Anti-Thrombogenicity of Styrene-Butadiene-Styrene Triblock Copolymer Grafted with Poly(ethylene glycol)s

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ABSTRACT: We transformed hydrophobic/hydrophobic styrene/butadiene/styrene tri-block copolymer (SBS) to hydrophobic / hydrophilic microphase-separated surfaces by grafting with hydrophilic poly(ethylene glycol) (PEG) on poly(butadiene) (PB) domain via hydrocarboxylation and hydrobromination and investigated the anti-thrombogenicity of these surfaces. In the case of SBS cast film from toluene solution, PEG was densely grafted because of the development of an unevenness on the order of several 10 nm on the surface, which had a huge surface area in comparison with poly(butadiene) rubber with its uniformly smooth surface. Grafted PEG (molecular weight = 600) was found to clearly inhibit adhesion and activation of platelets and coagulation of the whole blood component, which is indicative of anti-thrombogenicity. These properties correspond to a surface coated by a copolymer of 2-methacryloyl-oxyethyl phosphorylcholine and *n*-butyl methacrylate, which is well known to be the best excellent anti-thrombogenic material in the world. Melt-molded SBS film, which also has an uneveness on the order of several 10 nm, showed similar excellent anti-thrombogenicity. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2462–2476, 2009

Key words: SBS; PEG; graft; microphase-separation; anti-thrombogenecity

INTRODUCTION

Okano et al.[1] synthesized a block copolymer of hydrophilic 2-hydroxy ethyl methacrylate and hydrophobic styrene and these blocks formed microphase-separated domains. They found that this copolymer surface had an excellent anti-thrombogenicity because of the different adhesive protein molecules on each of the domain surfaces that maintained the normal state of the cells.

Segmented polyurethanes (SPUs) forming the microphase-separated domains were widely used as biomedical materials, because of showing excellent mechanical properties and good anti-thrombogenicity.[2] However, it was clarified that the soft segments of SPUs are degraded by macrophages.[3,4] Many investigators therefore, have studied surface modification of SPUs such as introducing of heparin,[5] and sulfonate groups,[6] blending of tri-block copolymer (PEG-PEO-PEG),[7] and coating of blood-compatible polymer[8] on the surface of SPUs. Ishihara and Iwasaki et al.[9–13] synthesized a copolymer of 2-methacryloyloxyethyl phosphorylchloline (MPC) and *n*-butyl methacrylate (BMA), which is similar to bio-membranes, and they found that the copolymer is the best excellent anti-thrombogenic material in the world, and SPUs coated the copolymer shows the improved blood compatibility.[14]

Sefton et al.[15] prepared a styrene-butadiene-styrene tri-block copolymer (SBS), which has also excellent mechanical properties and is able to mold at melt state, grafted with hydrophilic heparin molecules on the poly(butadiene) (PB) domains of SBS. After approximately two decades, Yang et al.[16,17] grafted vinyl pyridine by radiating ultraviolet light on the surfaces of PB domains of SBS cast film and found that this surface reduced the adhesion of protein molecules and resulted in good anti-coagulation property of blood. They also grafted poly(hydroxyl ethyl methacrylate) on the PB domain surface of SBS and found that this showed better anti-coagulation properties of blood than the vinyl pyridine.[18]

In contrast, Mori et al.[19] grafted poly(ethylene glycol) (PEG) molecules on poly(vinyl chloride) surface not having a microphase-separated structure, and they found that the adsorption of blood elements decreased with increasing PEG chain length. Since then, many researchers have studied on the anti-thrombogenicity of the materials having PEG chains on their surfaces.[20–25]

In the present study, we transformed hydrophobic/hydrophobic styrene/butadiene/SBS into hydrophobic/hydrophilic microphase-separated surfaces by grafting with hydrophilic PEG onto a PB domain via hydrocarboxylation and hydrobromination and

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TABLE I
Characterization of SBS

Properties	Values
PS/PB (wt/wt)	24/76
(vol/vol)	18/82
Specific gravity	0.94
M_w (g/mol)	1.34×10^5
M_n (g/mol)	1.28×10^5
$d = M_w/M_n$	1.05
Polymerization degree of PS	170
Polymerization degree of PB	2070

investigated the relationship between anti-thrombogenicity, which based on the coagulation and adhesion properties of platelets and whole blood components, the hydrophobic/hydrophilic microphase-separated surface, and the volume restriction effect. These results were then compared with those of poly(butadiene) rubber (BR) having uniformly smooth surface grafted with PEG.

MATERIALS AND METHODS

Materials and sample preparation

SBS block copolymer TR2827 supplied from JSR was used as an original material. The characterization of the SBS is shown in Table I; the molecular weight was measured by gel permeation chromatography and the fraction of each component was measured by ^{1}H-nuclear magnetic resonance using a AVANCE400 (Blocker) at magnetic field of 400 MHz.

Sample preparation

SBS 5 wt % tetrahydrofuran (THF) solution was precipitated into acetone for purification, and the purified SBS 5 wt % toluene solution was cast on a Teflon laboratory dish at room temperature. The melt-molded film was prepared by molding at 10 MPa after deairing at 2 MPa at 150°C.

BR supplied from JSR was purified by precipitation of THF solution into acetone, and then the purified BR 5 wt % toluene solution was then cast on a Teflon laboratory dish at room temperature.

The glass plate coated with copolymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) and BMA (poly(MPC-*co*-BMA) (PMB) was used as a control film. Poly(styrene) (PSt) as the thrombogenic material was used also as another control film. PSt was molded in film at 150°C.

It has been reported that diene groups of PB can hydrocarboxylate using Pd catalyst.[26–28] We therefore, attempted hydrocarboxylation using this method. Each SBS cast film, melt-molded film, and BR cast film was immersed in the mixture of dimethyl sulfoxide (40 mL) and H_2O (500 mL). Carbon oxide and oxygen gases (15 mL/min) were babbled with $PdCl_2$ (0.13 g) as a catalyst and $CuCl_2$ (026 g) and HCl (1000 μL) as an activator for 1 day. The SBS and BR films were then immersed in acetic anhydride at 60°C for 3 h to transform from the carboxylic acid to maleic acid.[27,29] These films were dipped in a liquid state of three different molecular weights (M) (400, 600, and 1000) of PEG at 80°C for 24 h. These treated films were washed with pure water to remove unreacted PEG and were dried in vacuum. Every chemical was purchased from Wako Jyunyaku Kogyou, Japan and used without any purification. The above procedure is shown in Figure 1.

It has been reported that diene groups of PB can hydrobrominate using bromine acid.[29] We therefore, attempted hydrobromination by using this method. SBS cast film, melt-molded film, and BR cast film were hydrobrominated by immersion in bromine acid for 60 s. Each of these films was dipped in the liquid state of three different molecular weights (400, 600, and 1000) of PEG at 80°C for 24 h. These treated films were washed by pure water to remove the unreacted PEG and dried in vacuum. This method can be carried out quickly and cheaply in comparison with the hydrocarboxylation method.

The above procedure is shown in Figure 2.

The codes for each of the samples are named in Table II.

Methods

Infrared spectra were measured using a Shimadzu Fourier transform infrared spectrometer (FTIR 8400) set with an attenuated total reflection (ATR) attachment (Spectra Tech) (FTIR-ATR) at a resolution of

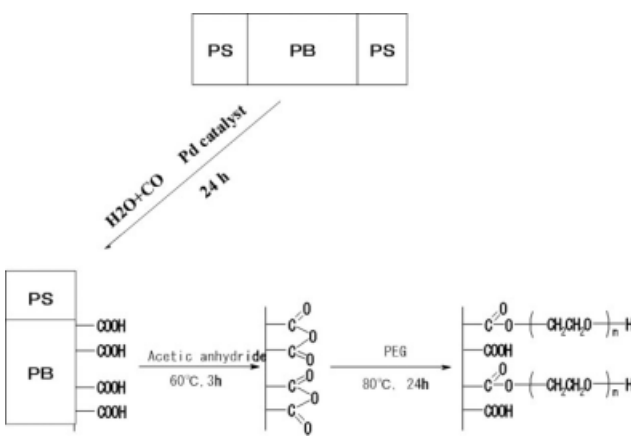

Figure 1 Scheme of hydrocarboxylation and grafting of PEG.

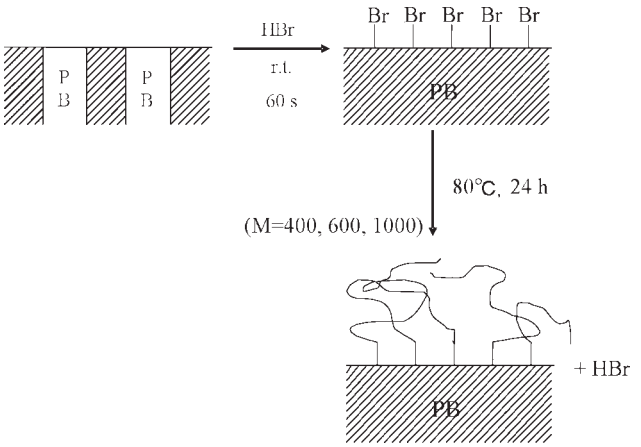

Figure 2 Scheme of hydrobromination and grafting of PEG.

4 cm^{-1}, a repeating number of 128, and measuring range of 4600–500 cm^{-1}.

X-ray photoeletron spectroscopy (XPS) was measured using an Axis-Ultra DLD XPS (Krato) width range of 1160 eV at a center of 570 eV, and was also measured for each O 1s, C 1s, Si 2p of oxygen, carbon, and silicon atoms. Before measuring, the film surface was wiped with ethanol to avoid contamination with dust.

The contact angle was measured using a CV-VP (Kyowa Interface Science, Japan) by dropping method using 50 μL of distilled water. The values reported are averages of 10 measurements on different parts of the film.

Atomic force microscopy was carried out with a SPM-9500 J3 (Shimazu, Japan) using optical beam deflection to monitor the displacement of micro-fabricated silicon cantilever SI-DF40 (Seiko Instruments, Japan). Measurements were performed with the dynamic phase mode (DFM) at 1.0 Hz. The DFM images (2 × 2 μm^2) were acquired with a pixel number of 512 × 512 by scanning the films in air under ambient laboratory condition (20°C).

The amount of grafted PEG was calculated from eq. (1),

$$\text{Grafted amount of PEG} = (w_f - w_i)/A \qquad (1)$$

where, w_i, w_f are the weight of the untreated film and the weight of the film after grafting PEG, and A is the area calculated from the width, height, and thickness of the film.

Evaluation of anti-thrombogenicity

Every film was first rinsed with ethanol, and the disk-shaped films were also placed in 24 flat-bottom wells. PBS was allowed to stand in the wells for 3 h to equilibrate the surface.

The evaluation procedure for the adhesion of platelets was as follows. Citrated whole blood was immediately centrifuged for 15 min at 1000 rpm to obtain citrated platelet-rich plasma (PRP). Five hundred microliters of PRP was then added to each well. The films were incubated for 1 h at 37°C.[30] After the PRP was removed, and the films were rinsed six times with PBS. The adhesive platelets were cross-linked with 2.5 vol % glutaraldehyde PBS solution. The platelets were freeze-dried after exchanging one after another of 50, 60, 70, 80, 90, 95, 100 vol % ethanol aqueous solution and then two

TABLE II
Code Names of SBS Films

Sample	Code name
SBS cast film	Cast
Carboxyl SBS by using Pd catalyst	Cast-carbo
SBS grafted by PEG (M = 400) after carboxyl	Cast-carbo-400
SBS grafted by PEG (M = 600) after carboxyl	Cast-carbo-600
SBS grafted by PEG (M = 1000) after carboxyl	Cast-carbo-1000
Cast SBS treated by HBr	Cast-HBr
SBS grafted by PEG (M = 400) after treatment of HBr	Cast-HBr-400
SBS grafted by PEG (M = 600) after treatment of HBr	Cast-HBr-600
SBS grafted by PEG (M = 1000) after treatment of HBr	Cast-HBr-1000
SBS melt film	Melt
Carboxyl melt SBS by using Pd catalyst	Melt-carbo
Melt SBS grafted by PEG (M = 600) after carboxyl	Melt-carbo-600
BR cast film	BR-cast
Carboxyl melt BR by using Pd catalyst	BR-carbo
BR grafted by PEG (M = 400) after carboxyl	BR-carbo-400
BR grafted by PEG (M = 600) after carboxyl	BR-carbo-600
BR grafted by PEG (M = 1000) after carboxyl	BR-carbo-1000
Glass plate coated by poly(MPC-*co*-BMA)	PMB
Poly(styrene)	PSt

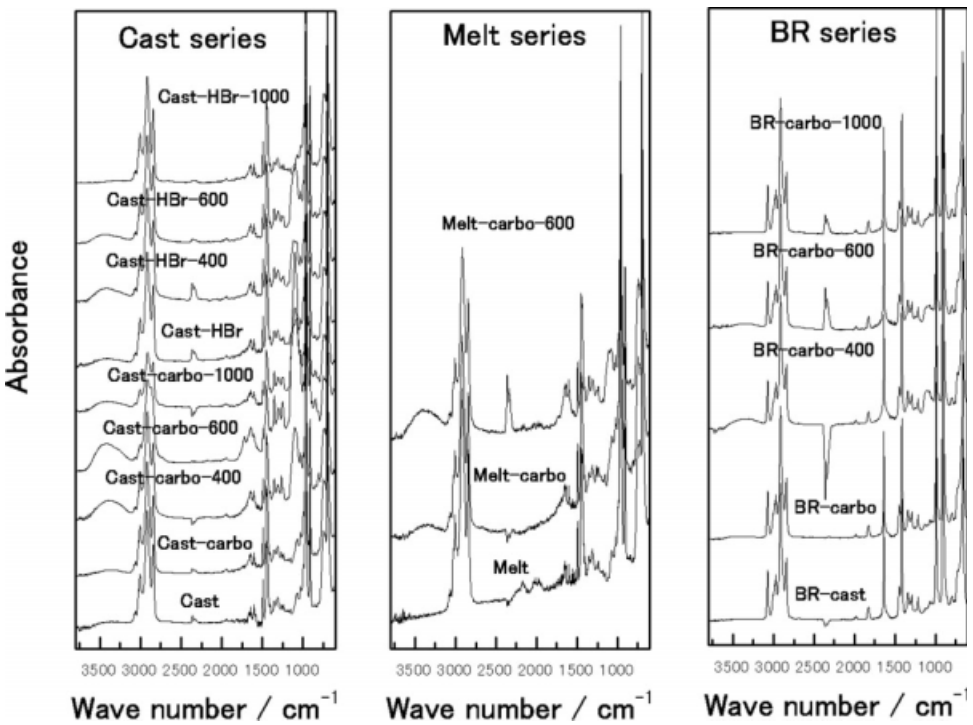

Figure 3 FTIR-ATR spectra of various kinds of SBS and BR films, (a) cast SBS, (b) hydrocarboxyl SBS, PEG-grafted SBS after hydrocarboxylation [(c) $M = 400$, (d) $M = 600$, (e) $M = 1000$], (f) hydrobrominated SBS, PEG-grafted SBS after hydroboration [(g) $M = 400$, (h) $M = 600$, (I) $M = 1000$), (j) melt-molded SBS, (k) hydrocarboxyl melt-molded SBS, (l) PEG ($M = 600$)-grafted SBS via hydrocarboxylation, (m) cast BR, (n) hydrocarboxyl BR and PEG-grafted BR via hydrobromination [(o) $M = 400$, (p) $M = 600$, (q) $M = 1000$).

exchanges with *n*-butanol. The freeze-dried platelets were coated with platinum and examined in a scanning electron microscope (Hitachi N-2380, Japan) at 20V and ×1.5 k to evaluate the adhesion of platelets. LDH assay method was also used to evaluate the platelet adhesion from LDH activity. After incubation, six films of each sample were dipped in a 0.5 vol % Triton X100 PBS solution for 24 h to dissolute LDH, and then LDH present in the supernatant of the samples was determined with a LDH kits purchased from Wako Jyunyaku Kogyou by measuring absorbance at 590 ± 10 nm.

The evaluation procedure for coagulation of blood components was as follows.[31,32] Whole blood (8.7 mL) was mixed with acid citrate dextrose solution (ACD-A) (1.3 mL) which was a mixture of sodium citric acid 11 g, citric acid 4 g, glucose 2.2 g, and 500 mL of distilled water. After removing PBS, the mixture of whole blood and ACD-A solution of 500 μL was added to every well. Fifty microliters of 0.1*M* calcium chloride solution was added to the mixture to promote the coagulation of blood components, namely activation of coagulation, and the films were incubated for 1 h at 37°C. After the incubation, 50 μL of 0.1*M* sodium citric acid was added to stop the coagulation reaction. The macroscopic coagulated materials on the surfaces of the films were removed by hand, and washed with distilled water. The coagulated components, which included platelets, fibrin, and red and white blood corpuscles were cross-linked with 2.5 vol % glutaraldehyde PBS solution. Each blood component was also freeze-dried and examined by the scanning electron microscope as well as the evaluation of the adhesion of platelets. Quantitative amount of coagulated components was observed by using a Bio-Rad's detergent compatible protein assay. This assay is improved over the original Lowry assay as color development. The coagulated components were dipped in a 0.5 vol % Triton X100 PBS solution for 24 h to dissolute protein. The obtained solutions were mixed with reagents A and B. After 15 min, the absorbance values of the solutions were measured at 750 nm.

RESULTS AND DISCUSSION

FTIR-ATR measurements

Figure 3 shows FTIR-ATR of every film.

The spectra of both the SBS cast film (a) and the hydrocarboxyl SBS cast film (b) are similar, and there is no absorption peak corresponding to a carbonyl group (C=O) at 1720 cm^{-1}. Every film dipped in PEG after hydrocarboxylation (c–e) shows absorption peaks of a C=O group and ether group (C—O—C) at 1100 cm^{-1}, but no peak corresponding

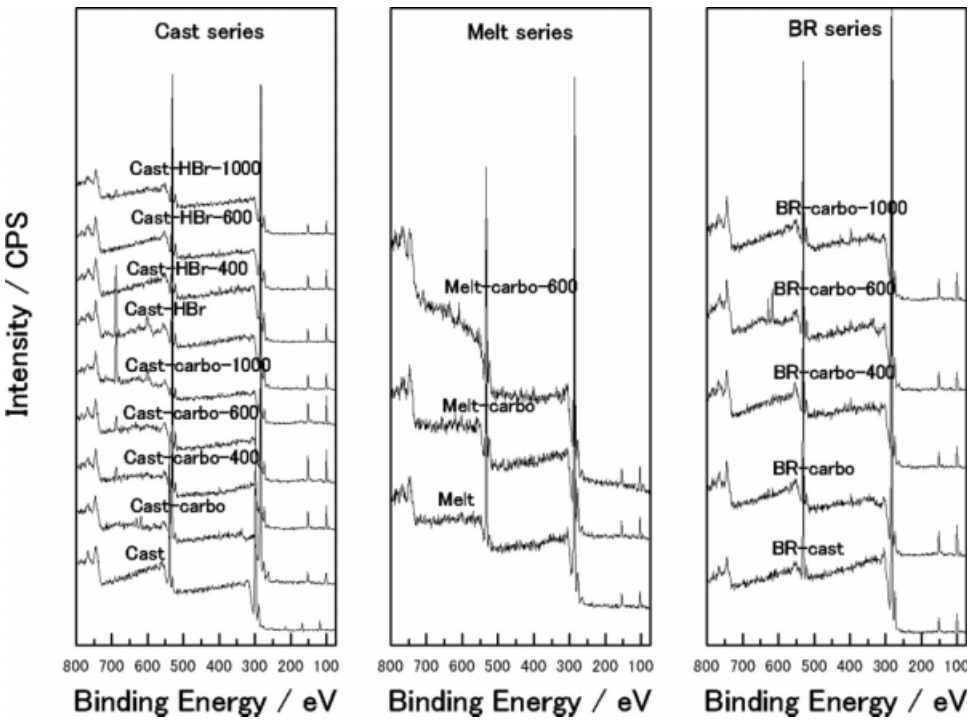

Figure 4 XPS patterns of various kinds of surfaces of SBS and BR films [alphabetical symbols are same to Fig. 3].

to a hydroxyl group (OH) can be clearly observed. These results indicate that PEG molecules were grafted on PB domain surfaces.

The hydrobrominate SBS cast film (f) shows an absorption peak of OH at 3400 cm^{-1}, which indicates that there was an immediate transformation from a hydrobrominate group to an OH group. Each film was dipped in three different molecular weights of PEG (g–i) after hydrobromination, and there also shows absorption peaks corresponding to a C—O—C group at 1100 cm^{-1}. These results clearly indicate that the hydrobrominate SBS cast film is also directly grafted with PEG.

The melt-molded film (j) also shows absorption peaks corresponding to C=O and C—O—C groups. These results indicate that a carboxyl group (COOH) was formed by oxidation in molding at 150°C in atmosphere because of a lack of artificial hydrocarboxylation. In contrast, the hydrocarboxyl melt-molded SBS film (k) shows absorption peaks corresponding to C=O and OH groups and the dipped PEG (M = 600) film (l) after hydrocarboxylation shows an absorption corresponding to a C—O—C group at 1100 cm^{-1}. It is therefore, clear that the melt-molded SBS film is also grafted with PEG.

Absorption peaks corresponding to C=O and OH groups were not observed for BR cast film (m) and the hydrocarboxyl film (n). The BR cast films (o–q) dipped in three different molecular weights of PEG, however, showed an absorption peak corresponding to C—O—C group. These results indicate that the BR cast film is also grafted with PEG.

XPS measurements

We did not observe the clear evidence of FTIR spectra of the hydrocarboxyl and the hydrobrominate films. This lack of evidence probably indicates that only information from the several micrometers of layers from the surface of each film is contained in the FTIR spectra, namely lots of the information regarding atomic groups without hydrocarboxylation and hydrobromination. Therefore, our XPS measurements only provided information corresponding to a very thin layer from the surface.

Figure 4 shows a wide range of XPS spectra of SBS and BR films. Peaks of C 1s at around 290 eV, O 1s at around 530 eV, and Si 2p at around 100 eV were observed in every spectra and F 1s at around 690 eV for cast films. These peaks are slightly deviated from previously reported data,[33-35] because of the electron charge on the surface. Silicon atoms are not contained in the purified SBS or the hydrocarboxyl and hydrobrominate SBS. The Si 2p peak was therefore likely because of contaminated silica in the measurement after wiping with ethanol. The presence of fluorine atoms was probably because of contamination from the Teflon seat in casting.

The left side column of Table III shows the relative values of oxygen atoms versus carbon atoms

TABLE III
Atomic Percentages of Elements and Percentages of Species Obtained from C1s Curve Fitting of SBS and Br Films

Code name	O/C	CH (%)	C—O (%)	C=O (%)	COO (%)
Cast	0.081	87	13		
Cast-carbo	0.23	63	17	15	5
Cast-carbo-400	0.23	73	21	4	2
Cast-carbo-600	0.27	72	23	3	2
Cast-carbo-1000	0.29	78	19	2	2
Cast-HBr	0.22	78	16	2	5
Cast-HBr-400	0.24	69	26	2	3
Cast-HBr-600	0.3 30	61	32	2	5
Cast-HBr-1000	0.3 30	60	33	4	4
Melt	0.2 20	81	12	4	4
Melt-carbo	0.22	78	14	4	4
Melt-carbo-600	0.23	74	20	4	3
BR-cast	0.11	88	9	2	2
BR-carbo	0.23	77	15	2	6
BR-carbo-400	0.24	76	17	6	2
BR-carbo-600	0.24	75	18	4	3
BR-carbo-1000	0.24	70	22	4	3

calculated from the area of O 1s and C 1s peaks corresponding to the index of hydrocarboxylation and the grafted PEG in the very thin surface layer in comparison with untreated films. The values corresponding to the hydrocarboxyl SBS and the hydrobrominate SBS cast films, the melt-molded, and the hydrocarboxyl melt-molded films, and the hydrocarboxyl BR cast film were larger than those for untreated films. In the case of hydrocarboxyl SBS cast films, hydrocarboxyl melt-molded films, and hydrocarboxyl BR cast film, these larger values mean that the increases in oxygen ratios correspond to the presence of COOH bonded to the very thin surface layer. The increase in hydrobrominated SBS cast film supports the bonding of OH groups as speculated based on the results of the FTIR. The increase in melt-molded SBS film supports the oxidation by heating in the mold process, as also speculated based on the FTIR results.

Figure 5 shows curve-fitting of the C 1s peaks of cast, hydrocarboxyl, and SBS grafted with three different molecular weights of PEG.[33,34] Table III also contains the data regarding the percentages of CH at 285 eV, C—O of 286.5 eV, C=O of 288.0 eV, and COO of 289.3 eV calculated from curve-fitting of each C 1s peak. We can see the data correponding to the COO group in the cases of hydrocarboxyl SBS cast and hydrocarboxyl melt-molded films and hydrocarboxyl BR cast film. Carbon peroxide was also formed by hydrocarboxylation. After grafting the PEG, the percentage of the CH group decreases, whereas the percentage of the C—O group increases for every film. These results indicate that the C—O—C group increases and the CH group relatively decreases with grafting PEG. Although the data corresponding to the percentages of C=O and COO groups randomly changes by $\sim$ 2–3%, the number of C=O group should not change before and after grafting. Therefore, the random data must be because of the deviation of the values calculated from the curve-fitting. The C—O group of hydrobrominate SBS cast film shows larger than hydrocarboxyl SBS and BR cast films. These results can be explained as follows. The C—O group is due to the C—OH group formed on the PB domain, because the bromine atom bonded to PB by the

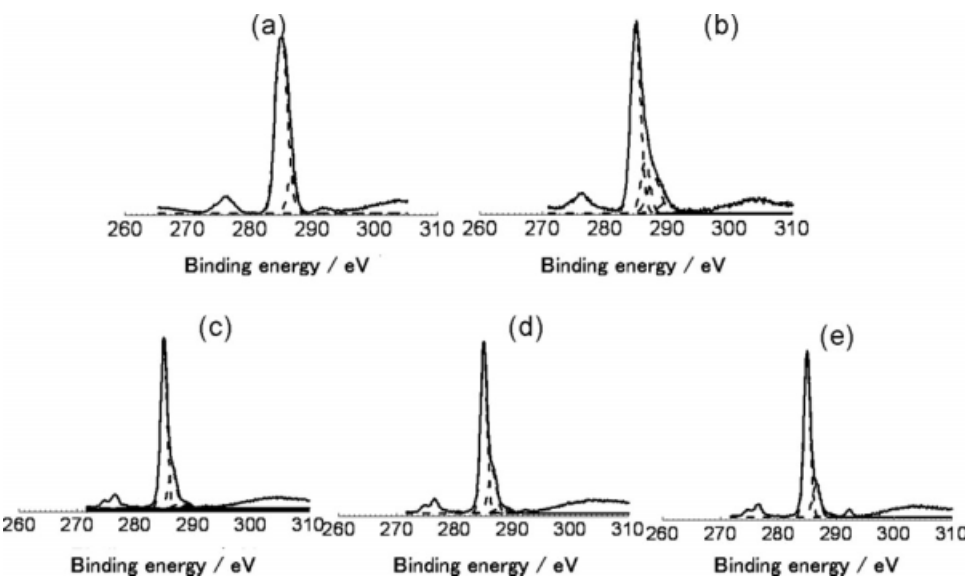

Figure 5 Curve-fitting of C 1s peak of [(a) cast SBS, (b) PEG-grafted SBS via hydrcarboxylation [(c) M = 400, (d) M = 600, (e) M = 1000).

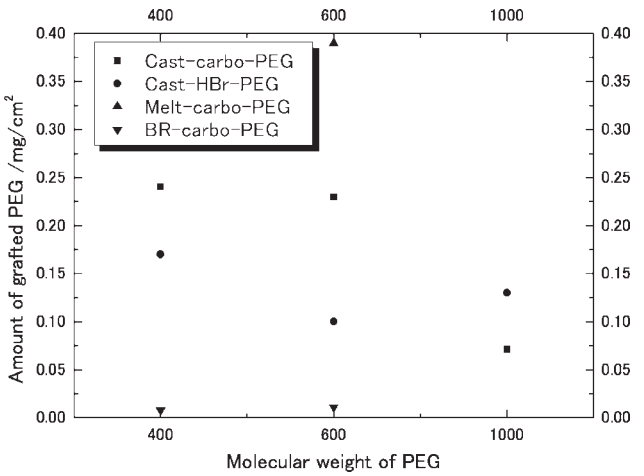

Figure 6 Dependence of amount of grafted PEG on molecular weight of PEG.

hydrobromination immediately transforms to an OH group, as described above.

Amount of grafted PEG

Figure 6 shows the amount of grafted PEG.

An increase in weight was observed after grafting of PEG for every SBS cast film. The grafted amount of PEG decreases with increases in the molecular weight. This result means that the grafted amount is affected by the relative decrease in the reaction site number and the increase in the steric hindrance of the PEG molecule itself by the increase in the molecular length of PEG. The amount of grafted PEG via hydrobromination is smaller than that via hydrocarboxylation. As such, the OH group transformed from bromine does not act as a reaction site with PEG molecules.

Very little PEG is grafted onto the BR cast film in comparison with SBS films. The amount of the grafted PEG for the SBS cast film was about 20 times of that of the BR cast film. This reason for this difference will be explained by the AFM observations in the next chapter.

Contact angle

Figure 7 shows the results regarding the contact angle of every film.

The contact angles of the SBS cast film and the melt-molded film showed the following tendencies;

cast film and melt-molded film > hydrocalboxyl film > PEG-grafted film

These findings indicate that the contact angle decreases in response to bonding with a hydrophilic group such as a carboxyl group and PEG molecules on the surfaces, and that the hydrophilic properties of the PEG molecule are stronger than those of the carboxyl group. The contact angle of the higher molecular weight of PEG-grafted film is larger than that of the PEG with lower molecular weight. This result means that the small grafted amount for the higher molecular weight of PEG is insufficient for providing hydrophilic properties.

The contact angle of PEG on the BR cast film is very low, although there is a very small amount of grafted PEG in comparison with the SBS films. The reason for this discrepancy will also be explained by AFM observations in a later chapter.

SPM observation

Figure 8(A) shows the SPM image of surface of cast and melt-molded SBS samples. Both surfaces shows micro-phase separated domains, and particularly cast film.

Figure 8(B) shows inclined SPM images for typical films. Uneveness on the order of several 10 nm in addition to submicron order roughness were observed in each of the SPM images for the SBS cast and the melt-molded SBS films and these hydrocarboxyl films, and these PEG-grafted films via hydrocarboxylation and hydrobromination. In contrast, the BR cast film and the hydrocarboxyl BR cast film were uniform and very smooth.

These results indicate that the SBS film has a much larger surface area than BR film. Consequently, the PB domains of SBS surfaces have a large number of PEG grafting reaction sites via hydrocarboxylation and hydrobromination, which results in a much larger amount of grafted PEG than the BR surface. The smooth surface of BR probably causes a low contact angle, because the dropped water easily spreads out on the surface.

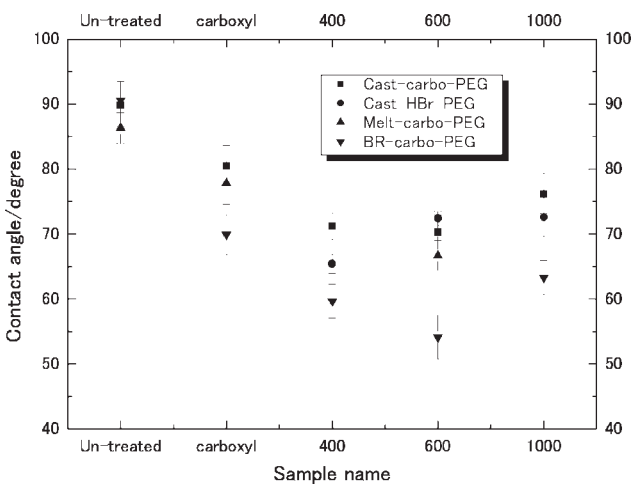

Figure 7 Contact angle of untreated and treated SBS films.

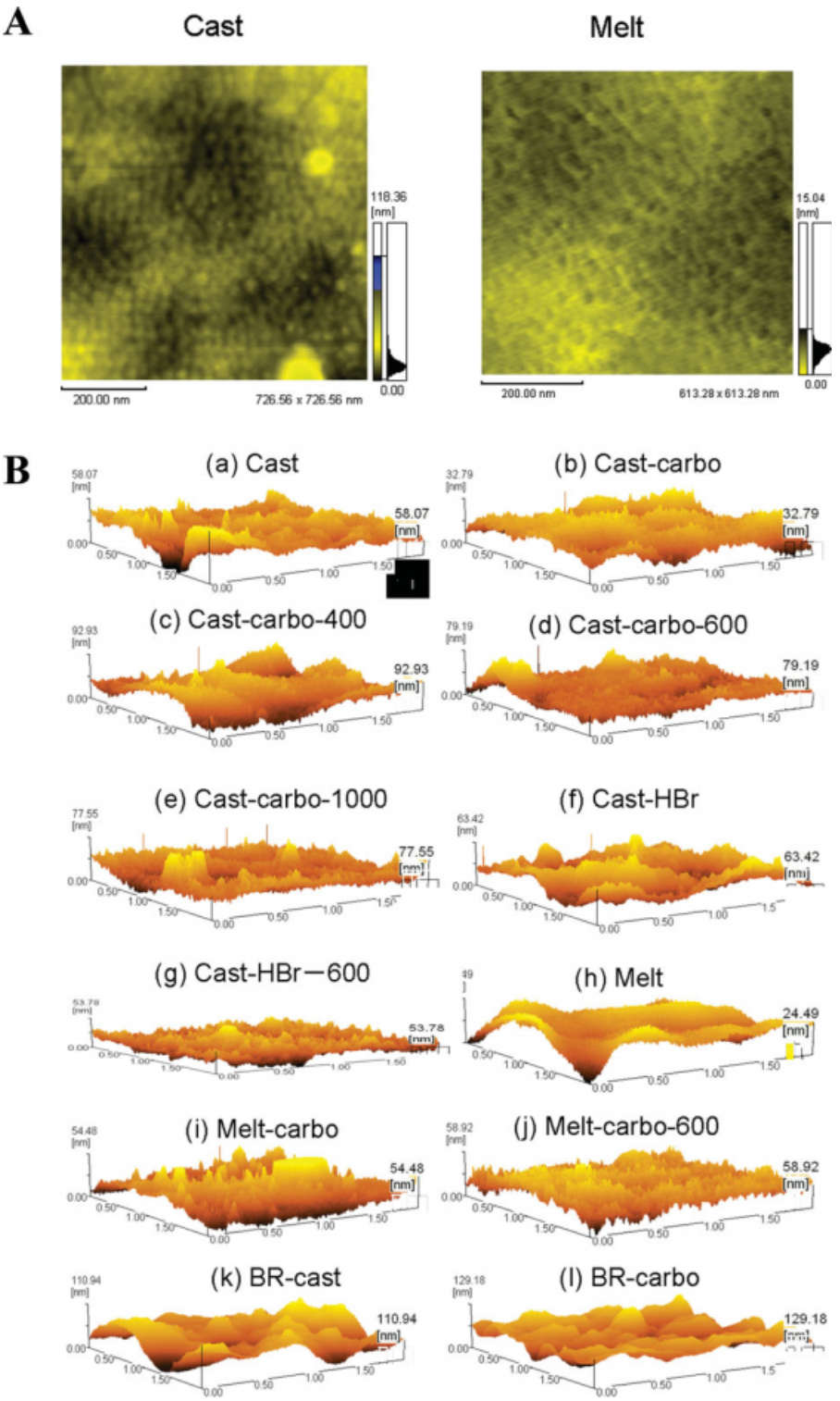

Figure 8 (A) SPM images of cast and melt-molded SBS. (B) SPM images of various kinds of SBS and BR films. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Evaluation of anti-thrombogenicity

Adhesion of platelets

Figure 9 shows SEM images of the adhesion of platelets on the surface of typical films.

PSt film (a) and PMB coated glass (h) were used as controls. Many platelets 2 μm in diameter can be observed to be adhered onto the PSt film, and the platelets transform into an angular shape with a pseudopodium, meaning that they are active cells.

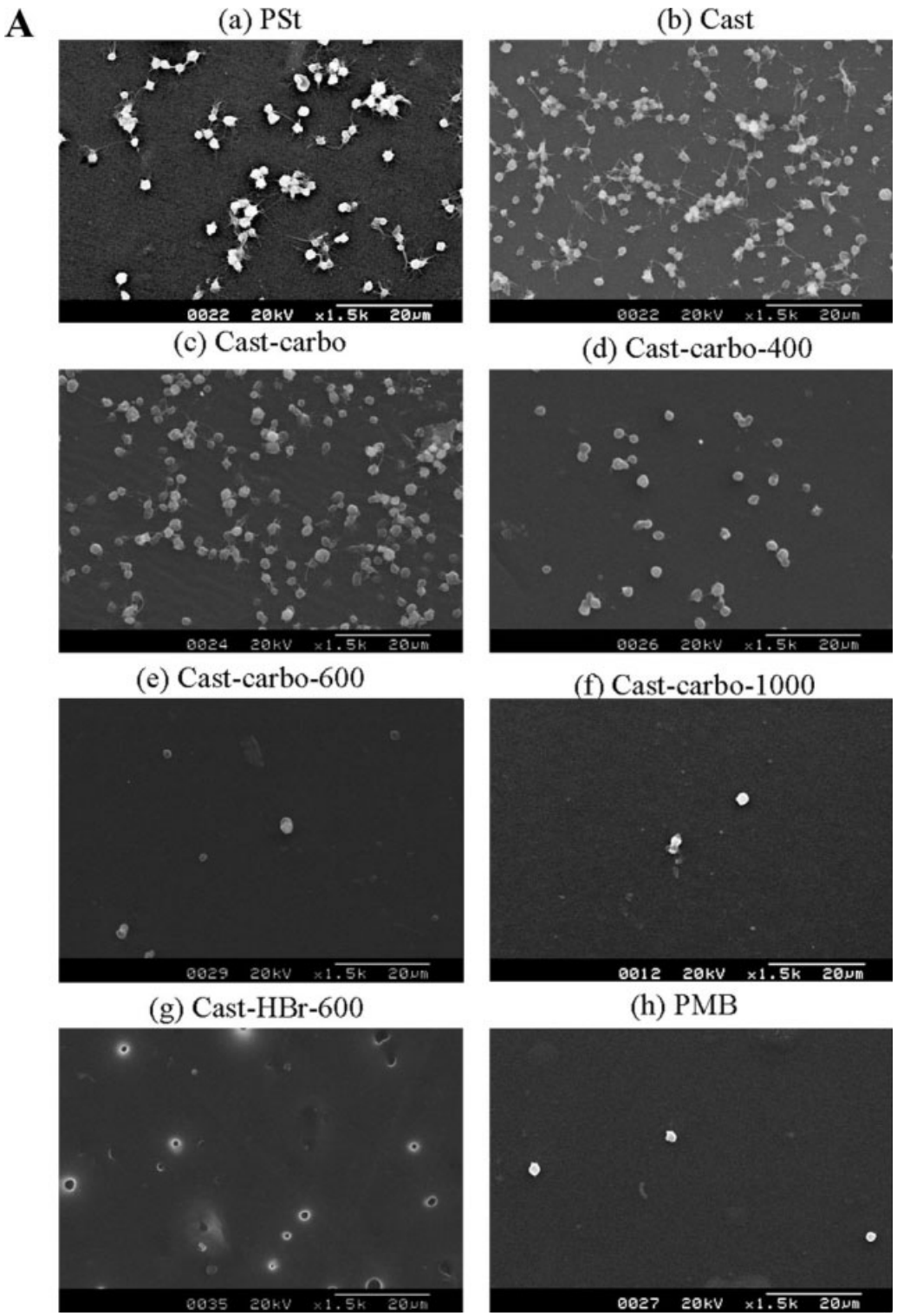

Figure 9 (A) SEM images (×1.5 k) of platelets adhered cast SBS film and melt-molded SBS film. (B) SEM images (×1.5 k) of platelets adhered cast SBS film and melt-molded SBS film.

In contrast, there are no platelets on the PMB-coated glass plate.

The number and shape of platelets adhered onto the SBS cast film (b) and the melt-molded SBS film (i) are similar to those on the PSt film. There was almost no change in the number of platelets after the hydrocarboxylation (c and j), though the number of platelets with pseudopodiums decreased. These results mean that the anti-activity effect against platelets is accompanied by modification from a hydrophobic/hydrophilic micro-phase separated domain structure to a hydrophilic/hydrophilic structure,

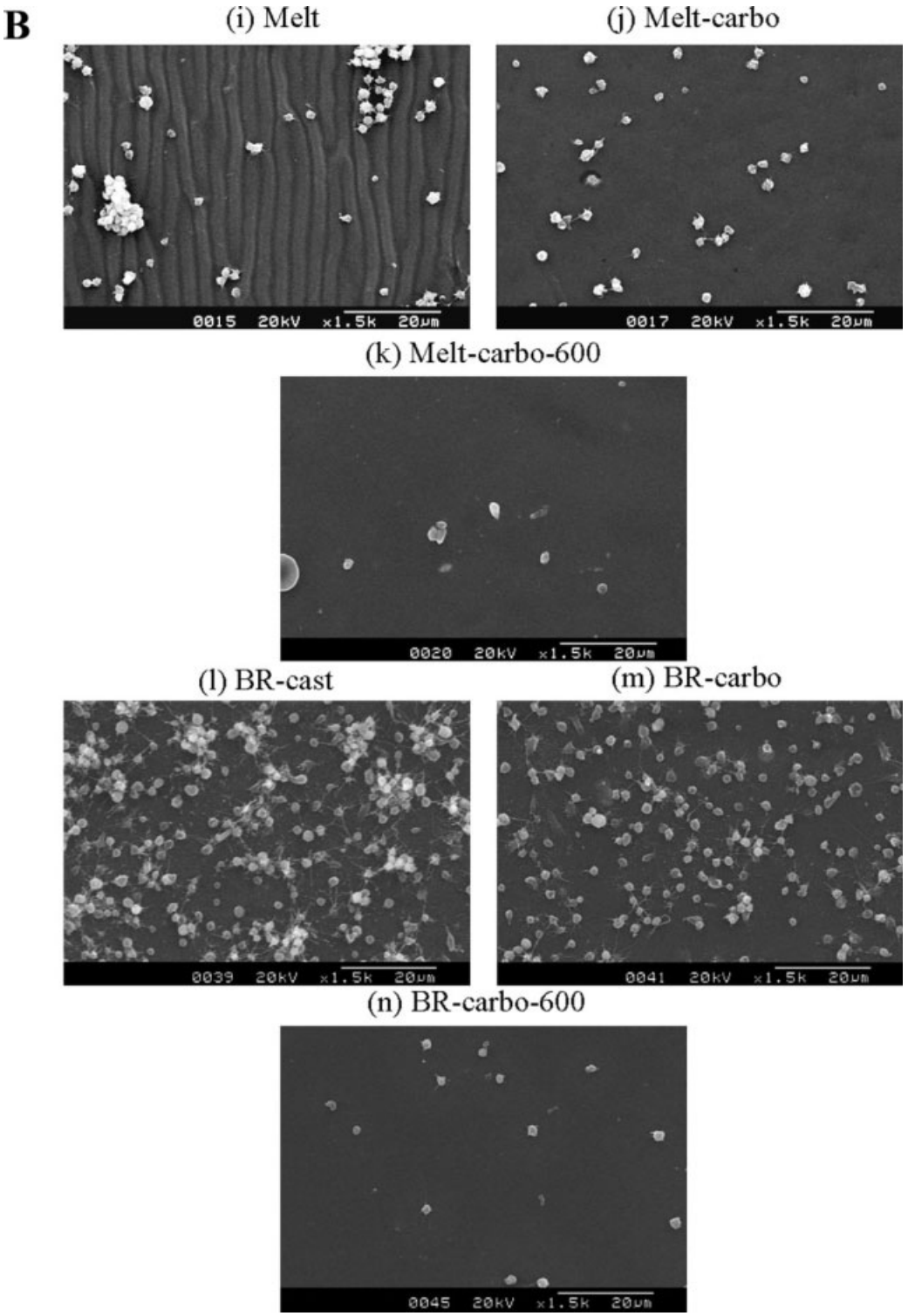

Figure 9 (*Continued from the previous page*)

although the effect is limited because of the short length of the hydrophilic atomic group. This effect is also observed for only the melt-molded SBS film because of formation of a carbonyl group by heating, as discussed above with the FTIR and XPS results.

The number of platelets drastically decreases in response to the grafting of PEG onto the SBS cast film (d–f), especially for $M = 600$ and the platelets did not have any pseudopodium. These results were also observed on the hydrobrominated SBS cast film (g) and the melt-molded SBS film (k) grafted PEG for $M = 600$. These phenomena are similar to the case of poly(MPC-*co*-MBA). This means that both of the SBS cast film and the melt-molded film grafted

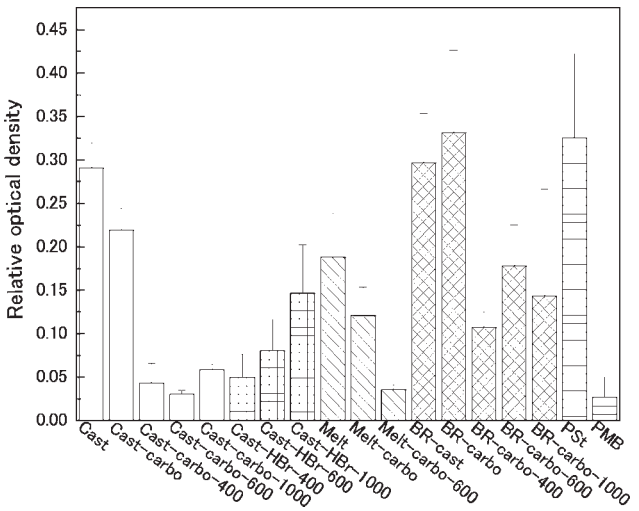

Figure 10 Results of LDH activity of platelets adhered on SBS and BR films.

PEG 600 effectively inhibit the adhesion and the activity of platelets as well as PMB.

The number of platelets adhered onto the BR cast film (l) was much greater than those on PSt, and this number did not decrease in response to the hydrocarboxylation (m). The number of platelets adhered onto the PEG-grafted BR cast film (n) surely decreased after hydrocarboxylation, though the pseudopodium was observed in many platelets. These results mean that the anti-adhesion and anti-activity effects of the uniform and smooth PEG-grafted BR cast film were less than those of the PEG-grafted SBS films having a hydrophilic/hydrophobic microphase-separated domain structure. Furthermore, the SBS surface with its unevenness on the order of several 10 nm, as shown in Figure 8, has a large number of reaction sites with PEG via hydrocarboxylation and the hydrobromination, which results in much denser grafting of PEG than occurs with the uniform and smooth BR. It therefore, can be conclude that the PEG-grafted SBS for $M = 600$ has a volume restriction effects, as reported by Yoshikawa et al.,[35,36] and that the hydrophilic/hydrophobic microphase-separated domain structure inhibits the activation of platelets.

Figure 10 shows relative optical density which means LDH activity of platelet. Higher LDH activity means stronger adhesion of platelets onto the film surface. Whole result corresponded to the results of SEM observation as follows. The LDH activity for PSt film was very high. In contrast, it was very low for PMB-coated glass plate. In the case of cast films, there was almost no change in the LDH activity of platelets after the hydrocarboxylation. The LDH activity of platelets drastically decreases in response to the grafting of PEG onto the SBS cast film, especially for $M = 600$. These results were also observed on the melt-molded SBS film grafted PEG for $M = 600$. These LDH activities are similar to the case of PMB coated glass. The LDH activity onto the BR cast film was similar to those on PSt, and this did not decrease in response to the hydrocarboxylation. The LDH activity onto the PEG-grafted BR cast film slightly decreased. It however, was evidently higher than the case of PMB coated glass. It is also clearly from the results of the LDH activity that both of SBS cast film and the melt-molded film grafted PEG for $M = 600$ most effectively inhibit adhesion of platelets in whole films as well as the results of SEM observation.

Coagulation of the blood component

Figure 11 shows SEM images for the coagulation of whole blood components on the surface of typical films. PSt film and PMB coated glass plate were used as control.

Almost no blood components exist on the PMB (h). In contrast, a great deal of fibrin is present, and some disk-shaped red blood corpuscles several micrometers in diameter along with a small number of white blood corpuscles of the same size adhere to the PSt film (a). However, no platelet is observed on the surface. Platelets are smaller than red and white blood corpuscles. As such, the platelets are probably hidden by the larger size of these corpuscles in relation to the depth of focus. It is definitely thought that fibrin and red blood corpuscles will cohere in an activated coagulation index system,[32] and that the surface of PSt (a) is a weak activator for the complement activity system due to its lack hydroxyl and carboxyl groups.

Blood components on the SBS cast film (b) are damaged by strong adhesion and are coagulated in the fibrin network sue to the weak inhibitory effects of the coagulation index system.

In case of the hydrocarboxyl SBS cast film (c), a small number of white blood corpuscles can be observed in addition to the damaged and coagulated red blood corpuscles in the fibrin network. These results mean that the surface weakly inhibits the effects of the coagulation index system because of the hydrophilic/hydrophobic microphase-separated domain structure and the complement activity system due to the existence of hydrophilic atomic groups.[32] These phenomena are also observed on only the melt-molded SBS film (i) because of the formation of the carbonyl group by heating, as discussed above with the FTIR and XPS results. Furthermore, the hydrocarboxyl SBS cast film (j) exhibits similar phenomena due to bonding with only the same carboxyl groups by hydrocarboxylation.

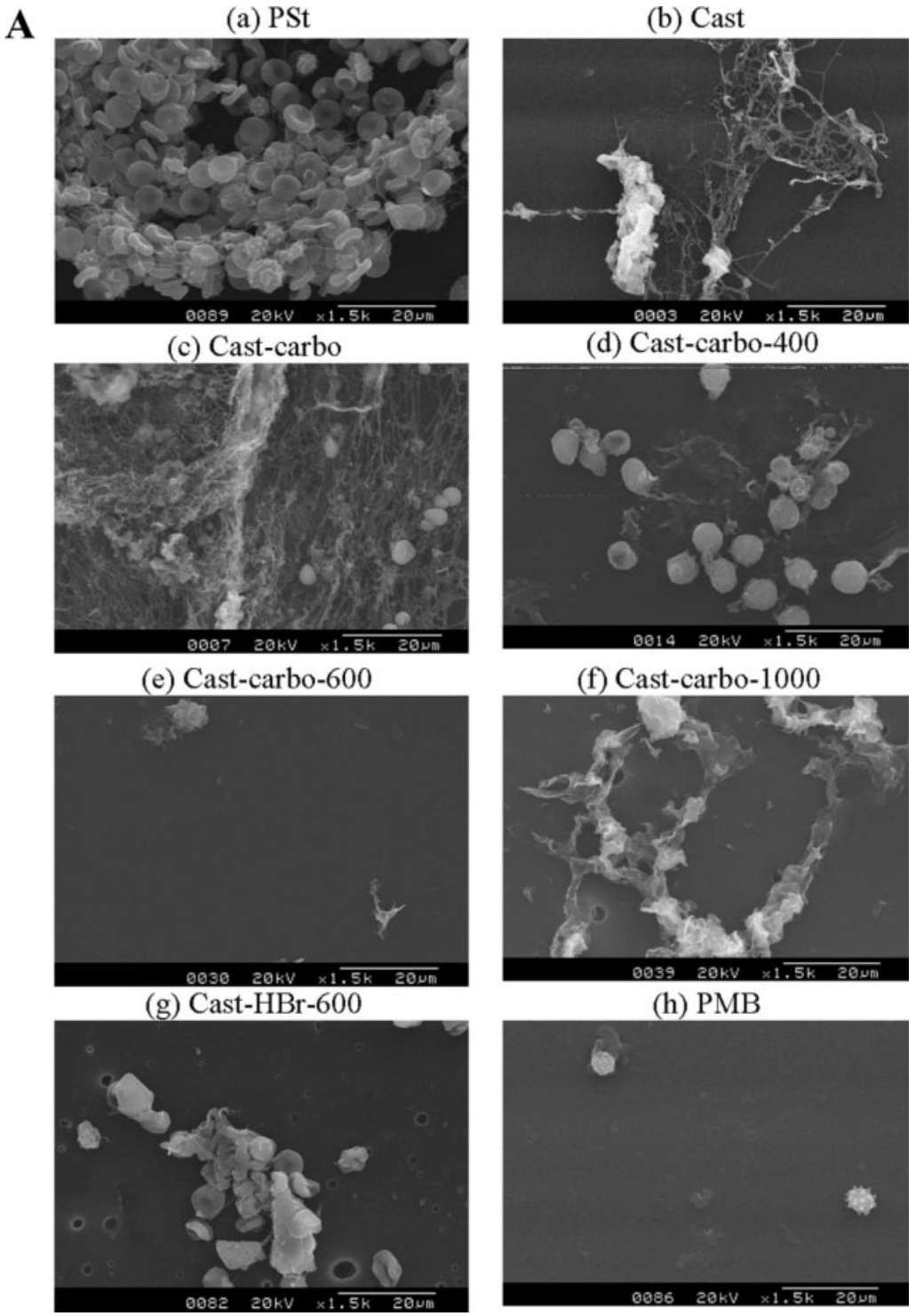

Figure 11 (A) SEM images (×1.5 k) of blood components coagulated on SBS and BR films. (B) SEM images (×1.5 k) of blood components coagulated on SBS and BR films.

It is clear that the amount of the coagulated blood component decreases on the PEG-grafted SBS cast films (d–f) and the PEG-grafted melt-molded SBS film (k) in comparison with hydrocarboxyl SBS cast film (c), and does not form a fibrin network, although a small number of white blood corpuscles can be observed. This decrease is remarkable for $M = 600$. Since this film was especially densely grafted with PEG on the PB domain, the volume restriction effect inhibited the coagulation index system. However, a small number of white blood corpuscles were observed. This probably caused the end group OH of PEG to have an activation effect on the complement system.

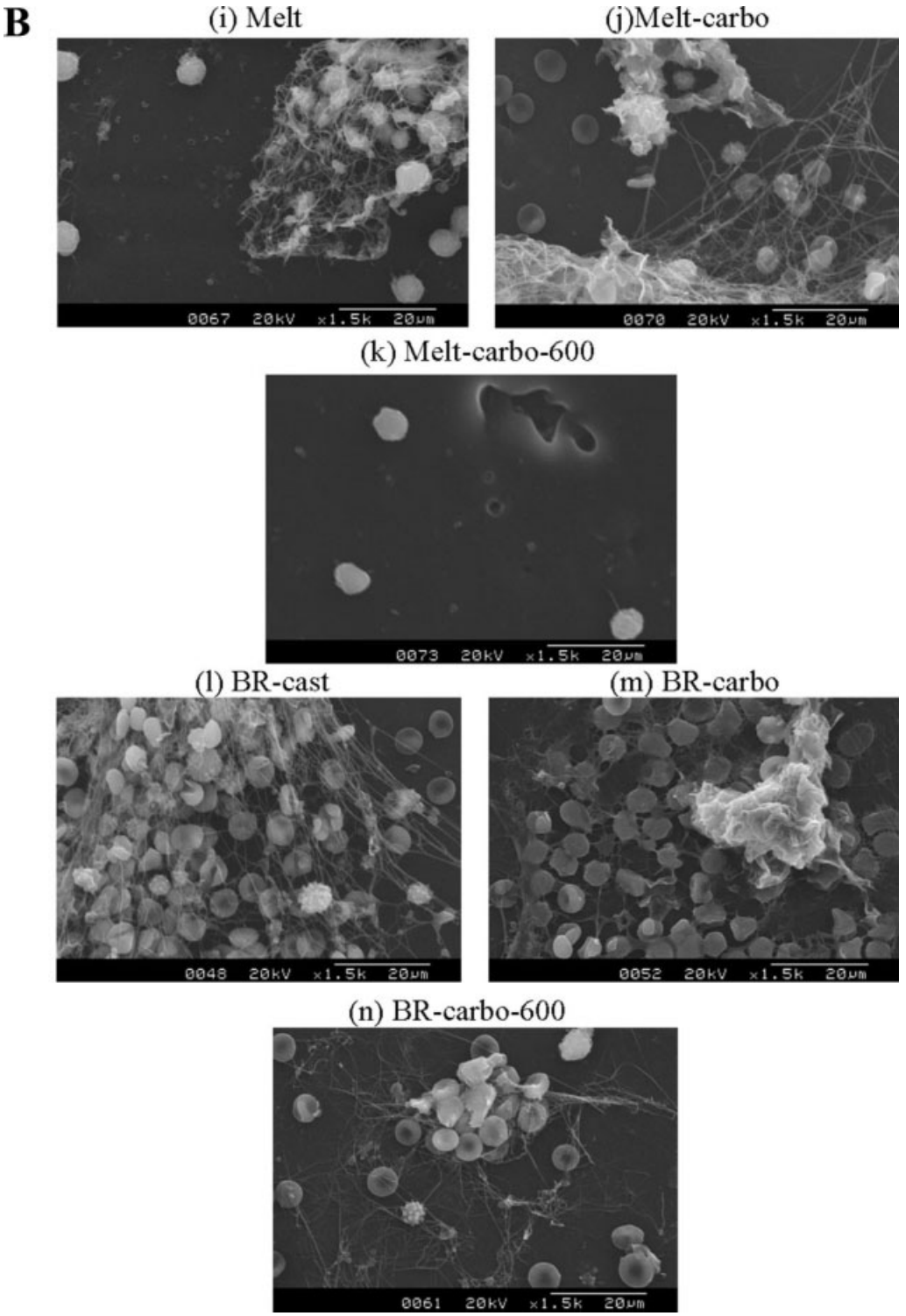

Figure 11 (*Continued from the previous page*)

PEG ($M = 600$)-grafted SBS cast film via the hydrobromination (g) also showed a slightly poor inhibition effect in relation to the coagulation of white blood corpuscles and the formation of a fibrin network in comparison with the PEG-grafted SBS cast film via the hydrocarboxylation (e), although this film was found to have excellent platelet anti-adhesion properties, as shown in Figure 9, because many OH groups that produce complement activity are formed by the hydrobromination, as described in the above FTIR results.

In the case of the uniformly smooth BR cast film, the amount of the coagulation of red and white blood corpuscles and the fibrin network showed following tendency;

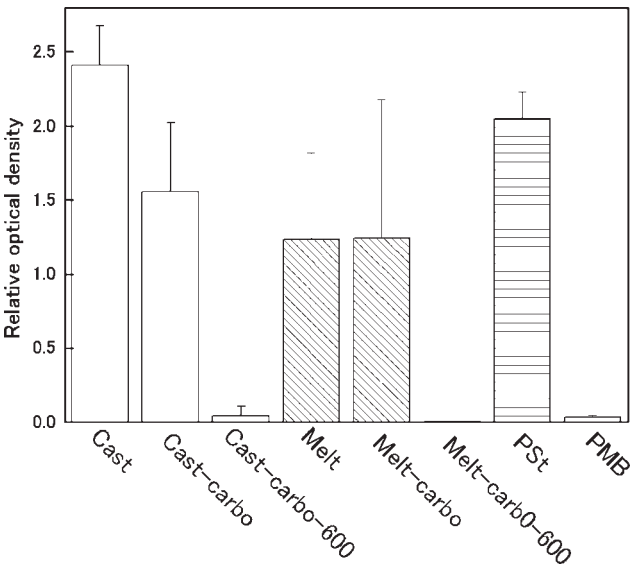

Figure 12 Quantitative amount of coagulated components of whole blood by using a Bio-Rad's compatible protein assay method.

BR cast film (l) > hydrocarboxylated BR cast film (m) > PEG-grafted BR cast film (n)

However, the PEG-grafted BR cast film, which showed the least amount of corpuscles and network in these films, poorly inhibited the effects of the coagulation of corpuscles and the formation of a fibrin network. Since the amount of grafted PEG is very small, as described above, this result supports the hypothesis that the dense PEG grafting is important to the volume restriction effect.[37]

Figure 12 shows quantitative amount of coagulated components by using a Bio-Rad's detergent compatible protein assay. It is clear that the amount of the coagulated blood component decreases on the PEG-grafted SBS cast films and the PEG-grafted melt-molded SBS film in comparison with hydrocarboxyl SBS cast film. This decrease is remarkable for $M = 600$ as well as results of SEM images.

In the case of the uniformly smooth BR cast film, the amount of the coagulated components showed following tendency;

BR cast film > hydrocarboxylated BR cast film > PEG-grafted BR cast film >PEG-grafted SBS cast film,

where, the PEG-grafted SBS cast film is nearly equal to the melt-molded SBS film. This tendency is similar to SEM images (Fig. 11).

CONCLUSIONS

In the case of SBS cast film from toluene solution, PEG was densely grafted because of the development of an unevenness on the order of several 10 nm on the surface, which had a huge surface area in comparison with BR with its uniformly smooth surface. Grafted PEG (molecular weight = 600) was found to clearly inhibit adhesion and activation of platelets and coagulation of the whole blood component, which is indicative of the best anti-thrombogenic material having a hydrophilic/hydrophobic microphase-separated domain structure. These properties correspond to a surface coated by a copolymer of 2-methacryloyl-oxyethyl phosphorylcholine and BMA, which is well known to be the best excellent anti-thrombogenic material in the world. Melt-molded SBS film, which also has an uneveness on the order of several 10 nm, showed similar excellent anti-thrombogenicity.

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