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Grafting of ethylene glycol–butadiene block copolymers onto dimethyldichlorosilane-coated glass by γ-irradiation

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Amphipathic ethylene glycol–butadiene block copolymers (PEG–PB) with different chain lengths of poly(ethylene glycol) (PEG) were synthesized by reacting poly(ethylene glycol methyl ether) (m-PEG, mol. wt = 350, 550, 750, 2000 and 5000) with telechelic polybutadiene (PB). The PEG–PB copolymers formed were covalently grafted to dimethyldichlorosilane-coated glass (DDS–glass) by γ-irradiation. The PEG-grafted surface was characterized by measuring advancing and receding contact angles, fibrinogen adsorption, the number of adherent platelets and the area of spread platelets. The grafting efficiency was measured indirectly from the ability of the surface to prevent platelet adhesion. The total dose of γ-irradiation necessary for grafting of PEG–PB onto DDS–glass in aqueous solutions was less than 0.24 Mrad at atmospheric pressure and ambient temperature. For successful grafting, the surface-adsorbed copolymers should be γ-irradiated in the presence of water. γ-Irradiation in the dried state did not result in copolymer grafting. The adsorption of copolymers for 30 min before exposure to γ-irradiation was enough for effective grafting. The grafting was equally effective whether or not DDS–glass was exposed to the air–copolymer solution interface when the DDS–glass was introduced into the copolymer solution. The copolymers were able to prevent platelet adhesion only when they were adsorbed onto DDS–glass at certain bulk concentrations. Too low or too high copolymer concentrations in the adsorption solution resulted in a surface where platelets could adhere and activate. The range of copolymer concentration which prevented platelet adhesion was larger as the PEG chain length of the grafted copolymers became longer. Our data indicate that platelet-resistant surfaces can be made by grafting PEG–PB onto chemically inert surfaces by a simple γ-irradiation process.

Keywords: Surface modification, poly(ethylene glycol), ethylene glycol–butadiene copolymer, gamma radiation

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One of the main problems in the use of biomaterials has been surface-induced thrombus formation, which is initiated by adsorption of certain plasma proteins and adhesion of platelets[1,2]. Appropriate surface modifications of existing biomaterials possessing desired physical properties are beneficial in improving biocompatibility without altering the bulk properties of the biomaterials.

Surface modification methods can be grouped into three general categories: physical adsorption, graft coupling and graft polymerization. In physical adsorption methods, amphipathic block copolymers have been commonly used for the prevention of protein adsorption and cell adhesion on hydrophobic surfaces[3,4]. Recently, a rubber-like material of ethylene glycol–butadiene diblock copolymer was coated on a hydrophobic surface to provide hydrophilicity resulting from the presence of poly(ethylene glycol) (PEG) tails[5]. The physical adsorption process is certainly the simplest method for surface modification. The adsorbed polymers, however, may be simply washed away by fluid or displaced by proteins which have higher affinity to the surface.

The conventional graft coupling process requires chemically reactive groups on the surface as well as on the polymer chains. For this reason, a series of prefunctionalization steps are necessary for covalent grafting[6–8]. The prefunctionalization of surfaces is usually done by using UV light, plasma, glow discharge, etc. Therefore, its application is limited only to the modification of the surfaces which can be exposed to these energy sources. Because most medical devices are made of chemically inert materials, a simple method which can directly modify the

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inert surface is very much desired. To accomplish this objective, it is necessary that the water-soluble polymer chains have the ability to react with inert surfaces. Recently, biocompatible polymers have been directly grafted onto chemically inert surfaces through simple photolysis of azides or aromatic ketones attached to the polymers[9–12]. Their dependency on UV light as an energy source, however, still limits their application, since UV light does not penetrate most materials. These methods are impractical for modifying the surfaces of fully assembled devices with complex shapes. Recently, we have also shown that azides can be activated by heat (~100°C) for grafting albumin to polypropylene[13]. This technique may have broader applications than the UV-induced photolysis method, as heat can conduct into the inner portion of the devices.

Of the many polymerization methods, free-radical polymerization has been used exclusively for surface modification[14–18]. The main disadvantage of this method is that a considerable amount of homopolymers may be formed in solution during free-radical polymerization, which eventually decreases the grafting efficiency. The polymerization can also proceed inside the material due to diffusion of monomers, so that the desirable mechanical properties of the materials may be altered. Before polymerization, the monomer solution should be completely degassed to remove oxygen. At the end of polymerization, removal of the unreacted monomers is also essential, since most monomers are highly toxic in nature. The graft copolymerization method is not particularly useful in grafting onto fully assembled devices with complex shapes.

For effective grafting of biocompatible polymers onto hydrophobic, chemically inert surfaces of fully assembled devices, the following criteria need to be met. First, multiple reactive groups should be introduced into the polymers to increase the probability of covalent bonding and the number of covalent bonds between a polymer chain and the surface. Second, the polymers should have high affinity to the surface. There must be a driving force which guarantees intimate contact between the reactive groups of the polymer and the surface. Third, the biocompatible part of the grafted polymers should extend into the aqueous solution to exert steric repulsion to proteins and cells. Fourth, the energy sources used for activating the reactive groups should be effective whether the surface is exposed or not. Fifth, the activated groups should have the ability to react even with chemically inert surfaces. Above all, the grafting procedure should be simple and cost-effective.

In this report, we introduce a simple method which fully satisfies all the above requirements to modify surfaces of fully assembled medical devices. The approach is to obtain a PEG surface by grafting an amphipathic ethylene glycol–butadiene copolymer (PEG–PB). Dimethyldichlorosilane-coated glass (DDS–glass) was used as a model surface for the grafting of PEG–PB, since the surface does not have any functional groups and is highly thrombogenic. The PEG-grafted DDS–glass was characterized by contact angle measurement, fibrinogen adsorption and quantitative determination of platelet adhesion and activation.

EXPERIMENTAL

Synthesis of PEG–PB

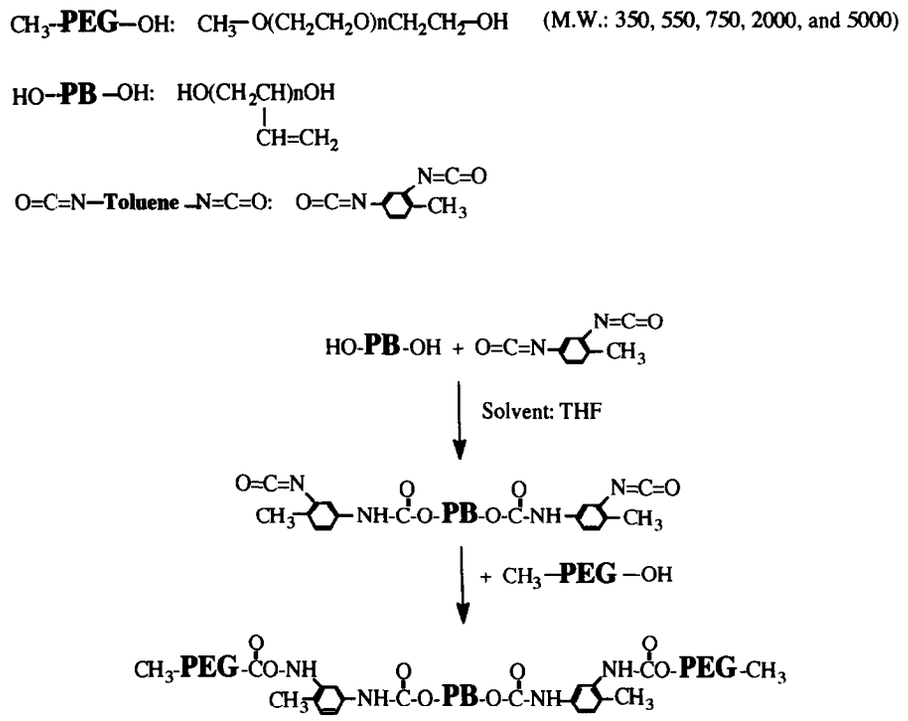
Tetrahydrofuran (THF; Aldrich Chemical Co., Milwaukee, WI) was purified by refluxing with lithium aluminium hydride ($LiAlH_4$; Aldrich) overnight and distilling from $LiAlH_4$ in dry nitrogen to remove water, peroxides, inhibitor and other impurities. It was used within a couple of days after the purification. Commercially available poly(ethylene glycol methyl ether) (m-PEG, mol. wt = 350, 550, 750, 2000 and 5000; Aldrich) was dried by azeotropic distillation in benzene overnight to remove adsorbed water, and the benzene was removed under reduced pressure. The dry m-PEG was used immediately. Hydroxy-terminated polybutadiene (PB; mol. wt = 700) was kindly provided by Nippon Soda Co., Ltd. and used as obtained. The content of 1,2-vinyl groups in PB was assayed by the manufacturer using the iodine monochloride (Wijs) method. The value was greater than 92%. Tolylene-2,4-diisocyanate (TDI; Aldrich) and dibutyltin dilaurate (Aldrich) were used as purchased.

PEG–PB was prepared by the following procedure. A 15% (w/v) PB solution in THF was prepared in a three-necked, round-bottomed flask under dry nitrogen. TDI and dibutyltin dilaurate as a catalyst were added to the PB solution. The molar ratios of TDI to PB and dibutyltin dilaurate to PB were 2.1 and 0.083, respectively. The reaction mixture was kept at 25°C while stirring. Two hours later, dry m-PEG (PEG: PB = 2:1) in THF (15% w/v) was added dropwise, and the reaction mixture was stirred continuously for 48 h under dry nitrogen. The product was twice precipitated with hexane from THF, and dried *in vacuo*. The chemical reaction involved in the above procedure is described in *Scheme 1*. The resulting PEG–PB copolymers from m-PEG350, m-PEG550, m-PEG750, m-PEG2000 and m-PEG5000 are referred to as 'COP350', 'COP550', 'COP750', 'COP2000' and 'COP5000', respectively.

The copolymers were characterized by using gel permeation chromatography, ^1H NMR and elemental analysis. Gel permeation chromatography measurements were carried out using a Toyo Soda model 802A high-speed liquid chromatograph equipped with a TSK G2000 Hs column and THF as eluent. Samples for NMR measurement were prepared in C_6D_6, and the ^1H NMR spectra were obtained using a Bruker WM270 NMR spectrometer (270 MHz). The elemental analysis data (C, H, N) were obtained from the microanalysis laboratory at Purdue University. The oxygen content was calculated from the data obtained.

Preparation of DDS–glass

Glass coverslips (9 × 35 mm, no. 1 thickness; Bellco, Vineland, NJ) were cleaned by soaking in chromic acid overnight and washed extensively in running distilled water. They were further rinsed with deionized distilled water for 2 h at room temperature and then dried at 80°C overnight. Glass tubing (i.d.



Scheme 1

2.50 mm; Kimble, Vineland, NJ), instead of glass coverslips, was used for the determination of the surface fibrinogen concentration using radiolabelled fibrinogen. The tubing was also cleaned as described above.

DDS–glass coverslips or tubing were prepared by immersing the clean coverslips or glass tubing in a solution of 5% DDS (Aldrich) in chloroform for 30 min. The DDS–glass was washed in chloroform and methanol in sequence twice and finally with water for 2 h before drying at 80°C.

Grafting procedures of COP2000 onto DDS–glass

Three different variables were examined to optimize the grafting efficiency of PEG–PB onto DDS–glass using COP2000. The variables included γ-irradiation dose, concentration of COP2000 solution and adsorption time. ^{60}Co with a dose rate of 0.0804 Mrad h^{-1} was used as a source of γ-irradiation. For convenience, the γ-irradiation time was used instead of the total dose. The aqueous COP2000 solution was freshly prepared for each use to avoid oxidation. The four different grafting procedures of COP2000 onto DDS–glass were designed and tested as follows.

Irradiation of COP2000-adsorbed DDS–glass immersed in COP2000 solutions

To determine whether an air–solution interface affects the grafting efficiency or not, the adsorption procedure was examined by carefully designed immersing methods of DDS–glass into polymer solutions.

DDS–glass without exposure to an air–solution interface. A DDS–glass coverslip was immersed in 4 ml of water in a diSPo culture tube (13 × 100 mm; Baxter, McGaw Park, IL). To this were added 4 ml of an aqueous COP2000 solution which had twice the concentration of the desired final concentration. The solution was gently mixed using a pipette. The final concentration was varied from 0.005 to 50 mg ml^{-1}, and the adsorption time was varied from 10 to 70 min. The solution containing the coverslip was then subjected to γ-irradiation at ambient temperature for 3 h. After the irradiation, the treated coverslip was taken out under running distilled water so that it did not go through the air–solution interface. Ungrafted COP2000 was removed by immersing the treated coverslip in an aqueous solution of 3% sodium dodecylsulphate (SDS; Bio-Rad Lab., Richmond, CA) at room temperature overnight, and the coverslip was washed with water in a sonicator for 5 min twice.

DDS–glass with exposure to an air–solution interface. DDS–glass coverslips were dropped into 8 ml of aqueous COP2000 solutions of different concentrations contained in diSPo culture tubes. COP2000 was allowed to adsorb onto the coverslips for various time periods. The concentration of the copolymer ranged from 0.005 to 50 mg ml^{-1}, and the adsorption time was varied up to 70 min. The solutions containing the coverslips were then subjected to γ-irradiation at ambient temperature for various time periods up to 12 h. After the irradiation, the coverslips were washed using the same procedure as described above.

In order to distinguish covalently bound COP2000 from physically adsorbed COP2000, DDS–glass coverslips were also immersed in COP2000 solutions of different concentrations for up to 12 h without γ-irradiation and washed using the same procedure. These surfaces were used as control samples.

Irradiation of COP2000-adsorbed DDS–glass immersed in water

COP2000 was adsorbed onto DDS–glass coverslips using the same procedure as described above. After the adsorption, the coverslips were dipped in distilled water and transferred to culture tubes containing 8 ml of water. The water containing the coverslips was then subjected to γ-irradiation for 3 h at room temperature. The treated coverslips were washed by the same procedure as described above.

Irradiation of COP2000-adsorbed DDS–glass in dry conditions

COP2000 was adsorbed onto DDS–glass coverslips as described above. After the adsorption, the coverslips were air-dried for 2 d and γ-irradiated for various time periods up to 12 h. The treated surfaces were also washed with SDS as described above.

Grafting procedures of PEG–PB with different PEG chain lengths

PEG–PB copolymers with different PEG chain lengths were adsorbed onto DDS–glass coverslips using the same procedure as described above. The PEG–PB concentration ranged from 0.005 to 50 mg ml^{-1} and the adsorption time was 1 h. The solution containing the coverslips was subjected to γ-irradiation at ambient temperature for 3 h. After the irradiation, the coverslips were washed using the same procedure as described above.

Platelet adhesion and activation

The efficiency of grafted copolymer in the prevention of platelet adhesion and activation was quantitated by measuring the number and spread area of adherent platelets[19]. Blood was obtained in heparinized containers (Vacutainers®, Becton-Dickinson, Rutherford, NJ) from healthy adult volunteers after informed consent. The heparin level in the heparinized container is unknown. All volunteers were kept free from aspirin or other drugs that might interfere with platelet functions. Diluted platelet-rich plasma (PRP) was obtained by centrifuging heparinized blood at 100g for 10 min at room temperature. The number of platelets in the PRP was diluted to one-quarter of its original concentration by mixing with platelet-poor plasma obtained by further centrifugation of PRP for 20 min. A perfusion chamber was made by placing parafilm spacers (0.013 cm thickness; Dow Corning, Midland, MI) between a sample coverslip and a glass slide (2.54 cm × 7.62 cm). The chamber was injected with phosphate-buffered saline (PBS; pH 7.4) for 10 min before 100 μl of dilute PRP was introduced to replace the PBS. Platelets were allowed to settle on the surface of the sample by gravity at room temperature for 1 h. After the unadherent platelets were removed by washing with PBS, the adherent platelets were fixed with 2% glutaraldehyde in PBS for 15 min. The glutaraldehyde solution was replaced with PBS and the fixed platelets were then stained with a 0.1% solution of Coomassie Brilliant Blue for 30 min. The stained platelets were observed with an inverted video microscope (Nikon Diaphot, Garden City, NY) and the microscope images were projected onto a video camera (Newvicon, Model 65, Dage-MTI, Michigan City, IN). The number of adherent platelets was counted in 24 separate video images by using a ×40 objective lens for each time point. The microscope images were directed to a computer for image analysis. The spread area of platelets adherent on the surfaces was measured using software obtained from Computer Imaging Applications (Madison, WI). The area of spread platelets was measured in eight separate microscope fields by using a ×100 objective lens at each time point.

Protein adsorption

Human fibrinogen (Sigma, Type I, St. Louis, MO) was purified by the Laki method[20]. A 2 mg ml^{-1} solution of the purified fibrinogen in PBS was prepared and subdivided into 1 ml portions in small plastic vials. These vials were stored at −70°C. Bovine albumin (Sigma) was used as received. The protein concentration was measured from the absorbance at 280 nm using absorptivities of 1.506×10^3 and 5.8×10^2 $cm^2 g^{-1}$ for fibrinogen and albumin, respectively[21,22]. In order to quantitate surface fibrinogen concentration, fibrinogen was labelled with ^{125}I (Amersham, Arlington Heights, IL) using Enzymobead reagent (Bio-Rad, Rockville Center, NY). The specific activity of fibrinogen was 2.7×10^7 cpm mg^{-1}.

The aqueous COP2000 or COP5000 solution of 0.5 mg ml^{-1} was allowed to adsorb onto DSS–glass tubing for 1 h, and the tubing containing the solution was then subjected to γ-irradiation at ambient temperature for 3 h. After the irradiation, the treated tubing was washed using the same procedure as described earlier.

A protein solution which contained 2 mg ml^{-1} albumin and 0.1 mg ml^{-1} fibrinogen was prepared. In order to avoid surface–air contact, the treated tubings were filled with PBS before the addition of the protein solution. The proteins were allowed to adsorb onto the surfaces of the tubings for 1 h at room temperature, and then the tubings were rinsed with PBS. The surface fibrinogen concentration was determined by measuring the radioactivity of ^{125}I-labelled fibrinogen using a gamma counter (Gamma 5500B, Beckman, Arlington Heights, IL). Eight samples were used for the calculation of the surface fibrinogen concentration.

Dynamic contact angle measurements

Advancing and receding contact angles of modified DDS–glass coverslips were determined by the Wilhelmy plate method[23]. These contact angle data were evaluated with an Autotensiomat® surface tension analyser (Fisher Model 215) at a controlled speed of approximately 2.54 mm min^{-1} at constant temperature (20°C) and humidity (30% relative humidity).

RESULTS

Characterization of PEG–PB

In *Figure 1*, the gel permeation chromatogram of m-PEG2000 is overlaid with that of COP2000. m-PEG2000 was eluted at 1042 counts, while COP2000 appeared at a lower count value (950) due to an increase in molecular weight. The chromatogram of COP2000 indicates that residual homopolymers of PEG and PB are absent in the synthesized copolymer.

In the ^{1}H NMR spectra of the PEG–PB copolymers, the signals from the protons of the vinyl groups of PB and the benzene ring of TDI were very small compared to the signal from the protons of PEG. This was more evident when the chain length of PEG became longer. The ^{1}H NMR spectrum of COP750, which has a small PEG chain, is shown in *Figure 2* as an example. The strong signal from the protons of the PEG backbone was seen at δ3.51. The two signals between δ4.8 and 5.8 were attributed to the protons in the vinyl groups of the PEG-attached PB. The NMR signal from C_6D_6 impurity appeared at the usual position. The three small signals between δ6.8 and 8.2 were due to the protons of the benzene rings of TDI attached to copolymers. It is apparent that PEG–PB copolymers were successfully prepared using the TDI coupling agent.

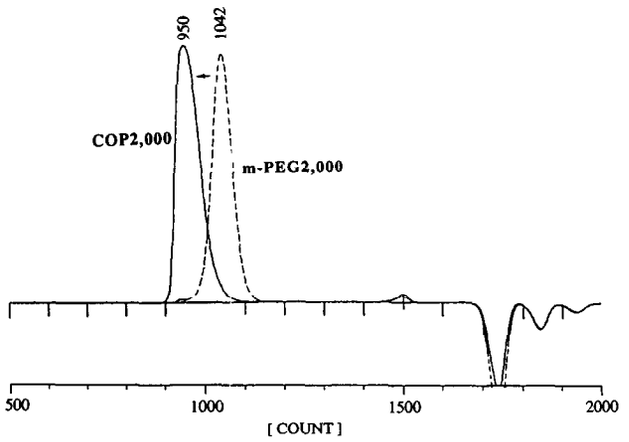


Figure 1 Gel permeation chromatograms of m-PEG2000 (– – –) and COP2000 (——).

The analytical results from elemental analysis and ^{1}H NMR are compared with the values calculated theoretically on the basis of ABA-type copolymers (PEG–PB–PEG) in *Table 1*. Since the average molecular weights of the reactants provided by the suppliers were used for the calculation, a slight discrepancy between the values obtained theoretically and experimentally was expected. Nevertheless, as shown in *Table 1*, they showed good agreement. Thus, it can be concluded that most of the synthesized copolymers are triblock copolymers of ethylene glycol and butadiene.

Effect of irradiation time on the prevention of platelet adhesion

To study the effect of irradiation time on the grafting efficiency, three sets of samples were prepared using COP2000 as follows. DDS–glass coverslips were immersed in COP2000 solutions passing through the air–solution interface. After the adsorption, the coverslips were γ-irradiated for various time periods while immersed in the bulk solution (the bulk set). For the preparation of the second set, the COP2000-adsorbed DDS–glass coverslips were dried before being subjected to γ-irradiation (the dry set). As a

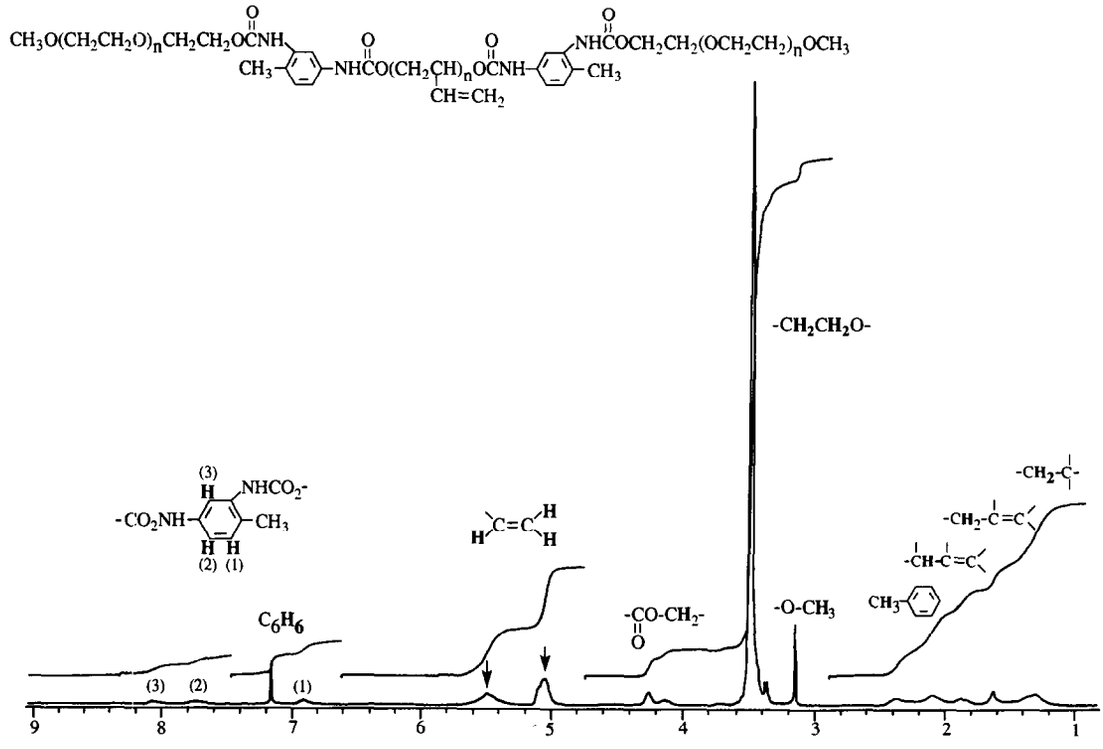


Figure 2 ^{1}H NMR spectrum of COP750 in C_6D_6.

Table 1 Quantitative analysis of the compositions of poly(ethylene glycol)–polybutadiene block copolymers by elemental analysis and ^{1}H NMR spectroscopy

Sample	Elemental analysis				NMR
	C (%)	H (%)	N (%)	O (%)	PEG content (%)
COP350	66.07	8.34	3.46	22.13	41.92
	(67.40)*	(8.87)	(3.20)	(20.53)	(39.24)
COP550	61.56	9.00	2.81	26.63	51.98
	(65.00)	(8.39)	(2.61)	(23.46)	(50.33)
COP750	61.99	9.10	2.36	26.55	58.40
	(63.35)	(8.97)	(2.20)	(25.48)	(58.01)
COP2000	57.53	9.31	1.26	31.90	77.32
	(58.98)	(9.07)	(1.11)	(30.85)	(68.63)
COP5000	57.16	9.45	0.94	32.45	92.25
	(56.55)	(9.12)	(0.51)	(33.82)	(90.19)

*Values in parentheses are theoretical values calculated based on the ABA-type copolymers (PEG–PB–PEG).

control set, COP2000 was adsorbed on DDS–glass coverslips for various time periods up to 12 h without γ-irradiation. *Figure 3* shows the relationship between platelet adhesion and γ-irradiation time for these three sets of samples. The number of platelets adherent on the bulk set decreased sharply from 49/1000 to 6/1000 μm^2 even after only 1 h of irradiation, and platelets could not adhere at all to the surfaces when the irradiation time was 3 h or longer. On the contrary, the numbers of platelets adherent on both the control set and the dry set were not significantly different from that of the untreated DDS–glass (zero irradiation time). The *P*-values are 0.82 for the former and 0.32 for the latter. The data indicate that the total dose required for grafting of PEG–PB in an aqueous solution was less than 0.24 Mrad (0.08 Mrad $h^{-1} \times 3$ h).

Effect of COP2000 concentration on the prevention of platelet adhesion and activation

In an attempt to establish a grafting process with as little limitation as possible in terms of the grafting condition, four different grafting methods were designed, as described in the Experimental section. *Figure 4* shows the dependence of platelet adhesion on

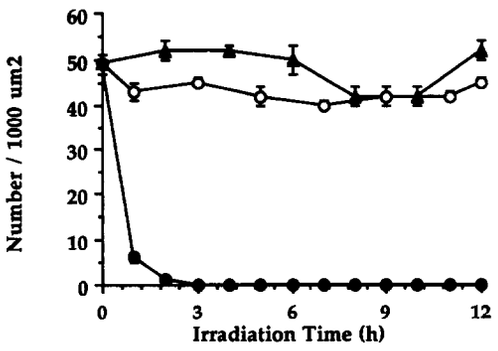


Figure 3 Changes in the number of platelets adherent on the control surface (▲), and on the surface irradiated either in dry conditions (○) or in a bulk solution (●) as a function of the γ-irradiation time. COP2000 was adsorbed from its 0.5 mg ml^{-1} solution for 1 h. Platelets in PRP were allowed to adhere for 1 h at room temperature. Mean ± s.e.m.

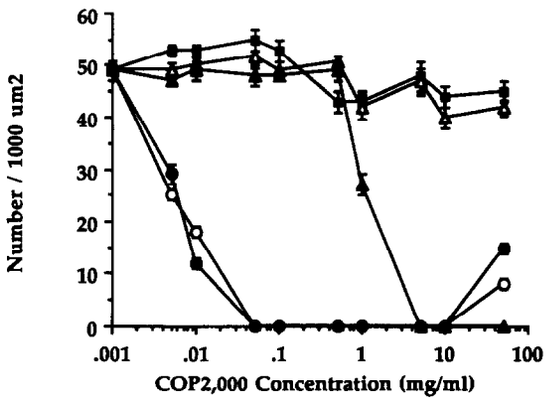


Figure 4 Changes in the number of platelets adherent on the control surface (■), and on surfaces irradiated in dry conditions (△), in water (▲), and in the bulk solution with (○) and without (●) passing through the air–solution interface as a function of the COP2000 concentration used for adsorption. COP2000 was adsorbed for 1 h. γ-Irradiation time was 3 h. Platelets in PRP were allowed to adhere for 1 h at room temperature. Mean ± s.e.m.

the bulk concentration of COP2000 used for adsorption. As a control, DDS–glass coverslips were immersed in COP2000 solutions of different concentrations for 4 h without being exposed to γ-rays. When the bulk concentration was increased up to 0.05 mg ml^{-1}, the number of platelets adherent on the DDS–glass irradiated in the bulk solution decreased sharply from 49/1000 μm^2 to zero, whether the DDS–glass passed through the air–solution interface or not. Platelets could not adhere to these surfaces when the concentration of the copolymer for adsorption was between 0.05 and 10 mg ml^{-1}. When the concentration of the copolymer was increased to 50 mg ml^{-1}, the numbers of platelets adherent on the surfaces with and without exposure to the air–solution interface were 8/1000 μm^2 and 15/1000 μm^2, respectively.

The numbers of platelets adherent on the DDS–glass irradiated in water and on the untreated DDS–glass were not significantly different ($P = 0.6354$) when the concentration of COP2000 was lower than 1 mg ml^{-1}. When the concentration was higher than 5 mg ml^{-1}, platelets could not adhere to these surfaces at all. Regardless of the concentration, however, the numbers of platelets adherent on both the control surfaces and the surfaces irradiated under dry conditions were not significantly different from that on the untreated DDS–glass ($P = 0.56$ and 0.22, respectively). The results indicate that the grafting of PEG–PB should be performed in the presence of water, either in the bulk polymer solution or in pure water. No significant difference ($P = 0.46$) was observed between the surfaces with and without exposure to the air–solution interface during the adsorption process. In both cases, however, the grafting efficiency was not high at high copolymer concentrations. The data demonstrate that amphipathic PEG–PB can be effectively grafted onto DDS–glass at polymer concentrations between 0.05 and 10 mg ml^{-1}. The concentration has to be higher than 5 mg ml^{-1} in the adsorption solutions if the samples are irradiated in water.

Effect of adsorption time on the prevention of platelet adhesion

Figure 5 shows the dependence of platelet adhesion on the adsorption time of COP2000 on DDS–glass. Only two platelets could adhere to 1000 μm^2 of the surfaces even with only 5 min of adsorption time for all three grafting methods. After 30 min of adsorption, platelets could not adhere to these surfaces at all. The results indicate that PEG–PB copolymer can easily adsorb onto DDS–glass, probably due to the hydrophobic interaction between the PB segment of the copolymer and the surface.

Effect of PEG–PB with different PEG chain lengths on the prevention of platelet adhesion and activation

Although the same bulk concentration was used for all PEG–PB copolymers, the polymer concentrations in the air–solution interface may differ depending on the PEG chain length of the copolymer. Therefore, the irradiation process was carried out in the bulk solution without passing through the air–solution interface for further studies. The number of platelets adherent on the copolymer-grafted DDS–glass is plotted against the molar concentrations of PEG–PB with different PEG chain lengths in *Figure 6A*. Each curve in this figure can be divided into roughly three portions: negative-slope portion, zero-slope portion and positive-slope portion. In the negative-slope portion, the number of platelets adherent on the copolymer-grafted DDS–glass dropped very sharply as the concentration of PEG–PB in the adsorption solution increased. The zero-slope portion, where platelet adhesion was negligible, was present only within a certain concentration range. If the concentration of the adsorption solution exceeded a certain level, the number of adherent platelets started rising again. In the negative- and positive-slope portions, the number of adherent platelets decreased with increasing PEG chain length

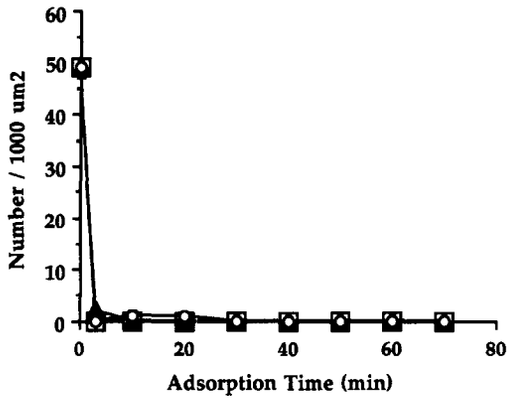


Figure 5 Changes in the number of platelets adherent on the surfaces irradiated in water (□), and in a bulk solution with (▲) and without (○) passing through the air–solution interface as a function of adsorption time. The COP2000 concentration was 0.5 mg ml^{-1} in the case of irradiation in the bulk solution and 10 mg ml^{-1} for irradiation in water. γ-Irradiation time was 3 h. Platelets in PRP were allowed to adhere for 1 h at room temperature. Mean ± s.e.m.

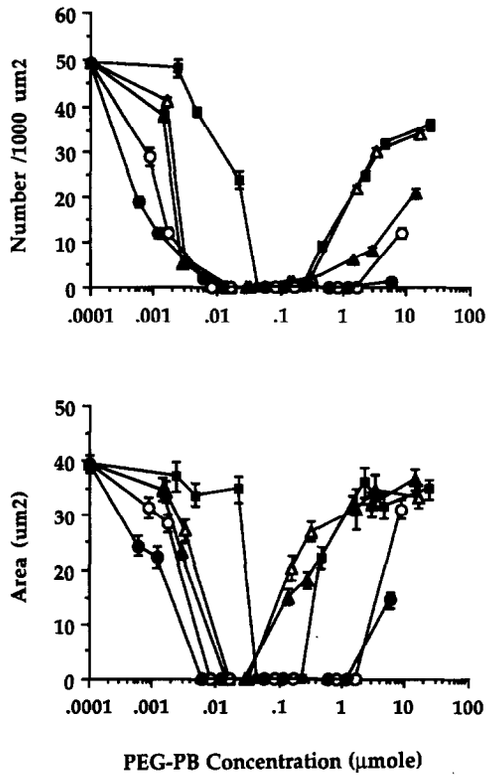


Figure 6 Changes in, **a**, the number, **b**, and the spread area of platelets adherent to the surfaces irradiated in the bulk solutions of COP350 (■), COP550 (△), COP750(▲), COP2000 (○) and COP5000 (●) as a function of the PEG–PB molar concentration used for adsorption. The PEG–PB was adsorbed for 1 h without exposure to the air–solution interface. γ-Irradiation time was 3 h. Platelets in PRP were allowed to adhere for 1 h at room temperature. Mean ± s.e.m.

of the copolymer, if the same concentration of the adsorption solution was used for all the copolymers. It was also observed that as the chain length of the PEG segment of the copolymer increased, the concentration range which yielded no platelet adhesion became significantly broader.

Figure 6B shows the spread area of the platelets adherent on the surfaces as a function of the molar concentrations of the copolymers with different PEG chain lengths. It also shows a similar trend, that the activation of the adherent platelets decreased in the low concentration range and increased in the high concentration range as the concentration of the adsorption solutions increased. When the chain length of PEG increased, the activation of adherent platelets decreased at the same concentration. Thus, it appears that, when the molecular weight of PEG is between 350 and 5000, the longer PEG chains are more effective in the prevention of platelet adhesion and activation than the shorter ones. One thing that should be noticed here, however, is that PEG chains are effective in the prevention of platelet adhesion and activation, regardless of the molecular weight, if they are adsorbed under the optimum conditions. For example, all the PEG chains used in this study are effective if they are adsorbed at the bulk concentration of 0.1 mg ml^{-1} (*Figure 6*).

Effect of COP2000 and COP5000 grafting on fibrinogen adsorption

Figure 7 shows the surface concentrations of fibrinogen on DDS–glass, COP2000-grafted DDS–glass and COP5000-grafted DDS–glass. After 1 h of protein adsorption, the surface fibrinogen concentration was 0.108 $\mu g\ cm^{-2}$ for DDS–glass, 0.016 $\mu g\ cm^{-2}$ for COP2000-grafted DDS–glass and 0.018 $\mu g\ cm^{-2}$ for COP5000-grafted DDS–glass. It is obvious that the adsorption of fibrinogen on the surfaces decreased significantly when DDS–glass was grafted with COP2000 or COP5000.

Contact angles of the surfaces grafted with PEG–PB with different PEG chain lengths

Figure 8A shows the advancing contact angle of DDS–glass grafted with PEG–PB with different PEG chains as a function of the molar concentration of the adsorption solution. Irrespective of the concentration and chain length of PEG, the measured advancing angles were between 80° and 90°, and were not significantly different from that of the untreated DDS–glass. The receding contact angles are shown in *Figure 8B*. As the concentration of the adsorption solution increased, the receding contact angle decreased at the beginning and increased with a further increase in concentration. However, the receding contact angles of DDS–glass grafted with PEG–PB with longer PEG chain lengths were higher than that with shorter ones.

DISCUSSION

The amphipathic PEG–PB copolymers have two components in their structure: a hydrophobic PB segment and one or two hydrophilic PEG segments. The hydrophilic PEG segments make the copolymer dissolve in aqueous solution via hydrogen bonding

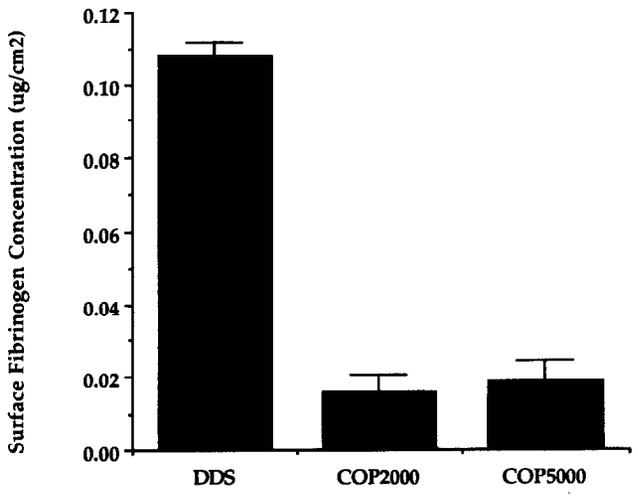


Figure 7 Surface fibrinogen concentration on DDS–glass, COP2000-grafted DDS–glass and COP5000-grafted DDS–glass. COP2000 and COP5000 were adsorbed from 0.5 mg ml^{-1} solutions for 1 h. γ-Irradiation time was 3 h (0.804 Mrad h^{-1}). The bulk concentration of radiolabelled fibrinogen was 0.1 mg ml^{-1} and the albumin concentration was 2 mg ml^{-1}. The proteins were adsorbed on the surfaces for 1 h at room temperature. Mean ± s.d.

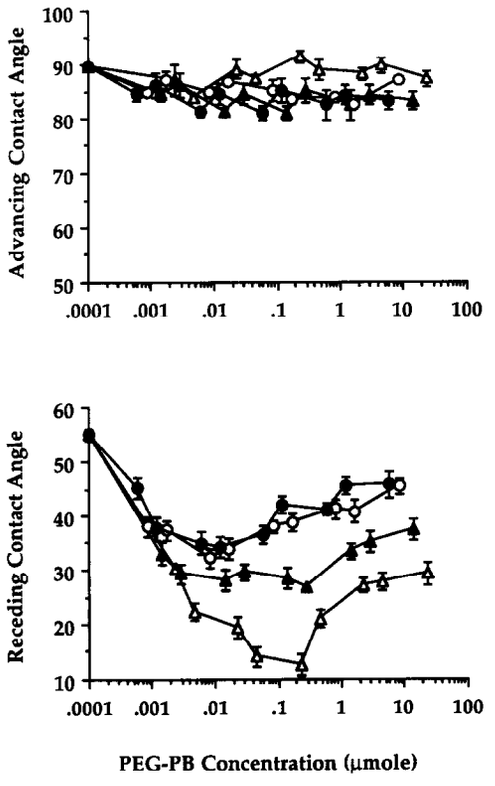


Figure 8 Changes in, **a**, advancing, and **b**, receding contact angles of the surfaces grafted with COP350 (△), COP750 (▲), COP2000 (○) and COP5000 (●) as a function of the PEG–PB molar concentration used for adsorption. The surfaces were adsorbed with PEG–PB for 1 h and γ-irradiated for 3 h (0.0804 Mrad h^{-1}) in the copolymer solutions. Mean ± s.e.m.

between the ether oxygen of PEG and water[24]. After the copolymers adsorb on the surface, the surface-bound PEG segments also confer biocompatibility by extending their chains into aqueous solutions from the surface to exert steric repulsion to proteins and platelets[25–27]. On the other hand, the hydrophobic PB segment serves as a driving force for spontaneous adsorption of PEG–PB onto hydrophobic DDS–glass in aqueous solution due to its incompatibility with water and hydrophobic interaction with the surface.

A double bond is one of the groups which is particularly sensitive to radiation-induced reaction[28]. Incorporation of double bonds into the hydrophobic PB segment can enhance the radiation sensitivity of PEG–PB. The energy from the incident radiation can be captured directly by the double bonds or indirectly by water molecules, which provide beneficial effects on activating the double bonds[29]. The adsorption of hydrophobic PB segments onto hydrophobic surfaces in an aqueous solution also enhances the intimate contacts of double bonds with the surfaces. Such intimate contacts eliminate long-range migration of the radicals on double bonds to the surface and increase the possibility of coupling between the copolymer and the surface. In dry conditions, however, not only may the double bonds lose intimate contact with the surface, but also there are less pathways for energy transfer or energy trapping than in aqueous solution. Consequently, the possibility of covalent bond

formation between the double bonds and the surface is low in dry conditions. γ-Rays, which can penetrate most materials, are a good energy source for modification of fully assembled devices, and the total dose required for grafting PEG–PB in the presence of water is less than 0.24 Mrad, which is weak enough not to affect the bulk properties of most materials.

PEG–PB can be grafted onto DSS-glass through different grafting procedures. One of the advantages of irradiating in water is that the copolymer solution can be recycled, since the polymer molecules in the solution are still intact. On the other hand, when irradiating the bulk solution, the copolymer solution cannot be utilized again. However, due to the high efficiency of grafting, even a very dilute copolymer solution can be used. Thus, either method can be used to suit various conditions and situations for practical applications.

It is believed that the mechanism as well as the adsorption kinetics of amphipathic copolymers depends on the polymer concentration in the bulk solution[30,31]. At concentrations well below the critical micelle concentration, amphipathic copolymers exist as individual molecules and their adsorption may not cover the whole surface. On the other hand, at concentrations well above the critical micelle concentration, the adsorption is dominated by micelles. The PEG chains, which are exposed to the water molecules, may block the attraction between the PB segment and the surface[32]. As a result, the grafting efficiency is expected to decrease and the micelles on the surface are easily removed. Therefore, at the very low or high concentrations of PEG–PB used for adsorption, the grafting on the surface may not be uniform or may form 'islands' of adsorbed block copolymers. Then, much of the surface will be exposed for protein adsorption and platelet adhesion. It appears that the adsorption of individual PEG–PB molecules from a polymer solution in the intermediate concentration range gives a homogeneous coverage of the surface and high grafting efficiency.

The decreases in platelet adhesion and activation on the copolymer-grafted surfaces may also be attributed to the decrease in the adsorption of thrombogenic proteins such as fibrinogen. Fibrinogen can displace loosely adsorbed polymers or proteins such as albumin from the surface[33,34]. If the surface fibrogen concentration is equal to or higher than 0.02 $\mu g\ cm^{-2}$, adherent platelets are fully activated[34]. In this study, the fibrinogen concentration was lower than 0.02 $\mu g\ ml^{-1}$ after DDS–glass was grafted with COP2000 or COP5000 when fibrinogen was adsorbed from a binary protein solution.

The high advancing contact angles for all treated DDS–glass samples can be ascribed to the hydrophobic CH_2 groups of PEG–PB, which are exposed to air to minimize the surface free energy. As mentioned above, extremely low or high concentrations of PEG–PB in the adsorption solution resulted in a low surface density of the grafted PEG–PB. The uncovered portion of the surface may contribute to higher receding contact angles than that of relatively densely-packed PEG–PB. The copolymer-grafted DDS–glass, however, exhibited an unusual phenomenon: the receding contact angle, which measures hydrophobicity, increased with the PEG chain length of the grafted PEG–PB. It was shown in *Figure 6* that PEG of longer chain length can prevent platelet adhesion and activation better than PEG of shorter chain length. It is generally believed that the hydrophilic surface reduces platelet adhesion and activation. Considering these facts, one may conclude that the data on the PEG chain length and the contact angles contradict each other. This may be explained by the difference in density of PEG on the surfaces. Increase in chain length of PEG can cause a decrease in copolymer adsorption due to the steric repulsion between molecules. The surface density of long PEG chains may be lower than that of short PEG chains, and this results in higher receding contact angles. The increase in PEG length, however, increases the thickness and the mobility of the grafted layer, which increases the prevention of protein adsorption and platelet adhesion by steric repulsion[35,36]. On the other hand, the short PEG chains may not be long enough to completely prevent the contact of large proteins or platelets with the underlying hydrophobic surfaces, although the surfaces possess low receding contact angles.

In summary, the data obtained from this study suggest that amphipathic PEG–PB copolymers with different PEG chain lengths of molecular weights between 350 and 5000 have high affinity to hydrophobic surfaces. PEG can be terminally grafted onto the surface through the activation of the double bonds along the PB segment by γ-irradiation. The major advantage of this method is that this procedure can be used to modify virtually any solid surfaces regardless of their composition and shape. Above all, this method is very simple, effective and cost-effective.

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