of 150 μm/sec to measure advancing and receding contact angles. In order to examine the effect of hydration on modified surfaces, contact angle determinations were performed on the same films after 1 day hydration in triple-distilled water.

Electron Spectroscopy for Chemical Analysis (ESCA): The changes in chemical structure of film surfaces by the copolymers were investigated by ESCA. ESCA spectra for each sample were obtained on a spectrometer ESCA 2803-S (SSI, USA) with AlK_α X-ray. For determining O/C and S/C stoichiometries, a collecting factor of 2.50 and 1.80 was used for O_{1s} and S_{2p3}, respectively. Binding energies were referenced to the hydrocarbon component peak at 285.0 eV. After smoothing the C_{1s} spectra, the subpeaks were deconvoluted using a curve-fitting method from a series of Gauss-Lorentzian curves.

Swelling Property and Water Stability of Copolymers: The swelling property of the copolymer blended PU films was examined by measuring the water adsorption content. The films (1×3 cm^2) were weighed after thorough drying (W_{dry}) and immersed in purified water. After 1 day, the films were taken out from the water, wiped dry with tissue paper, and weighed again immediately (W_{wet}). Water adsorption was determined as follows : % swelling = $[(W_{wet}- W_{dry})/W_{dry}] \times 100$. To examine the stability of the 10 wt% copolymer blended PU films, the films were immersed in water for 7 days with continuous shaking. After that, the films were taken out from the water, rinsed with fresh water, dried to constant mass in vacuum oven, and then weight again ($W_{dry,\ final}$). The extraction of the copolymers from the films was determined as follows : copolymer extraction (%) = $[(W_{dry}- W_{dry,\ final})/W_{dry}]\times 100$.

***In Vitro* Platelet Adhesion:** Copolymer coated glass beads (0.5 g) were carefully weighed into plastic disposable 5 mL syringes and equilibrated with 2 mL phosphate buffered saline (PBS, pH = 7.4, 0.1 M) for overnight. Prior to adhesion studies, the buffer was removed from the syringe and 2 mL of human platelet rich plasma (PRP, platelet no. 5.2×10^5 cells/μL) was added into the syringe. The syringe was then tapped to remove air bubbles, sealed, and rotated in a shaking incubator (120 rpm) at 37 °C. By this method, the beads were constantly exposed to PRP. Therefore, only surface/platelet interactive influences were observed. A set of syringes was arranged for adhesion times of 15, 30, 60 and 120 min PRP incubation. At each time point, the syringes were quickly removed from the shaking incubator, and counted immediately depleted platelets in the PRP with the coulter counter or hemacytometer (Spencer Bright-Line, Fisher, NJ). The amount of platelets that adhered upon the specimen was calculated by subtracting the number of unadhered platelets from the number of diluted platelets that were initially incubated. Platelet adhesion test of blended films (1×1 cm^2) was performed by a similar procedure to that of beads.

***In Vitro* Protein Adsorption:** A bicinchoninic acid (BCA) protein assay was performed to determine the amount of proteins adsorbed onto copolymer-coated surfaces. Platelet

poor plasma (PPP), obtained from a healthy adult, fibrinogen (bovine serum fibrinogen, BSF) and albumin (bovine serum albumin, BSA) were used for *in vitro* study of protein adsorption. After equilibration with 5 mL of PBS (0.1 M, pH 7.4), each of samples (1 × 1 cm^2) was incubated in 5 mL of human plasma protein, BSF (40 $\mu g\ mL^{-1}$), and BSA (460 $\mu g\ mL^{-1}$) solutions (0.1 M PBS, pH 7.4), respectively, at 37 °C for the scheduled times (5 min, 15 min, 30 min, 1 h, and 2 h). After washing the samples three times with 10 mL of PBS solution (0.1 M, pH 7.4), the adsorbed protein on each surface, which was sonicated for 1 h, was eluted with sodium dodecylsulfate (SDS) solution (1wt% SDS + 1 mM EDTA + 0.1 M Tris, pH 7.4). The amount of adsorbed protein was measured with a BCA protein assay kit (Product No. 23231, 23232, and 23234, Pierce Chemical Co., Rockford, IL, USA).

***In Vitro* Bacterial Adhesion:** Two species of bacteria commonly implicated in implant infections, *S. epidermidis* (KCTC 1917) and *E. coli* (KCTC 2441) were used for the adhesion studies. All strains were obtained from KCTC (Korea Collection for Type Cultures, Daejon, Korea). Bacteria were grown in media for 20 h, centrifuged at 1,500 rpm for 30 min, washed and resuspended in PBS. Test films (1 × 1 cm^2) were incubated in a particular medium inoculated with 1 × 10^8 CFU/mL for 24 h at 37 °C with constant swirling. After the incubation period, the films were rinsed in sterile PBS to remove the nonadherent bacteria and were fixed in 10% neutral buffered formalin overnight at 4 °C. The fixed bacteria were observed by SEM. In addition, the bacteria were eluted from the surfaces using an ultrasonic cleaner. The number eluted was then measured by colony-forming unit (CFU) counts. Statistical analysis exhibited significant differences ($p < 0.05$ by students t test) in the adhering CFU between control and the copolymer-coated surfaces. Media (tryptic soya broth, TSB or brain heart infusion, BHI, and fresh citrated (0.37%) human plasma containing media) were utilized to examine each strain. The plasma was diluted to 67% with TSB to provide sufficient nutrients for bacterial growth.[17]

Results and Discussion

Characterization of Copolymers. Sulfonation of PEG1KMA monomer was performed with sodium metal and subsequently propane sultone. Sulfonation was confirmed by the presence of SO_2 at 1030 cm^{-1} in the IR spectrum (Figure 2) and by the methylene group of propane sultone (2.2-3 ppm) in the NMR spectrum (Figure 3), respectively.

Poly(PEG1K-SO_3MA/OMA) copolymers with various compositions were synthesized by conventional radical polymerization. Copolymers were characterized by FTIR, NMR, and EA. The typical NMR spectra of PEG1KMA and PEG1K-SO_3MA monomer were shown in Figure 3. ^{1}H-NMR spectra of the copolymers demonstrated the typical

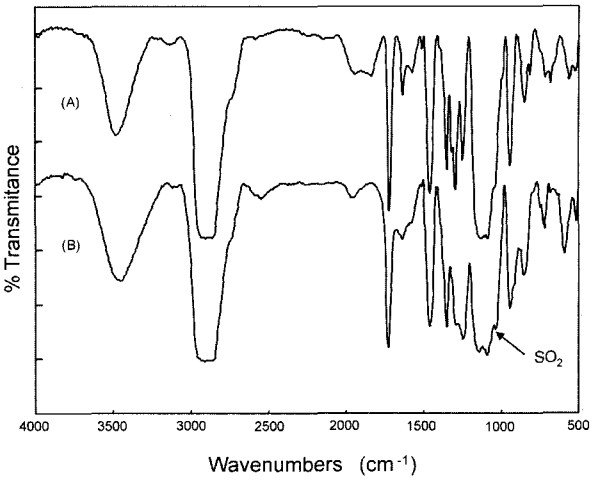

Figure 2. FT-IR spectra of copolymers. (A) Poly(PEG1KMA/OMA) = 5.3/4.7 and (B) poly(PEG1K-SO_3MA/OMA) = 4.4/5.6.

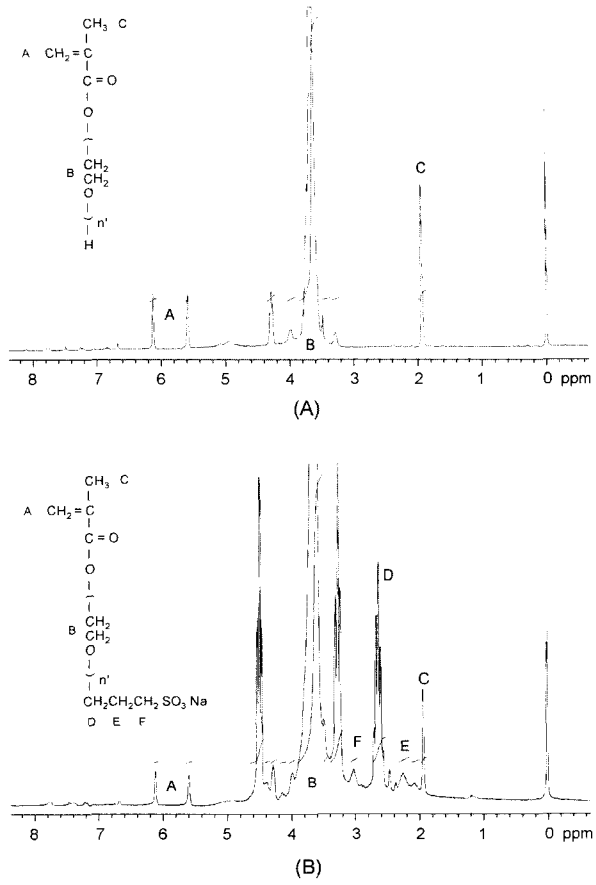

Figure 3. ^{1}H-NMR spectra of PEG1KMA and PEG1K-SO_3MA monomers. (A) PEG1KMA and (B) PEG1K-SO_3MA.

peaks of corresponding monomers, respectively (data not shown). The compositions of the copolymers were estimated from the peak integrals of NMR spectra (Table I).

The results of the copolymerization of PEG1KMA/PEG1K-SO_3MA and OMA are summarized in Table I.

The copolymer composition estimated by NMR demonstrated differences when compared to feed ratio of monomers. The water solubilities of poly(PEG1KMA/OMA) and poly(PEG1K-SO_3MA/OMA) depend on the content of hydrophilic component of PEG of the copolymers. Water-insoluble copolymers were utilized for coating using chloroform or blending and further evaluations.

Surface and Bulk Properties.

Dynamic Contact Angle: Copolymer coated PE or PU surfaces were characterized by water contact angle measurement. Table II summarizes the results of the dynamic contact angle measurements of the copolymer coated-PU and -PE surfaces and blended-PU surface, respectively.

Dynamic contact angle measurements from copolymer-coated PU and PE surfaces showed that the hydrophilicity in general, significantly increased (i.e., contact angles decreased) as compared to control surface. The PEG1K/PEG1K-SO_3 chain density effect on surface hydrophilicity of copolymer surfaces demonstrated that hydrophilicity increased as the PEG or PEG-SO_3 content increases. In addition, after 1 day

Table I. The Copolymerization of Poly(PEG1KMA/OMA) and Poly(PEG1K-SO_3MA/OMA)

Copolymer	Feed Ratio (mole ratio)	Copolymer[a] Composition	Water[b] Solubility	η_{inh}[c] [dl/g]	T_m[d] [°C]
Poly(PEG1KMA/OMA)	1 / 9	1.3 / 8.7	IS	0.373	41.4
Poly(PEG1KMA/OMA)	3 / 7	3.7 / 3.9	IS	0.548	41.9
Poly(PEG1KMA/OMA)	5 / 5	5.3 / 4.7	IS	0.728	42.4
Poly(PEG1KMA/OMA)	7 / 3	N.D.	S	N.D.	N.D.
Poly(PEG1KMA/OMA)	9 / 1	N.D.	S	N.D.	N.D.
Poly(PEG1K-SO_3MA/OMA)	1 / 9	0.6 / 9.4	IS	0.365	40.3
Poly(PEG1K-SO_3MA/OMA)	3 / 7	3.0 / 7.0	IS	0.884	41.0
Poly(PEG1K-SO_3MA/OMA)	5 / 5	4.4 / 5.6	IS	0.894	44.4
Poly(PEG1K-SO_3MA/OMA)	7 / 3	N.D.	S	N.D.	N.D.
Poly(PEG1K-SO_3MA/OMA)	9 / 1	N.D.	S	N.D.	N.D.

[a]Calculated by ^{1}H-NMR spectra. [b]IS: water-insoluble copolymer, S: water-soluble copolymer.
[c]Determined at 25 °C in 0.5 wt% $CHCl_3$ solution using an Ubbelohde viscometer. [d]Measured on DSC thermograms.
N. D. is not determined, because of water-soluble copolymers.

Table II. Contact Angle Data of Copolymer-treated Surfaces

Surfaces	θa	θr	θa*	θr*
PU control	90 ± 0.7	56 ± 1.0	82 ± 0.3	52 ± 1.1
PU coated with				
Poly(PEG1KMA/OMA)=1.3/8.7	81 ± 2.3	38 ± 3.6	86 ± 2.6	30 ± 2.3
Poly(PEG1KMA/OMA)=3.7/6.3	77 ± 3.7	35 ± 2.6	84 ± 3.9	28 ± 1.2
Poly(PEG1KMA/OMA)=5.3/4.7	71 ± 1.9	31 ± 3.6	80 ± 2.7	26 ± 3.9
Poly(PEG1K-SO_3MA/OMA)=0.6/9.4	71 ± 3.3	36 ± 3.1	87 ± 2.1	26 ± 3.8
Poly(PEG1K-SO_3MA/OMA)=3.0/7.0	65 ± 3.3	30 ± 3.2	84 ± 3.1	22 ± 3.3
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	62 ± 2.0	22 ± 2.8	82 ± 0.9	14 ± 3.2
PE control	99 ± 0.8	75 ± 1.6	104 ± 1.5	77 ± 1.7
PE coated with				
Poly(PEG1KMA/OMA)=1.3/8.7	97 ± 3.7	36 ± 3.7	92 ± 3.7	28 ± 1.7
Poly(PEG1KMA/OMA)=3.7/6.3	77 ± 2.6	35 ± 3.7	87 ± 1.1	27 ± 2.7
Poly(PEG1KMA/OMA)=5.3/4.7	53 ± 0.8	35 ± 1.7	87 ± 2.2	26 ± 1.6
Poly(PEG1K-SO_3MA/OMA)=0.6/9.4	94 ± 3.0	39 ± 1.9	83 ± 3.7	31 ± 2.5
Poly(PEG1K-SO_3MA/OMA)=3.0/7.0	89 ± 3.8	34 ± 1.5	95 ± 3.6	28 ± 2.4
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	80 ± 3.3	34 ± 1.9	93 ± 3.4	24 ± 1.5
PU control	90 ± 0.7	56 ± 1.0	82 ± 0.3	52 ± 1.1
PU blended with				
Poly(PEG1KMA/OMA)=5.3/4.7	73 ± 1.6	45 ± 1.2	85 ± 0.4	34 ± 2.0
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	67 ± 1.5	32 ± 1.8	80 ± 2.8	20 ± 0.8

Unit: degree, mean ± S. D., n=4. θa: advancing contact angle, θr: receding contact angle.
*: measured after hydration for 24 hrs.

hydration, similar trend was observed and all copolymer surfaces demonstrated lower receding angle compared to non-hydrated surfaces, which is due to the surface orientation and extension of hydrophilic PEG chain into the water phase. Sulfonated PEG surfaces show slightly higher wettability (lower contact angle) when compared to PEG surfaces. It is interesting to note that advancing angles of hydrated surfaces increase as compared to those of dried surfaces. This aspect should be subjected to further study.

In the case of copolymer blended PU film, contact angles decreased when compared to PU control, suggesting that hydrophilic PEG1K or PEG1K-SO_3 chains of copolymer additives extended into the water phase as described in copolymer coated surfaces above. It is known that amphiphilic copolymers are surface-active and when these copolymers are blended to polymer matrix they may diffuse and accumulate to surfaces.[19,20]

ESCA of Copolymer Coated Surface: The changes in chemical structure on the PU and PE film surfaces by the treated poly(PEG1KMA/OMA) and poly(PEG1K-SO_3MA/OMA) were analyzed by ESCA.

As seen in ESCA carbon 1S spectra (Figure 5), 285.0 eV was chosen as the binding energy for the C-H group of the C_{1s} peak components. Dilks gave the carbon binding energies in the following groups: [21]

-C-O- : 286.6 0.2 eV,

-C=O : 287.8 0.2 eV,

-O-C=O : 289.0 0.2 eV.

Figure 5 showed that ether carbon (-C-O-) peaks increase significantly in the poly(PEG1KMA/OMA) and poly (PEG1K-SO_3MA/OMA) coated PU surfaces as compared with the control, which is similar to the copolymer coated/ blended PE surfaces (data not shown).

Table III summarizes the ESCA results of control, poly (PEG1KMA/OMA), and poly(PEG1K-SO_3MA/OMA) coated-PU and -PE surfaces.

From the Table III, the poly(PEG1KMA/OMA) and poly (PEG1K-SO_3MA/OMA) coated surfaces showed increased atomic percentages of oxygen or oxygen to carbon ratio with

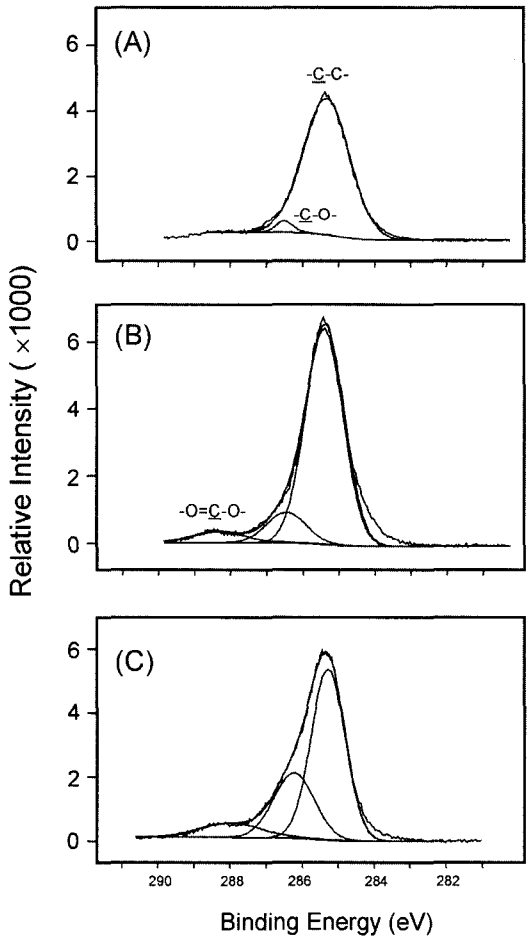

Figure 4. High-resolution C_{1S} XPS peaks of the copolymer coated PU film. (A) Control film, (B) poly(PEG1KMA/OMA) = 5.3/4.7, and (C) poly(PEG1K-SO_3MA/OMA) = 4.4/5.6.

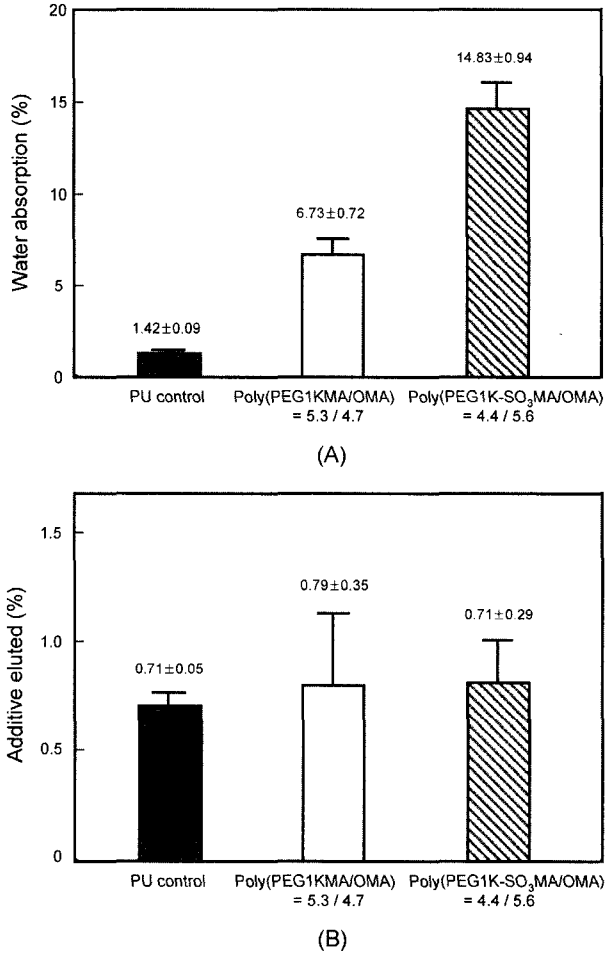

Figure 5. Water absorption and copolymer elution of copolymer-blended PU films. (A) Water absorption of 10 wt% copolymer-containing PU films after immersion in water for 24 hrs (n=4).

Table III. Surface Elemental Compositions of Copolymer-treated Surfaces

Surfaces	C^a	O^a	S^a	O/C^b	S/C^b
PU control	78.30	19.40	-	0.248	-
PU coated with					
Poly(PEG1KMA/OMA)=1.3/8.7	88.15	11.85	-	0.134	-
Poly(PEG1KMA/OMA)=3.7/6.3	86.51	13.49	-	0.156	-
Poly(PEG1KMA/OMA)=5.3/4.7	85.65	14.35	-	0.168	-
Poly(PEG1K-SO_3MA/OMA)=0.6/9.4	83.56	15.82	0.62	0.189	0.007
Poly(PEG1K-SO_3MA/OMA)=3.0/7.0	83.20	15.86	0.94	0.191	0.011
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	80.56	18.12	1.32	0.225	0.016
PE control	98.02	1.91	-	0.019	-
PE coated with					
Poly(PEG1KMA/OMA)=1.3/8.7	82.93	17.07	-	0.206	-
Poly(PEG1KMA/OMA)=3.7/6.3	82.59	17.41	-	0.211	-
Poly(PEG1KMA/OMA)=5.3/4.7	78.19	21.81	-	0.279	-
Poly(PEG1K-SO_3MA/OMA)=0.6/9.4	84.90	14.86	0.24	0.175	0.003
Poly(PEG1K-SO_3MA/OMA)=3.0/7.0	80.49	19.09	0.42	0.237	0.005
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	79.47	19.96	0.57	0.251	0.007
PU control	78.30	19.40	-	0.248	-
PU blended with					
Poly(PEG1KMA/OMA)=5.3/4.7	88.30	11.70	-	0.133	-
Poly(PEG1K-SO_3MA/OMA)=4.4/5.6	85.84	13.36	0.79	0.156	0.009

[a] Unit: mol %. [b] Surface molar ratio.

the increasing PEG1K chain density of the copolymers. For the poly(PEG1K-SO_3MA/OMA), sulfur content as well as oxygen of the surfaces also increased as the PEG1K-SO_3 chain density of the copolymers increased. In the case of the copolymer-blended surfaces, the oxygen content of the poly(PEG1K-SO_3MA/OMA) is more increased than that of the poly(PEG1KMA/OMA).

Swelling Property and Water Stability: The swelling property and stability of the blended films may be important parameters in many applications. The swelling property of copolymer blended PU film was examined by measuring the water content after immersion in distilled water. As seen in Figure 6(A), the water adsorption of the copolymer-blended films increased compared to that of control film, probably due to the hydration and reorientation effect of the PEG1K/PEG1K-SO_3 chain. In addition, PEG1K-SO_3 copolymer containing film showed larger water uptake than the film containing PEG copolymer. Increased water uptake of PEG1K-SO_3 relative to PEG1K is due to negatively charged sulfonate group of the copolymer-blended film.

The stability of copolymer additives entrapped in PU films was also examined by measuring weight changes of the films after immersion in water for 7 days. As shown in Figure 6(B), both copolymer films demonstrated no significant amount of extraction and the extraction amount is almost same as control PU films, suggesting the stability of the copolymer blended PU film.

Platelet Adhesion: The result of platelet adhesion onto the PU films blended with 10wt% of poly(PEG1KMA/OMA) = 5.3/4.7 and poly(PEG1K-SO_3MA/OMA) = 4.4/5.6 is shown in Figure 7.

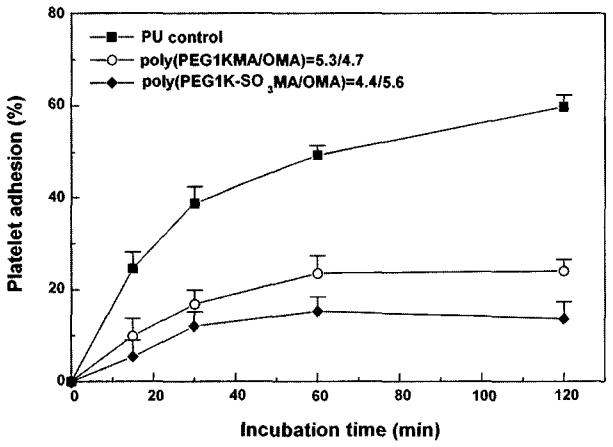

Figure 6. Platelet adhesion on PU films blended with copolymers. (The data are expressed as % of the number of adhered platelet with respect to the total number of platelets).

All copolymer surfaces demonstrated less platelet adhesion than control ($p < 0.05$ by students t-test). poly(PEG1K-SO_3MA/OMA) blended surface showed the lower amount of platelet adhesion than poly(PEG1KMA/OMA) surface.

In addition, platelet adhesion on the poly(PEG1KMA/OMA) copolymer coated PU surfaces decreased as the chain

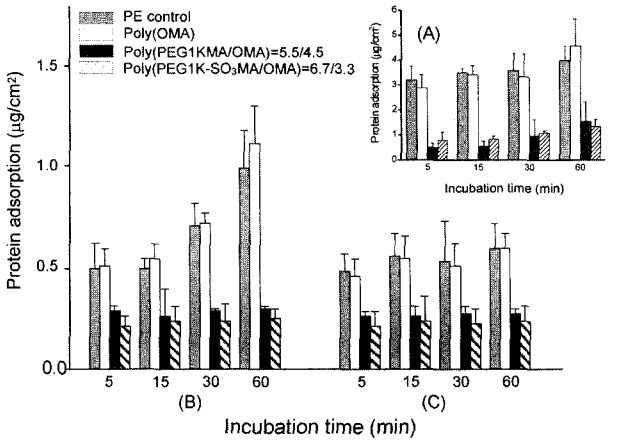

Figure 7. Protein adsorption on PE films coated with copolymers. (A) human plasma protein adsorption, (B) fibrinogen adsorption, and (C) albumin adsorption. (Mean ± SD, n=4).

density of PEG (the molar ratio of PEG1KMA) increases (data not shown). It is well known that PEG surface repels protein adsorption and platelet adhesion and the resultant biological behaviors are dependent on the PEG chain density.[22,23] The current explanation for PEGs passivity of surfaces with more dense PEG chains include low interfacial free energy with water, hydrophilicity, dynamic motion of PEG, extended chain conformation of PEG, and unique solution properties of PEG as discussed earlier. The film surfaces containing the copolymers with more dense PEG chains were significantly effective for the prevention of platelet adhesion, which is consistent with other reports.[5] For the platelet adhesion on poly(PEG1K-SO_3MA/OMA) copolymer coated surfaces with various PEG1K-SO_3 chain densities, less platelet adhered on the copolymer surfaces with higher density (higher molar ratio) of PEG1K-SO_3MA (data not shown).

From this study, it is expected that the copolymer surfaces containing the higher density of PEG/PEG-SO_3 can prevent platelet activation, which is supported by SEM views of platelets morphology on the copolymer (data not shown). In the case of PEG1K-SO_3 copolymer surface, both the hydrophilic and mobile PEG and the negatively charged SO_3 group seem to be affected in a synergistic way to exhibit less adhesion and activation of platelets, resulting in enhanced blood compatibility, both surfaces coated with copolymers and blended with copolymers.

Obtained results suggest the usefulness of this copolymer as coating or surface modifying additives to improve the blood compatibility of blood contacting devices.

Protein Adsorption: When blood contacts an artificial surface, the hemostatic system may respond in diverse ways depending upon the nature and duration of the stimuli. It is well known that foreign surfaces placed in contact with blood will rapidly adsorb plasma proteins at the blood-materials interface, which will mediate subsequent thrombotic events. Fibrinogen is the major protein adsorbed, and many other plasma proteins, including albumin and gamma-globulin, are also adsorbed.

Protein adsorption studies were performed with human PPP, BSF, and BSA. Each point represented an average of four samples. Dye binding methods to quantify bound protein remaining on SDS washed copolymer surfaces demonstrated negligible nonspecific binding to copolymer surfaces. This suggest that the amount of protein remaining on the surfaces after SDS treatment (1% v/v) was below quantifiable amounts and that SDS solution was effective in removing almost all of the adsorbed protein.

Figure 7(A) shows the adsorption of plasma protein on copolymer surfaces after each time incubation interval. In general, poly(PEG1KMA/OMA) demonstrated lower protein adsorption than control PE surface and hydrophobic poly(OMA) surface ($p < 0.05$ by students t-test). There were no significant differences between PEG and PEG-SO_3 copolymers. In addition, similar protein adsorption behaviors have been observed in total protein adsorption as well as single proteins (fibrinogen and albumin) adsorption analysis in Figure 7, (B) and (C). These results are consistent with platelet adhesion ones, which can be explained by the fact described above in platelet adhesion.

Bacterial Adhesion: Bacterial adhesion and colonization onto biomaterial surfaces and subsequent infectious complications are frequent reason for the failure of many medical devices and implants such as cardiovascular implants, catheters and urinary tract access.

S. epidermidis adhesion on poly(PEG1KMA/OMA) and poly(PEG1K-SO_3MA/OMA) copolymers surfaces in TSB and plasma was shown in Figure 8, respectively. Significant difference was seen between *S. epidermidis* adhesion in broth and plasma on modified surfaces. Both copolymer surfaces

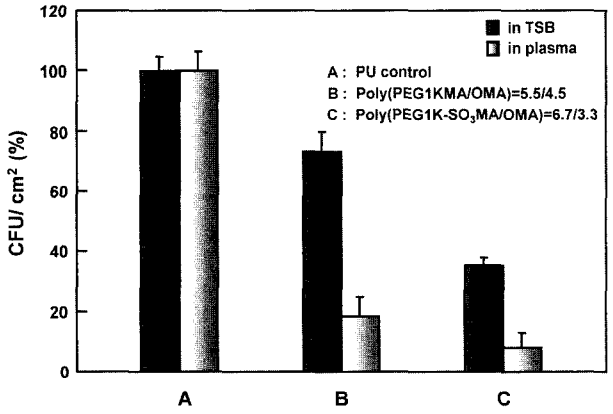

Figure 8. *S. epidermidis* adhesion on copolymers coated PU films in TSB and plasma, respectively (Mean±SD, 100% indicates to 2.1×10^7 and 2.0×10^7 CFU for TSB and plasma, respectively). (A) Poly(PEG1KMA/OMA) and (B) poly(PEG1K-SO_3MA/OMA).

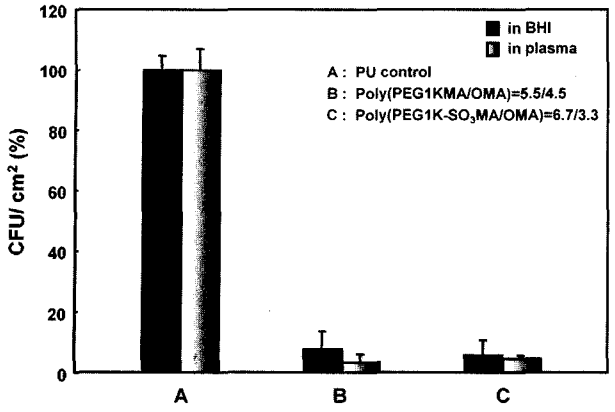

Figure 9. *E. coli* adhesion on copolymers coated PU films in BHI and plasma, respectively (Mean ± SD, 100% indicates to 1.8×10^7 and 2.1×10^7 CFU for BHI and plasma, respectively). (A) poly(PEG1KMA/OMA) and (B) poly(PEG1K-SO_3MA/OMA).

reduced *S. epidermidis* adhesion in both media as compared to PU control. However, reduction in plasma is more significant compared to that in broth. No significant difference was observed between poly(PEG1KMA/OMA) and poly (PEG1K-SO_3MA/OMA) copolymers in plasma. In contrast, the degree of *S. epidermidis* adhesion on sulfonated PEG surfaces is lower than that on PEG surfaces in broth.

Figure 9 shows *E. coli* adhesion on copolymers in BHI and plasma, respectively. Compared to PU control, all poly (PEG1KMA/OMA) and poly(PEG1K-SO_3MA/OMA) copolymer surfaces demonstrated consistently lower bacterial adhesion in both media. Difference between *E. coli* adhesion in BHI and plasma was also small on both copolymer surfaces. These results suggest that in general, both copolymers are more effective to repel *E. coli* in both media than *S. epidermidis*.

A variety of methods have been utilized in an attempt to reduce the incidence of infections. Prevention of bacterial adhesion or at least significant lowering of the number of viable bacteria seems to be the effective way to minimize foreign body infections. There are two principal approaches to preventing foreign body infections by influencing the interaction between biomaterial and bacterium: 1) development of polymers or polymer surfaces with antiadhesive properties,[24-29] 2) development of polymers or polymer surfaces with antimicrobial properties. Numerous agents such as surfactant, antimicrobial agents, heparin, nonsteroidal antiinflammatory agent and silver formulations have been applied in an attempt to prevent the bacterial adhesion.

Previously we have reported *S. epidermidis* and *E. coli* adhesion onto PU surfaces grafted by PEG chains with various terminal groups such as -OH, -NH_2, and -SO_3 (same PEG chain density and chain length on each surface) and PEG-heparin respectively.[17] All PEG grafted surfaces reduced bacterial adhesion significantly and the adhesion level differs depending on surfaces as well as media. Obtained results from this study are correlated with the previous report. Overall, bacteria adhere more on hydrophobic surface than hydrophilic one. This may be related to the observed low affinity of proteins and cells for PEG surfaces.[19] It is known that PEG surfaces in general, repel protein on the surface due to PEGs unique characteristics such as high chain mobility, a large excluded volume and hydrophilicity as described earlier. In addition, the plasma proteins, such as fibrinogen are known to play an important role in the adhesion of bacteria strain on these materials. To elucidate the mechanism of bacterial adhesion and colony formation on the polymer surfaces, protein adsorption study is under investigation. In summary, these copolymer surfaces may be useful in reducing bacterial-associated infections in medical devices.

Conclusions

The PEG1K/PEG1K-SO_3 methacrylate copolymers have been synthesized and characterized to utilize as coating or blending materials for biomedical applications. The modified surfaces using methacrylate copolymers demonstrated increased hydrophilicity, possibly due to the hypothesized reorientation of hydrophilic PEG1K/PEG1K-SO_3 chains into water phase.

All PEG1K/PEG1K-SO_3 methacrylate copolymer surfaces demonstrated less platelet and bacterial adhesion and protein adsorption than control. In addition, platelet adhesion on copolymer surfaces decreased as the chain density of PEG in copolymers increases. The PEG1K/PEG1K-SO_3 methacrylate copolymer surfaces may be useful as coating and blending materials to improve the blood compatibility of conventional biomedical devices which come in contact with blood.

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