Albumin grafting on to polypropylene by thermal activation

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4-azido-2-nitrophenyl albumin (ANP-albumin) was prepared by reacting 4-fluoro-3-nitrophenyl azide with albumin. The thermal decomposition kinetics of phenyl azide of ANP-albumin was studied at various temperatures by Fourier-Transform Infrared (FTIR) spectroscopy. The decomposition rate of the phenyl azide increased with temperature. The activation energy for the first-order decomposition of the phenyl azide was 128.0 kJ/mol. Albumin was grafted on to polypropylene (PP) films by thermolysis of the azido groups of ANP-albumin with no premodification of the PP surface. The albumin-grafted surface was characterized by electron spectroscopy for chemical analysis (ESCA) and by quantitative determination of platelet adhesion and activation. The bulk concentration of ANP-albumin used for adsorption varied from 0.001 to 30 mg ml^{-1}, and the albumin-adsorbed PP films were incubated at 100°C for up to 7 h. The carbon and nitrogen peaks resulting from the grafted albumin were used to compare the surface albumin concentrations as a function of the concentration of ANP-albumin in the adsorption solution. When the PP film was adsorbed with ANP-albumin at the concentration of 5 mg ml^{-1} or higher and incubated at 100°C for longer than 5 h, the surface became resistant to platelet adhesion. The ANP-albumin can be grafted on to chemically inert surfaces such as PP surface through simple thermolysis of azido groups to prevent platelet adhesion and activation.

Keywords: Albumin, polypropylene, surface modification, thermal activation

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One of the main problems associated with blood-contacting medical devices is surface-induced platelet adhesion and subsequent thrombus formation[1]. It is generally accepted that the thrombus formation is mediated by thrombogenic proteins, such as fibrinogen or fibronectin, which adsorb to the artificial surface and enhance the interaction of the surface with platelets[2,3]. The presence of other proteins, such as albumin, on the surface is believed to inhibit the adsorption of thrombogenic proteins to the surface and decrease platelet adhesion and activation[4].

One way to make a blood-compatible surface is the formation of an albumin layer on the surface, formed by physical adsorption of albumin[5,6], enhancing the albumin affinity of the surface by grafting long carbon chains[7,8], cross-linking of the adsorbed albumin with glutaraldehyde[9-11] and photografting of albumin to the surface[12,13]. When a surface is exposed to blood, the adsorbed proteins are believed to be exchanged or displaced by blood proteins[14,15]. Covalent grafting of albumin to the surface is necessary to maintain the surface passivating effect of albumin.

Most medical devices are, however, made of chemically inert materials such as polyethylene and polypropylene (PP), and premodification of the surfaces and long complicated grafting procedures are always required. To be practical, the grafting procedure should be simple, and albumin should have the ability to react with inert surfaces. We know that azide can generate chemical intermediates, nitrenes, capable of coupling to various bonds including C-H bonds[16].

In our previous studies, PEG and albumin were bound to chemically inert dimethyldichlorosilane-coated glass (DDS-glass) through activation of azide by UV irradiation[12,17]. In this study, albumin was functionalized by attaching azide to its amino groups and covalently bound to PP films through thermolysis of the azido groups. PP was chosen as a model polymer, since it is widely used for making blood-contacting medical devices, but is highly thrombogenic[5]. The albumin-grafted surfaces were characterized by electron spectroscopy for chemical analysis (ESCA) and by platelet adhesion and activation.

EXPERIMENTAL

Synthesis and characterization of ANP-albumin

The synthesis of 4-azido-2-nitrophenyl albumin (ANP-albumin) was carried out by dissolving human serum albumin (HSA, Sigma, 1X, crystallized) in 12.5 mM sodium borate buffer (pH 9.8) to make the final concen-

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tration of 10 mg ml^{-1}. 4-fluoro-3-nitrophenyl azide in ethanol (30 ml at 33 mg ml^{-1}) was added dropwise to 100 ml of the albumin solution under stirring at 40°C. After 20 h, the reaction mixture was filtered through a disposable sterile filter with 0.45 μm nylon membrane (Corning, Inc., Corning, NY, USA). The filtrate was then dialysed against 2 l of deionized distilled water. During 3 d of dialysis, the water was changed six times. The solution was filtered and then freeze-dried. During all procedures, the samples were protected from light. The yield was 1 g. The presence of ANP bound to albumin was verified from the characteristic stretching band of azido group at 2118 cm^{-1} on the IR spectrum of ANP-albumin. The number of ANP groups in each albumin molecule was determined by measuring the absorbance of ANP at 460 nm. The absorptivity of 4-azido-*N*-methyl-2-nitroaniline at 460 nm, which is 4800, was used for the calculation[18]. It was found that each albumin molecule contains about 15 ANP groups.

Thermal decomposition of azide

The kinetics of thermolysis of phenyl azide of ANP-albumin was measured by incubating 5 mg ml^{-1} of ANP-albumin in water at different temperatures for various time periods. The samples were then freeze-dried to obtain IR spectra. The freeze-dried samples were taken as KBr pellets with a Nicolet 20SXc Fourier transform infrared (FTIR) system.

Grafting of ANP-albumin on to PP films

PP films (0.5 mm thick, Total Plastics Inc., Kalamazoo, MI, USA) were cut into 22 × 22 mm pieces and soaked in 2% Isoclean® solution at 60°C for 3 h. They were then rinsed with distilled water for 2 h and washed with acetone and ethanol in sequence twice in a sonicator for 15 min. The clean films were then dried *in vacuo*.

Concentrated ANP-albumin solution was added into the water, in which a film was immersed. The final bulk concentration was varied from 0.001 to 30 mg ml^{-1}. After albumin was adsorbed for 1 h at room temperature, the solution was diluted with running distilled water. The film was then moved to another glass tube which was filled with water and incubated at 100°C for various time periods up to 7 h. After incubation, ungrafted albumin was removed by washing with 1% sodium dodecyl sulphate (SDS, Bio-Rad Lab., Richmond, CA, USA) aqueous solution for 1 h at 100°C, then extensively with water. These films are called the albumin-grafted PP films. The PP films which were treated with albumin instead of ANP-albumin are referred to as the albumin-treated PP films.

ESCA analysis

ESCA studies of the films were performed using a Physical Electronics model 548 instrument. The source was a 10 kV, 40 mA X-ray beam from a magnesium anode. Survey scans were carried out at 100 eV pass energy on all samples. High resolution peaks for C_{1s} and N_{1s} scans were also obtained from each sample at 25 eV pass energy to determine the atomic composition of the surfaces. Surface charge build-up was corrected by shifting C_{1s} peak to 285 eV. The obtained peaks were normalized by dividing the area of each peak by the Scofield cross-section. The normalized values were then used to calculate the atomic ratios on the surfaces.

Platelet adhesion and activation

Platelet-rich plasma (PRP) was prepared from whole blood drawn from healthy adult volunteers[19]. A perfusion chamber assembled from a sample film, two silicone rubber spacers (0.013 cm thickness, Dow Corning, Midland, MI, USA), and a glass slide (2.54 × 7.62 cm) was used to introduce platelets to the film[20]. The chamber was filled with phosphate-buffered saline (PBS, pH 7.4), and PRP was introduced into the chamber by replacing PBS to avoid surface-air contact. The platelets were allowed to settle on the film by gravity at room temperature for 1 h. The unadhered platelets were removed by washing with PBS, and the adherent platelets on the surface were fixed with 2% glutaraldehyde in PBS for 15 min. After replacing the glutaraldehyde solution with PBS, the fixed platelets were stained with 0.1% solution of Coomassie Brilliant Blue for 1 h. The stained platelets were observed with an inverted light microscope (Nikon Diaphot, Garden City, NY, USA), and the microscope images were projected to a video camera (Newvicon model 65, Dage-MTI, Michigan City, IN, USA)[20]. The number of adherent platelets was counted in 24 separate video images by using ×40 objective lens for each time point. The microscope images were directed to a computer for image analysis, and the spread area of the adherent platelets was measured using a software obtained from Computer Imaging Applications (Madison, WI, USA)[20]. The area of spread platelets was measured in eight separated microscope fields at each time point by using a ×100 objective lens.

Scanning electron microscopy (SEM) observation

The samples for scanning electron microscopy (SEM) observation were prepared by fixing platelets on surfaces with 2% glutaraldehyde in PBS for 2 h, dehydrating in a series of ethanol, and critical point drying (Union, model CPD-020) using liquid CO_2 as a transition fluid. After coating with gold-palladium using Hummer 1 sputter-coater, the samples were observed with a Jeol JSM-840 scanning electron microscope at an accelerating voltage of 10 kV.

RESULTS

Thermal decomposition of phenyl azide

The kinetics of thermal decomposition of phenyl azide of ANP-albumin in aqueous solutions was measured from the FTIR spectra. After the sample was incubated at 80°C, the intensity of the azido band at 2118 cm^{-1} decreased slightly as the incubation time increased. *Figure 1* shows the thermal decomposition of azido groups at 90°C. The phenyl azide of ANP-albumin was completely decomposed after 7 h incubation at 100°C. To calculate the intensity change of the azido band, the IR band at 1392 cm^{-1} was used as an internal standard. Since the half bandwidth of the IR band of albumin did not change after chemical reaction or heating, this band

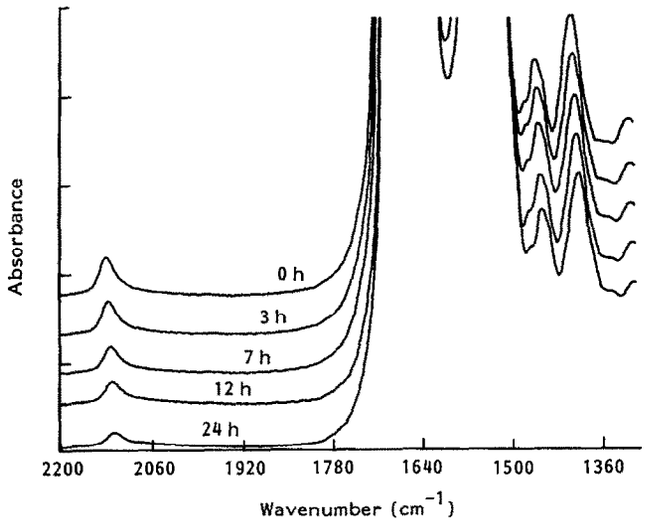

Figure 1 IR spectra of ANP-albumin. The aqueous ANP-albumin solution was incubated at 90°C for various time periods.

is believed to be due to the local vibrational mode and may be assigned to a methyl bending vibration[21]. The intensity ratio of the azido band at 2118 cm^{-1} to the band at 1392 cm^{-1} is plotted as a function of incubation time on a semilog paper. As shown in *Figure 2*, the intensity ratio decreased linearly with the increase in temperature and incubation time, indicating a first-order decomposition of azido groups. The decrease in the intensity ratio was most pronounced at 100°C. The rate constants at different temperature are listed in *Table 1*. The activation energy was calculated from the Arrhenius plot (*Figure 3*). The calculated activation energy for decomposition of phenyl azide of ANP-albumin in water was 128 kJ/mol.

ESCA studies

Figure 4 shows the ESCA survey scans of PP (a), albumin-treated PP (b) and albumin-grafted PP films (c).

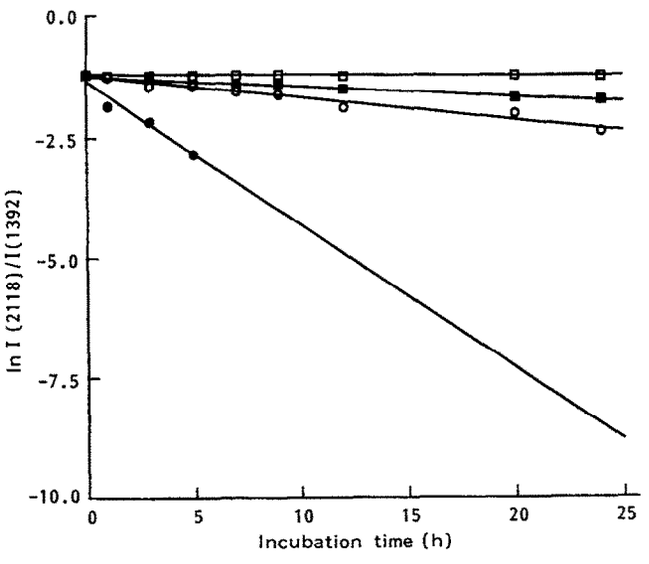

Figure 2 Changes in the intensity ratio of the azido band at 2118 cm^{-1} to the band at 1392 cm^{-1} (I(2118)/I(1392)) of ANP-albumin IR spectra as a function of incubation time: □, 60°C; ■, 80°C; ○, 90°C; and ●, 100°C.

Table 1 Thermal decomposition of phenyl azide of ANP-albumin in water

Temperature (°C)	k × 10^2 (h^{-1})	R^2	Relative rate
60	0.265	0.997	0.009
80	2.254	0.977	0.076
90	4.631	0.970	0.156
100	29.738	0.955	1.000

k: Rate constant.
R^2: Correlation coefficient.

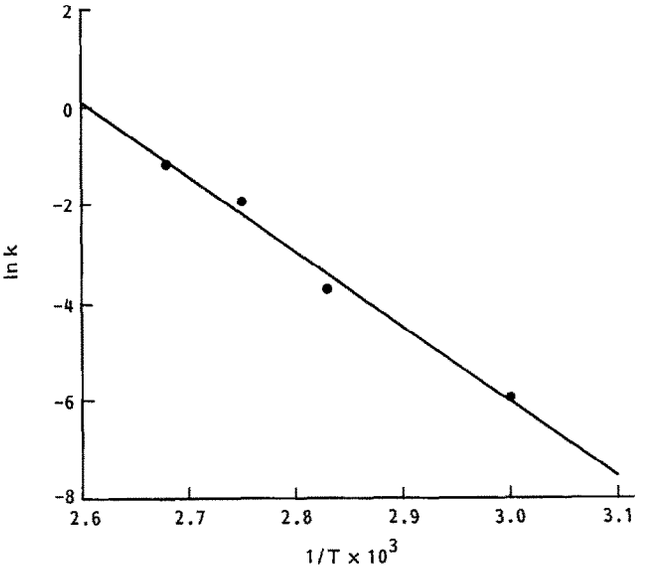

Figure 3 Arrhenius plot of the decomposition rates of phenyl azide of ANP-albumin in aqueous solution.

The atomic percentage of carbon, nitrogen and oxygen on the three surfaces are presented in *Table 2*. The concentration of albumin or ANP-albumin used for adsorption was 5 mg ml^{-1}, and the incubation time was 5 h. The survey scan of the PP film showed that only carbon and oxygen peaks were present on the surface. The atomic composition of the PP film was 88.5% carbon and 11.5% oxygen. After the PP film was grafted with ANP-albumin, a nitrogen peak became evident, and the intensity of oxygen peak increased. Typical atomic compositions for this sample were 76.1% carbon, 4.5% nitrogen and 19.4% oxygen. For albumin-treated PP film, the atomic composition was 81.0% carbon, 2.6% nitrogen and 16.4% oxygen.

The contributions of carbon and nitrogen atoms in the peptide bonds of albumin were analysed using high resolution spectra of C_{1s} and N_{1s}. The N_{1s} and C_{1s} spectra of PP (a), albumin-treated PP (b) and albumin-grafted PP films (c) are shown in *Figure 5*. For the C_{1s} spectra, differences in peak shapes were observed among the three surfaces, and the spectra could be resolved into three distinct peaks which corresponded to different carbon atomic environments. The tallest peak at 285 eV is attributed to the hydrocarbon (-C-C-) of the PP film. The peak at a slightly lower binding energy is probably due to carbon in -C-N- and -C-O- bonds. The most prominent feature is the peak at 288.1 eV, which is due to the carbon in peptide bond[22, 23] (-N-CO-). As shown in

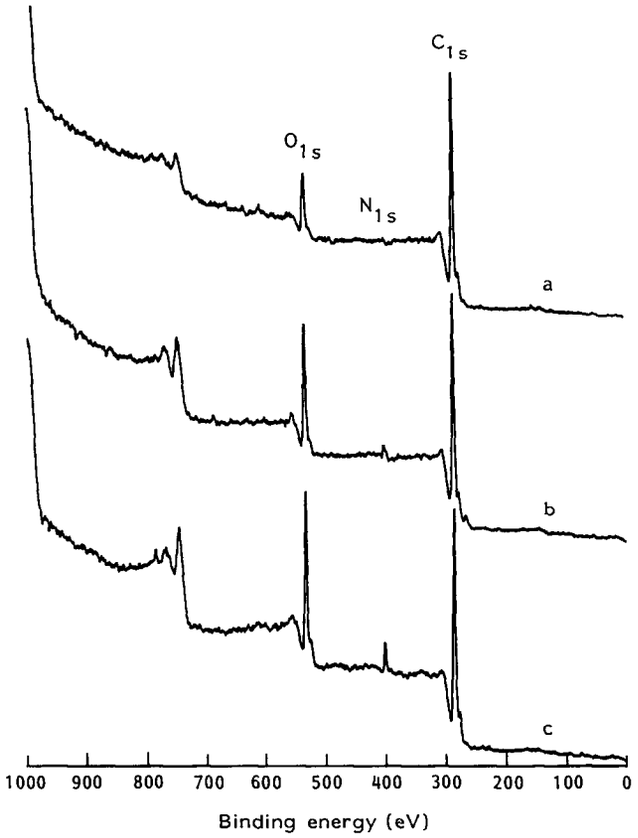

Figure 4 ESCA survey scans of the PP (a), albumin-treated PP (b) and albumin-grafted PP films (c). The concentration of albumin or ANP-albumin used for adsorption was 5 mg ml^{-1} and the incubation time was 5 h.

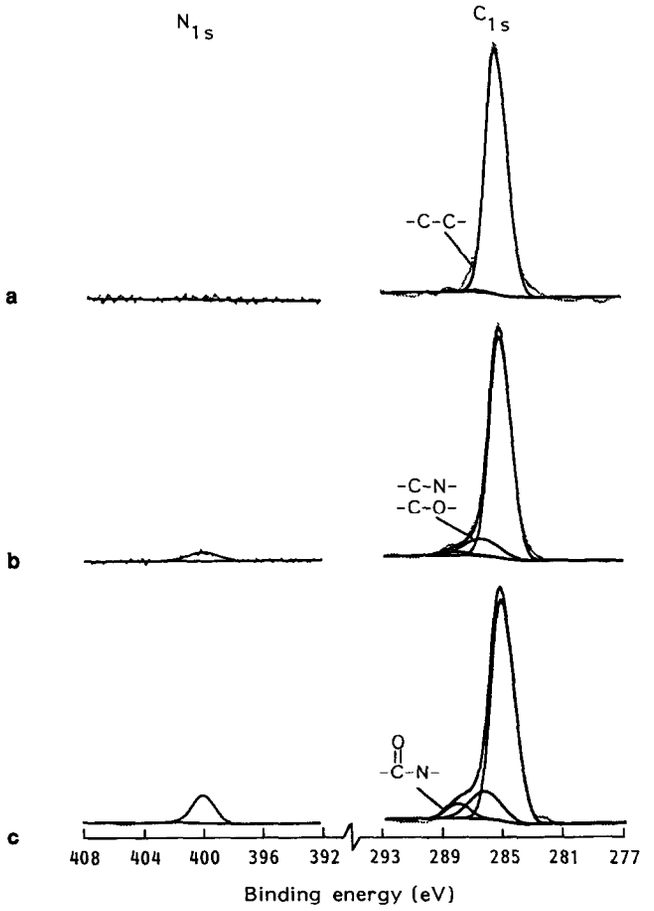

Figure 5 High-resolution ESCA C_{1s} and N_{1s} spectra of the PP (a), albumin-treated PP (b) and albumin-grafted PP films (c). The concentration of albumin or ANP-albumin used for adsorption was 5 mg ml^{-1}, and the incubation time was 5 h.

Table 2 Atomic compositions of PP, albumin-treated PP and albumin-grafted PP films

Surface	%C	%N	%O
PP film	88.5	0.0	11.5
Albumin-treated PP film	81.0	2.6	16.4
Albumin-grafted PP film	76.1	4.5	19.4

Figure 5, the C_{1s} spectrum of the PP film is dominated by a single hydrocarbon peak, whereas no peptide carbon peak was observed. Compared to a very weak peptide carbon peak in the spectrum of the albumin-treated PP film, a distinct shape and intensity of the peptide carbon peak was observed in the spectrum of the albumin-grafted PP film. In addition, the appearance of the nitrogen peak also indicates the presence of albumin on the surface.

As shown in *Figure 5*, no nitrogen peak was observed in the spectrum of the PP film. However, a distinct nitrogen peak appeared at 400.1 eV in the spectrum of the albumin-grafted PP film. The nitrogen peak in the spectrum of the albumin-treated PP film was much smaller than that of the albumin-grafted PP film. The significant differences in the intensities of peptide carbon and nitrogen peaks between the albumin-grafted PP film and the albumin-treated PP film indicate that the surface albumin concentration is much higher when albumin is grafted by thermolysis of azide. The presence of the nitrogen peak on the albumin-treated PP film indicates that SDS was not able to remove all albumin molecules from the surface, even though they were not covalently grafted.

The intensities of peptide carbon and nitrogen peaks were expected to be proportional to the amount of albumin on PP films. For quantitative evaluations of the grafted albumin, the peaks were normalized by dividing the area of each peak by the Scofield cross-section. The atomic ratios of peptide carbon at 288.1 eV and nitrogen at 400.1 eV to hydrocarbon at 285 eV were calculated using the normalized values. *Figure 6* shows the calculated atomic ratios as a function of the concentration of ANP-albumin used for adsorption. The relative surface albumin concentrations on the albumin-grafted PP film gradually increased with increasing concentration of the bulk ANP-albumin. These two sets of data, which are complementary, show that more ANP-albumin was grafted to the surface as the concentration of ANP-albumin in solution increased up to 10 mg ml^{-1}.

Platelet adhesion and activation studies

Since the ultimate goal of albumin grafting is to prepare blood-compatible surfaces, determination of platelet adhesion and activation is very important. Platelet adhesion and subsequent activation were quantitated by

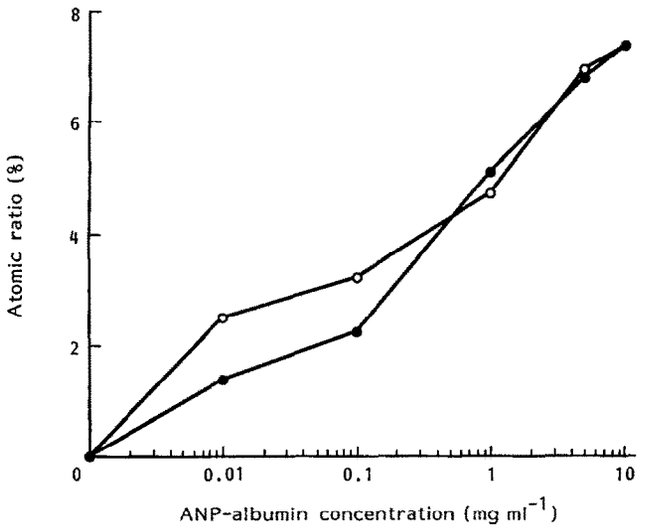

Figure 6 Atomic ratios of ●, peptide carbon to hydrocarbon and ○, nitrogen to hydrocarbon on the albumin-grafted PP films as a function of the ANP-albumin concentration used for adsorption. The adsorption time was 1 h and the incubation time was 5 h.

measuring the number and area of platelets adherent on the surface, respectively. ANP-albumin or albumin was adsorbed and incubated for various time periods. *Figure 7* shows the dependence of platelet adhesion and activation on the bulk concentration of ANP-albumin or albumin used for adsorption on PP films. The concentration in adsorption solution varied from 0.001 to 30 mg ml^{-1}, ANP-albumin was adsorbed for 1 h and incubated at 100°C for 5 h. To understand the effect of heat on the grafting of ANP-albumin, PP films were also treated with albumin at 100°C or with ANP-albumin at room temperature. The number of platelets adherent on the albumin-grafted surface decreased when the ANP-albumin concentration for adsorption was >0.05 mg ml^{-1}. The spread area of the adherent platelets reduced when the ANP-albumin concentration was >0.1 mg ml^{-1}. Platelets could not adhere on to the surface when the ANP-albumin concentration was increased to 5 mg ml^{-1}. Regardless of concentration, however, the number and area of platelets adherent on the two control surfaces did not seem to be significantly different from those on the untreated PP film. It indicates that activation of azide by heating was required for ANP-albumin grafting, and the concentration of ANP-albumin used for adsorption should be 5 mg ml^{-1} or higher.

Figure 8 shows SEM photographs of platelets adherent on albumin-treated PP (*Figure 8a*) and albumin-grafted PP films (*Figures 8b, c* and *d*). For albumin-treated PP film, the concentration of albumin used for adsorption was 5 mg ml^{-1} (*Figure 8a*). The concentrations of ANP-albumin used for grafting were 0.1 mg ml^{-1} (*Figure 8b*), 1 mg ml^{-1} (*Figure 8c*) and 5 mg ml^{-1} (*Figure 8d*). The control albumin-treated surface and the surface treated with 0.1 mg ml^{-1} of ANP-albumin showed fully activated platelets and small thrombi (*Figures 8a* and *b*). However, the number of platelets adherent on the latter surface was lower than that on the former surface. Only a few unactivated platelets were observed on the albumin-grafted PP film, when the concentration of ANP-albumin

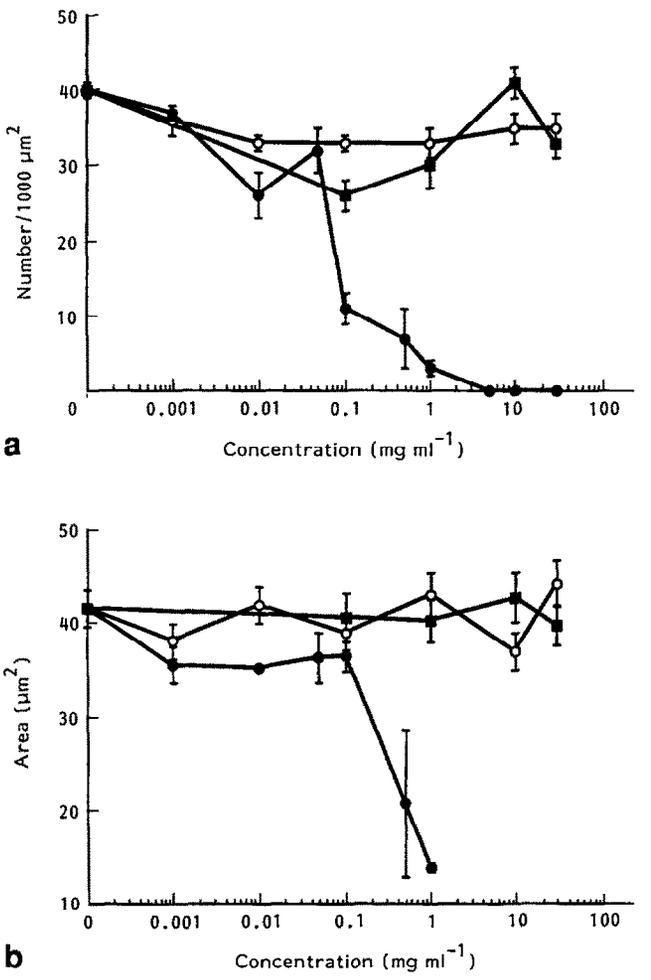

Figure 7 Changes in **a**, number and **b**, area of platelets adherent on the surfaces treated with ●, ANP-albumin at 100°C; ■, ANP-albumin at room temperature; and ○, albumin at 100°C as a function of the albumin concentration used for adsorption. Albumin or ANP-albumin was adsorbed for 1 h and incubated at 100°C for 5 h. Platelets in PRP were allowed to adhere for 1 h at room temperature. Average ± SEM (n = 48 for **a** and 16 for **b**).

was 1 mg ml^{-1}. As the concentration was increased up to 5 mg ml^{-1}, no platelets were able to adhere to the surface.

The effect of incubation time for albumin grafting on to PP films on the prevention of platelet adhesion and activation was also examined. Albumin or ANP-albumin was adsorbed on PP film for 1 h from 5 mg ml^{-1} and the surface was incubated at 100°C in water for various time periods up to 7 h. *Figure 9* shows the relationship between platelet adhesion and activation and incubation time. The number of platelets adherent on the PP film adsorbed with ANP-albumin decreased sharply after incubation for 1 h, and platelets could not adhere on the surfaces when the incubation time was 5 h or longer. A dramatic decrease in spread area was observed after 3 h of incubation of the adsorbed ANP-albumin. On the contrary, the number and area of platelets adherent on albumin-treated PP film remained almost the same as those on the untreated PP film, regardless of incubation time.

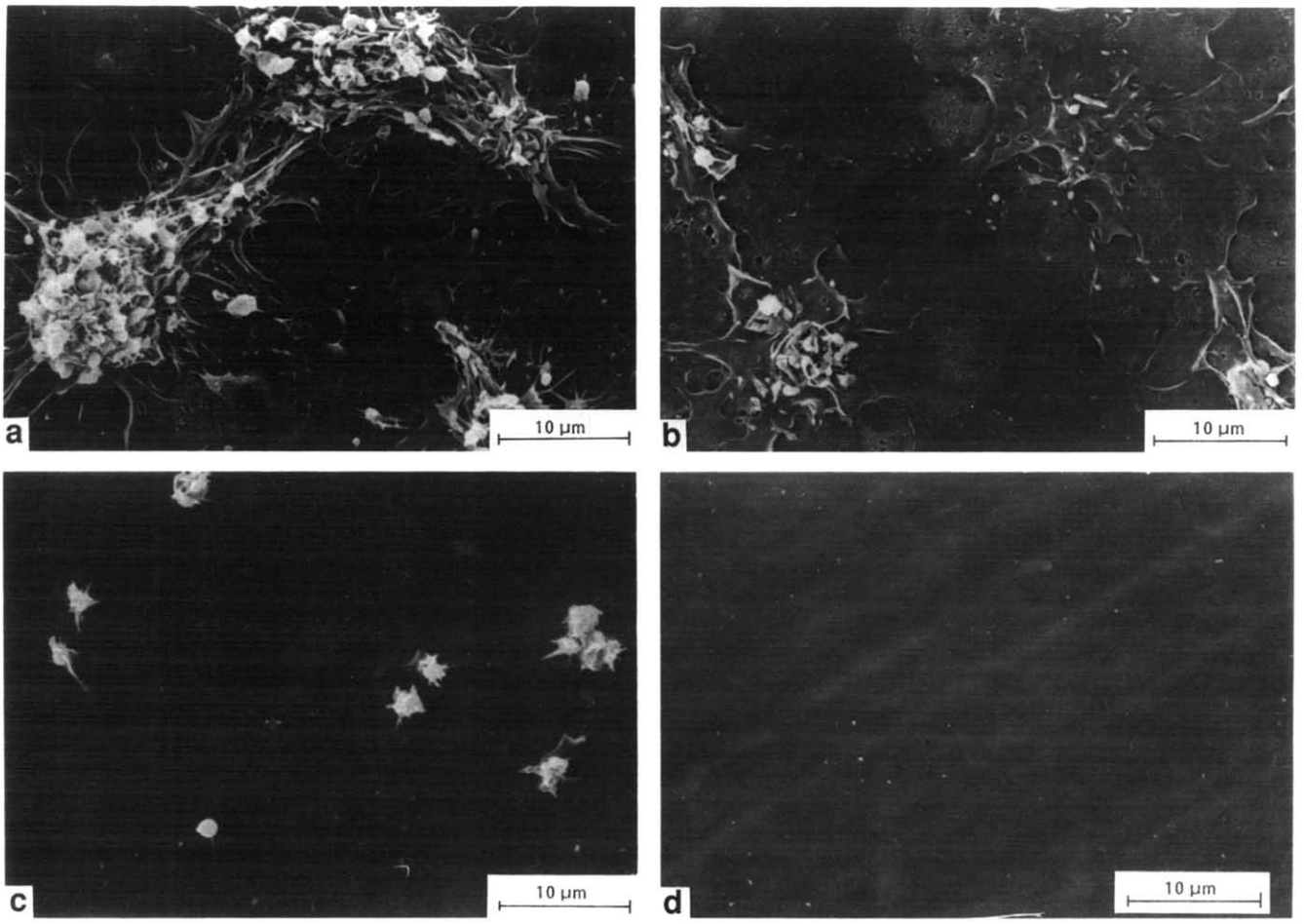

Figure 8 SEM photographs of platelets adherent on to **a**, albumin-treated PP and **b–d**, albumin-grafted PP films. The concentration of ANP-albumin used for adsorption was **b**, 0.1 mg/ml; **c**, 1 mg/ml; and **d**, 5 mg ml^{-1}. The concentration of albumin was **a**, 5 mg/ml. Albumin or ANP-albumin was adsorbed for 1 h and incubated at 100°C for 5 h. Platelets in PRP were allowed to interact with the surfaces for 1 h at room temperature.

DISCUSSION

Our previous study demonstrated that PEG or albumin with azido groups can be grafted on to DDS-glass through photolysis of azide. It also showed that the grafting efficiency of albumin was much greater than that of PEG, and no long adsorption time was necessary for albumin grafting[12, 17]. It is probably because the adsorption of albumin to hydrophobic surfaces occurs readily through the hydrophobic interaction and each albumin molecule contains many ANP groups. Grafting by photolysis, however, has its limitations. Since UV light does not penetrate most of the materials, the entire surface to be modified must be exposed to the UV light. For medical devices with a complicated shape, such as an oxygenator, it may be difficult to graft ANP-albumin by photoactivation. For those devices, it would be very convenient if heat could be used as an alternative energy source to activate azide.

Aryl azides are widely used for generating aryl nitrenes by thermolysis or photolysis. Aryl azides readily release two of the three nitrogens as N_2 and generate nitrenes[24]. The temperature range required for thermolysis of phenyl azides[16] is 130–180°C. It is reported that the decomposition rate of azides was accelerated by hydroxylic solvents[25]. Water, which was used as a solvent in this study, appears to act as an energy sink and a heat-transfer agent. As a result, the thermal decomposition reaction of phenyl azide readily proceeded, even at 100°C. In addition, it is known that an electron-donating parasubstituent causes an acceleration in decomposition rates of azido groups[25]. The electron-donating amino group of albumin as a parasubstituent should have an effect on the acceleration in the thermal decomposition of the phenyl azide of ANP-albumin.

Nitrenes can carry out a wide range of chemical reactions, including the reaction with carbon–hydrogen bonds through direct insertion or a hydrogen abstraction process[16]. It is believed that the latter path is more feasible for the reaction of nitrene with carbon–hydrogen bonds, and intermolecular insertion of aryl nitrenes into carbon–hydrogen bonds is a poorly competitive process[16]. It is generally accepted that rearrangement of nitrene generated from aryl azide does not occur as readily as that from alkyl azide, and consequently the nitrene obtained on thermolysis of phenyl azides has a longer lifetime. The probability of intermolecular reactions of phenyl nitrene is much higher[24]. In addition, it is known that the hydrophobic segment of albumin is tightly adsorbed on a hydrophobic surface through hydrophobic

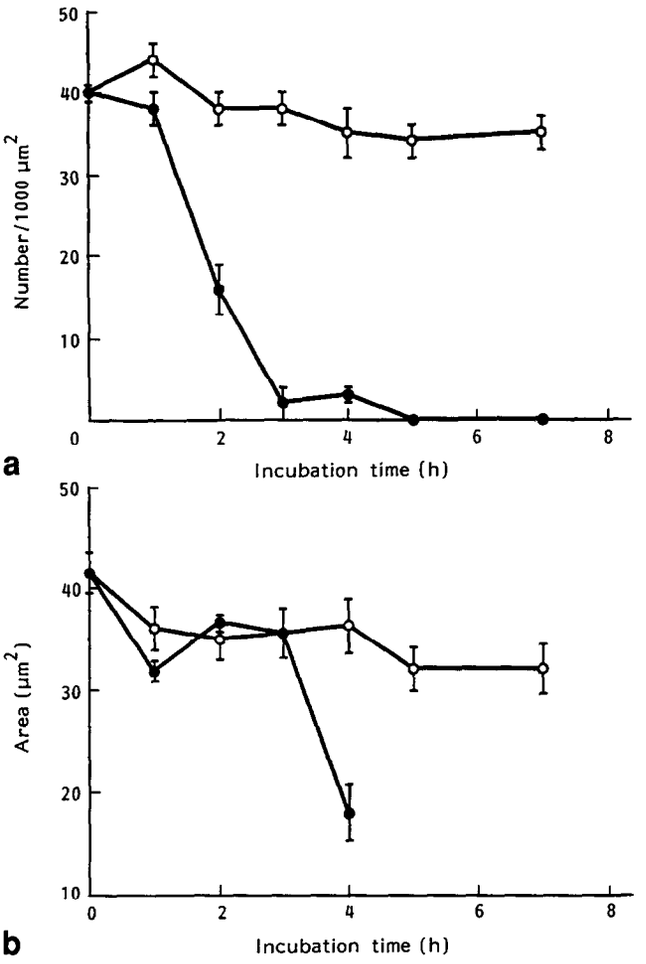

Figure 9 Changes in **a**, number and **b**, area of platelets adherent on to ○, albumin-treated PP and ●, albumin-grafted PP films as a function of incubation time. Albumin (5 mg/ml) or ANP-albumin (5 mg/ml) was adsorbed for 1 h at room temperature and incubated at 100°C for various time periods. Platelets in PRP were allowed to adhere for 1 h at room temperature. Average ± SEM (n = 48 for **a** and 16 for **b**).

interaction, and this may increase the possibility of the abstraction of hydrogen atom from PP surface by nitrene and the coupling of the radicals formed.

ESCA is generally regarded as a key technique for surface characterization. This technique provides elemental analysis of the top 1–5 nm layer of the surface. As shown in *Figure 4* and *Table 2*, the nitrogen and oxygen contents increased and the carbon intensity of the film decreased after albumin was grafted on to the surface. This indicates the presence of albumin molecules on the surface, since N_{1s} and O_{1s} peaks originate mainly from nitrogen or oxygen atoms in albumin molecules, whereas carbon peak is mainly from the hydrocarbon of the PP film. Although PP films do not contain oxygen atoms, an oxygen peak was observed on the control PP film. Oxygen may originate from many different sources, including oxygen adsorbed from the air and residual detergent molecules used for cleaning the surface. It is difficult to identify various kinds of oxygen in the ESCA spectra for quantitative evaluation of albumin on the surface. Therefore, no further analysis was made for the oxygen peak.

Although the C_{1s} peak is also complicated due to the wide variety of carbon environments, the overall profile could be fitted quite well with three Gaussian curves (*Figure 5*). The important feature is that it is possible to observe a C_{1s} contribution from the peptide bonds of the protein molecules at 288.1 eV binding energy. Similar ESCA results have also been obtained for albumin[22], haemoglobin[26] and fibrinogen[23]. In these cases, however, an additional very weak carboxyl peak was observed at 289.3 eV. The S_{2p} peak at 164.1 eV from the disulphide groups of the protein molecules was also distinguishable in the study of Lindberg *et al.*[22]. Nevertheless, neither peak was observed in our studies. It must be noted that the ESCA spectra of the proteins in their studies were recorded from a thick layer of the proteins adsorbed on a metal substrate. We were not able to detect the trace of sulphur from the disulphide bridge and the carbon from carboxyl residues of albumin, probably due to the relatively low surface albumin concentration on the films.

The adsorption of certain proteins on to hydrophobic surfaces in buffer solutions is known to be irreversible[27,28]. The physical adsorption of albumin to hydrophobic surfaces made it difficult to distinguish between covalently bound and physically adsorbed albumin on the surface. In addition, the adsorptivity of heat-denatured albumin is known to be greater than that of the native albumin[29]. It has been shown, however, that SDS is a powerful detergent and its protein elutability from polymer surface increases at elevated temperatures[28,30]. Although the films were boiled in SDS solution for 1 h at 100°C, physically adsorbed albumin could not be completely removed from the film, as shown by the ESCA spectra of the albumin-treated PP film. The ESCA spectra clearly showed weak peptide carbon and nitrogen peaks (*Figure 5b*). It is interesting that platelet adhesion and activation still occurred on the albumin-treated PP film despite the residual albumin on the surface (*Figures 7–9*). It may indicate that most of the loosely adsorbed albumin was removed from the surface by SDS boiling.

The decrease in surface albumin concentration may have resulted in the exposure of bare surface sites, large enough to activate platelets or to adsorb other thrombogenic proteins such as fibrinogen. It is also possible that the non-covalently adsorbed albumin was displaced by blood proteins. Our previous study showed that platelets could activate fully as long as a small fraction of the surface (2–15%) was covered with fibrinogen[31]. In addition, the PP film itself is highly thrombogenic, even without the adsorbed fibrinogen. Therefore, a completely covered albumin layer grafted on the surface is required for the prevention of platelet adhesion and activation. It appears that the number is more sensitive than the area of adherent platelets to the changes in the surface concentration of the grafted albumin (*Figures 7* and *9*). The critical surface albumin concentration for the prevention of platelet activation on DDS-glass was found to be 0.23 $\mu g/cm^2$, and this surface concentration can be easily obtained by adsorbing albumin at the bulk concentration[31] of 1 mg ml^{-1} for 1 h. As shown in *Figure 7*, when the concentration of ANP-albumin used for adsorption was 1 mg ml^{-1} or above, platelets could not adhere to the albumin-grafted PP film. Since the grafting procedure was performed in water after excess albumin had been

washed from the surface, the amount of the grafted albumin on the PP film was expected to be a monolayer. The conformation of albumin may be changed by introduction of phenyl azides and heat treatment. Would denaturation alter the thromboresistant properties of natural albumin? According to our study, the heated ANP-albumin was very effective in the prevention of platelet adhesion and activation after grafted on to the thrombogenic surfaces. The denatured albumin grafted on the surface is believed to be still highly hydrated in aqueous solution, so that its hydrophilic regions extend into the water and exert steric repulsion against platelets[32].

This study has demonstrated that azide can be activated by heat and react with inert surfaces to graft albumin. The major advantage of this method is that this procedure can be used to modify virtually any solid surface regardless of its composition and the shape. Covalently grafted albumin would not be displaced by other proteins and cells in blood. The event of immune reaction with the albumin-modified surface and the potential toxicity of phenyl azides, however, still needs to be examined. Our current efforts are directed toward lowering the activation energy of azide by using other kinds of azides instead of phenyl azide and attaching these azides to synthetic polymers instead of albumin.

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