Adsorption of Blood Proteins on Glow-Discharge-Modified Polyurethane Membranes

NESRIN KAYIRHAN,[1] ADIL DENIZLI,[2] NESRIN HASIRCI[1,3]

[1] Middle East Technical University, Department of Polymer Science and Technology, Ankara, Turkey

[2] Hacettepe University, Chemistry Department, Biochemistry Division, Beytepe, Ankara, Turkey

[3] Middle East Technical University, Department of Chemistry, Ankara, Turkey

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ABSTRACT: Polyurethanes are a class of polymers that have a wide range of applications in the medical field although their blood compatibility still needs improvement. In order to obtain medical purity, this study prepared membrane-form polyurethanes from toluene 2,4-diisocyanate (TDI) and poly(propylene ethylene glycol) without the addition of any ingredients such as solvents, catalysts, or chain extenders. The aim was to increase surface hydrophilicity and improve blood compatibility. Therefore, the prepared membranes were modified by treatment with oxygen or argon plasmas. Characterizations of the samples were achieved by contact-angle and water-uptake studies as well as from atomic force microscope (AFM) pictures. It was found that oxygen-modified samples were more hydrophilic than argon-modified samples. The AFM images showed that surface roughness increased with plasma treatment. The protein adsorption experiments carried out with single protein solutions demonstrated that the adsorption of bovine serum albumin and fibrinogen decreased drastically by increasing the applied power and exposure time of the glow discharge. A similar decrease in the adsorption of protein was also observed for human blood proteins. The alterations of the conformational structures of the adsorbed proteins were examined by fluorescence spectrophotometry. Similar spectra with the same maximum wavelength were observed for native and desorbed proteins. These results showed that no denaturation of the proteins occurred upon adsorption on the surfaces of the prepared membranes. J Appl Polym Sci 81: 1322–1332, 2001

Key words:

INTRODUCTION

A biomaterial is a substance used in prostheses or in medical devices designed for contact with the living body for the intended method of application and for the intended period.[1] Synthetic polymers, the most diverse class of biomaterials, are widely used in both medical and pharmaceutical applications, and they contribute significantly to the quality and effectiveness of health care. Their applications range from use in a variety of implants or other supporting materials (e.g., vascular grafts, artificial hearts, intraocular lenses, joints, mammary prostheses, and sutures) to utilization in extracorporeal therapeutics and other supporting devices (e.g., hemodialysis, hemoperfusion, blood oxygenation, intravenous lines, nee-

Correspondence to: A. Denizli, P.K. 51, Samanpazari 06242, Ankara, Turkey (denizli@eti.cc.hun.edu.tr).

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dle catheters, and blood-bags), controlled release systems (e.g., transdermal drug delivery patches, microspheres, and microcapsules for targeted drug-delivery devices for different routes of administration), and clinical diagnostic assays (mainly as carriers and supporting materials).[2]

To be biocompatible, materials used in medical applications must meet certain criteria and regulatory requirements. The surfaces of biomaterials are believed to play an important role in determining biocompatibility. For materials that come in contact with blood, the first event is the adsorption of blood proteins at the solid–liquid interface. Thereafter, processes such as the activation of intrinsic coagulation, the adhesion and aggregation of platelets, and the activation of the complement system may take place, depending on the composition and the conformation of the adsorbed protein layer. The adsorbed protein layer's composition usually changes as a function of exposure time. To obtain more insight into the relation between the character of the polymer surface and its blood compatibility, protein adsorption must be studied on a series of polymers with well-characterized surface structures.

Polyurethanes, a unique class of polymers, have a wide range of applications because their properties can be readily tailored by the variation of their components. Therefore, they are extensively used as foams, coatings, adhesives, elastomers, and fibers.[3] They are also widely used in blood- and tissue-contacting applications, including vascular prostheses, blood filters, catheters, insulation for pacemaker leads, heart valves, cardiac assist devices, and chambers for the artificial heart. These applications use this family of polymers because of the latter's physiological acceptability, relatively good blood-tolerability, stability over extended implant periods, and excellent physical and mechanical properties. However, if there are to be further biomedical applications, the blood compatibility of polyurethanes needs to be improved.[4–6]

The purpose of the present research was to gain an insight into the influence of glow-discharge treatment (i.e., oxygen and argon) on polymer surface characteristics such as hydrophilicity and adsorption of blood proteins. For this purpose, glow-discharge-treated polyurethane membranes were prepared, and the adsorption of proteins from aqueous solutions (i.e., noncompetitive adsorption) or from human plasma (i.e., competitive adsorption) was studied. In order to examine the effects of adsorption on conformational changes of albumin molecules, the fluorescence spectra of the desorbed and native proteins were obtained and compared.

EXPERIMENTAL

Materials

Toluene 2,4-diisocyanate (TDI) was obtained from Sigma Chemical Company, (St Louis, Missouri) and was purified with vacuum distillation. Poly(propylene ethylene glycol) was obtained from the Shell Company (The Netherlands; (trade name: Caradol SC 46-02) and used as received. Bovine serum albumin and fibrinogen were purchased from Sigma and used as received. All other chemicals were supplied from Merck (Darmstad, Germany) and used as received.

Methods

Preparation of Polyurethane Membranes

The experimental setup was composed of two parts. In the upper part, a 250-mL single-neck flask was connected to a burette through a valve. The lower part was the polymerization chamber. It was placed in an oil bath that sat on a heater/magnetic stirrer. This chamber was connected to the burette with a valve and also was connected to the vacuum line with another valve. Purified TDI was placed in the upper flask and maintained under a nitrogen atmosphere. The lower container, with glycol inside, was evacuated for 1 h at about 90°C in order to remove volatile chemicals, especially water, and the desired amount of TDI (TDI:glycol—4:20 v/v) was added dropwise onto the hot glycol. The mixture was stirred for 3 h at 90°C, producing a viscous solution. Then this solution was poured into glass Petri dishes, placed into a vacuum oven, heated for 3 h at 80°C, and stored in a vacuum until solid films were formed, as described previously.[7,8] This process took approximately 1 week. After solidification of the samples, the molds were placed in boiling water in order to eliminate unreacted isocyanates and to peel the membranes easily off the molds. In boiling water isocyanates are converted to crosslinked structures such as urea and biuret branching. The membranes were washed several times with distilled water and dried prior to glow-discharge applications.

Glow-Discharge Modification

The polyurethane membranes were treated with argon or oxygen plasma created in an RF glow-discharge reactor. For glow-discharge treatment, the membranes were placed on the sample holder in the reactor, which was flushed with dry nitrogen and then pumped down to a pressure of 0.01 Torr. The argon or oxygen was introduced at a constant flow rate of 50 mL/min. A radio frequency at 13.56 MHz was applied. The membranes were exposed to the plasmas for various exposure times (5–40 min) and glow-discharge powers (40–90 W). After the treatment the gas flow was stopped, and the pressure in the reactor was brought up to atmospheric pressure by backfilling with nitrogen. The plasma-treated membranes were kept in nitrogen atmosphere until use.

Characterization of Polyurethane Membranes

Water Content of Polyurethane Membranes: The water-uptake behavior of polyurethane membranes was determined in distilled water. Dry membrane pieces were placed in distilled water and kept at a constant temperature of 25±0.5°C until they reached equilibrium. Swollen membranes were removed and weighed by an electronic balance (Shimadzu, Japan, EB.280 $\pm 1.10^{-3}$ g). The water content of the swollen membranes were calculated by using the following expression:

$$\text{Water uptake\%} = [(W_s - W_o)/W_o] \times 100 \quad (1)$$

where W_o and W_s are weights of the sample before and after swelling, respectively.

Contact Angle Measurements: The glow-discharge-treated polyurethane membranes were characterized using the captive-bubble method underwater contact-angle measuring technique.[9] The device used consists of a traveling goniometer with ×15 eyepieces at variable-intensity light sources and a micrometer-adjustable X–Y stage vertically mounted on an optical bench. The stage contains a Plexiglas container in which a Teflon plate suspends. The polymer sample was held on the underside of the Teflon plate by means of small Teflon clips. The container was then filled with triple distilled water, and the plate with sample was lowered into the container until the sample was completely immersed. A bubble of air with a volume of about 0.5 μL was then formed at the tip of the Hamilton microsyringe, detached, and allowed to rise to the polymer–water interface. The air bubbles were photographed within 5 min after reaching equilibrium of contact with the samples. Experiments were carried out at at 25°C. The equilibrium contact angles (θ_{air}) were calculated from the height (h) and the width (b) of the air bubbles at the surfaces of polyurethane samples by using eq. (2). The mean value of 10 measurements was considered. The reproducibility of contact angles was ±2%.

$$\theta_{air} = \cos^{-1}[(2h/b) - 1] \quad \text{for } \theta_{air} < 90°\text{C} \quad (2)$$

Atomic Force Microscopy Studies: In order to observe the surface topography of the untreated and glow-discharge-treated membranes, atomic force micrographs were taken with a AFM (Topometrix TMX 2000 Explorer; in contact mode in air). Used with this microscope were a 130 μm tripod scanner and a pyramidal tip of the type used for topographic images. The force exerted by probe on the surface was 0.7 nN.

Protein Adsorption from Aqueous Solutions

Bovine serum albumin (BSA) and fibrinogen adsorption levels on the untreated and the glow-discharge-treated polyurethane membranes were studied batch-wise in K_2HPO_4–KH_2PO_4 buffer media at pH 7.4. The initial protein concentration was 1.0 mg/mL in each case. In a typical adsorption experiment, protein was dissolved in 25 mL of buffer solution, and 3 membrane pieces (1 cm in diameter) were added. These experiments were conducted at 25°C. At the end of the predetermined equilibrium period (i.e., 1 h), the membranes were removed from the solution. The amount of protein in the solution was determined spectrophotometrically at 280 nm, and the adsorbed amount was calculated from the following equation:

$$q = [(C_O - C_A).V]/A \quad (3)$$

where q is the amount of protein adsorbed (μg/cm^2); C_O and C_A are, respectively, the concentrations (μg/mL) of the protein in the solution initially and after the adsorption experiments; V is the volume of the aqueous phase (mL); and A is the total surface area (cm^2) of the used membranes.

Competitive Adsorption of Blood Proteins from Human Plasma

Adsorption of blood proteins (i.e., human serum albumin, γ-globulin, and fibrinogen) from human plasma on the untreated and glow-discharge-treated membranes was studied in a batch-wise reactor. The blood samples were obtained from healthy human donors and centrifuged at 500 g for 30 min at room temperature to separate the plasma. Then 10 mL of the freshly separated human plasma containing albumin (38.5 mg/mL), fibrinogen (2.43 mg/mL), and γ-globulin (18.4 mg/mL) was incubated with 3 pieces (1 cm in diameter) of the untreated or glow-discharge-treated membranes for 1 h. The temperature was kept constant at 25°C. HSA and fibrinogen concentrations were determined by using bromocresol green dye and the Clauss method, respectively. Total protein concentration was determined by the Lowry method. γ-globulin concentration was determined from the difference.[10]

Fluorescence Spectroscopy Studies for Conformational Changes

To examine the effects of adsorption on conformational changes of proteins, fluorescence spectra of the native albumin, heat-denatured albumin, and desorbed albumin were compared. Native albumin was heat-denatured by incubating the aqueous solution (3 mg/mL, pH: 7.4, ionic strength: 0.1) at 70°C for 90 min. Fluorimetric measurements were achieved with a Jasco FP-550 spectrofluorometer using 1 cm^2 quartz cells. Monochromatic readings were taken from a digital display with a 0.25-sec time constant and a 3-nm bandwidth on the excitation side and 5 nm on the emission side. Initial calibration was obtained for a standard solution of albumin in PBS at 280 nm fluorescence excitation and 342 nm emission wavelengths.

RESULTS AND DISCUSSION

Properties of Polyurethane Membranes

A variety of approaches have been taken to enhance the blood compatibility of polymer surfaces. Among them, of interest to us is the surface modification of existing polymers without changing bulk properties. These modifications are based on such factors as surface wettability, protein adsorption, platelet adhesion, and other blood coagulation factors.[11]

Glow-discharge treatment is an attractive technique to modify the chemical and physical properties of polymeric biomaterials.[12–15] This technique provides the advantage of changing surface properties without affecting those of the bulk structure. Glow-discharge treatment may be used to etch the polymeric surface (e.g., with argon or oxygen plasma), to form individual reactive functional groups (e.g., amine from ammonia, hydroxyl from water, etc.), or to deposit a polymer-like coating (using organic chemicals, polymerizable monomers, etc.). Since the surface is a determinant issue for biomaterials, in this study we attempted to create blood-compatible polyurethane surfaces utilizing this technology. Argon and oxygen were selected as the model gases to modify the membranes in a glow-discharge system. Both the exposure time and the glow-discharge power were varied in order to alter the number of argon- and oxygen-containing surface active groups.

As is well known, polymer surfaces undergo oxidation when exposed to oxidative plasma or brought into contact with air after exposure to gas plasmas. The extent of oxidation greatly depends on the composition of the gas, the polymer substrate, the reactor design, and the discharge conditions.[16] In this study polyurethane membranes were exposed to a glow-discharge plasma of oxygen or argon, and air–water contact angles were detected after these treatments using the captive-bubble method. The contact-angle results are given in Figure 1.

It was observed that the glow-discharge treatment of the polyurethane membranes reduced the contact angles for both the oxygen- and argon-plasma treatments. The contact-angle value of untreated polyurethane membrane was 63.2°. There was a significant decrease in the contact-angle values after glow-discharge treatment because of the oxidation of radicals created by the plasma process. The determined minimum contact-angle values were 52.5° for the argon-plasma-treated and 45.7° for the oxygen plasma-treated-samples. It should be noted that the oxygen-plasma-treated membranes have more hydrophilic structure than the argon-plasma-treated surfaces. It is interesting to note that surface hydrophilicity of the argon-plasma-treated membranes increased with increasing glow-discharge power and treatment time. This probably originated from oxidation of the radi-

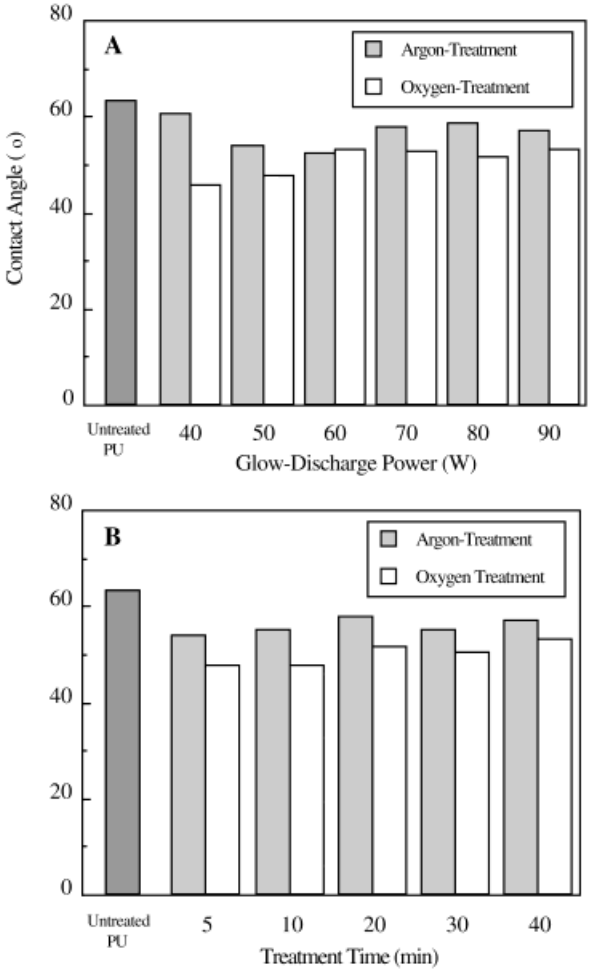

Figure 1 Effect of glow-discharge treatment on contact-angle values of polyurethane membranes: (a) glow-discharge power (treatment time: 5 min); (b) treatment time (discharge power: 50 W).

cals, which were created on the surface, with air/oxygen since the reactor was opened to atmosphere after plasma treatment. It is also worth noting that the contact angles were measured through the water phase. Relatively small contact angles indicate a relatively more hydrophilic structure. This shows that the hydrophilic character of the polyurethane surfaces increased with glow-discharge treatment in either case.

To define the effect of glow-discharge treatment on water-uptake properties, the water uptake of the untreated and oxygen-plasma-treated or argon-plasma-treated membranes were determined in distilled water. The equilibrium water uptake of the untreated polyurethane membrane was about 8.9% (w/w). But after glow-discharge treatment, the equilibrium water uptake increased because of the hydrophilization of the membranes. The water uptake values were 12.6% (w/w) and 14.8%, respectively, for argon-treated and oxygen-treated samples. These results, as do the contact-angle results, demonstrate that the oxygen-glow-discharge treatment provides more hydrophilic surfaces than the argon treatment. Surface hydrophilicity increased in the following order: oxygen-treated polyurethane > argon-treated polyurethane > untreated polyurethane.

Topography of the membrane surfaces were examined using atomic force microscopy. As can be seen in Figure 2, untreated polyurethane surfaces demonstrated high smoothness and homogeneity. It is apparent that the glow-discharge-treated polyurethane membranes have a rougher surface than the untreated membrane. The mean surface roughness (RMS) of untreated polyurethane membrane was found to be 25.08 nm when imaged in contact mode. The RMS increased with glow-discharge treatment. For example, the RMS values obtained for the argon- and oxygen-glow-discharge-treated membranes were 38.74 nm and 50.31 nm, respectively.

Protein Adsorption Studies

It has been advocated that adsorption of plasma proteins to polymer materials profoundly affects the interaction of blood cells with polymer materials and consequently the thrombus formation on the materials. In particular, fibrinogen is known to be a protein with high surface activity.[17] It plays an important role in the clotting system in both the plasma phase (intrinsic) and the cellular phase (platelet aggregation) in normal hemostasis and has also been implicated in thrombosis on foreign surfaces.[18] Fibrinogen adsorption is a well-known contributor to surface-induced thrombosis. Platelets contain a receptor site specific for fibrinogen, which is active only when platelets are activated. Meanwhile, thrombus formation decreases if the first adsorbed protein is albumin. Here the protein adsorption capacities of polyurethane surfaces treated with oxygen or argon plasmas were compared as functions of the applied glow-discharge power and exposure time.

Effects of Glow-Discharge Power

The BSA and fibrinogen adsorption capacities of the argon- or oxygen-treated polyurethane membranes at different glow-discharge powers are given in that order in Figure 3(a,b). In both cases,

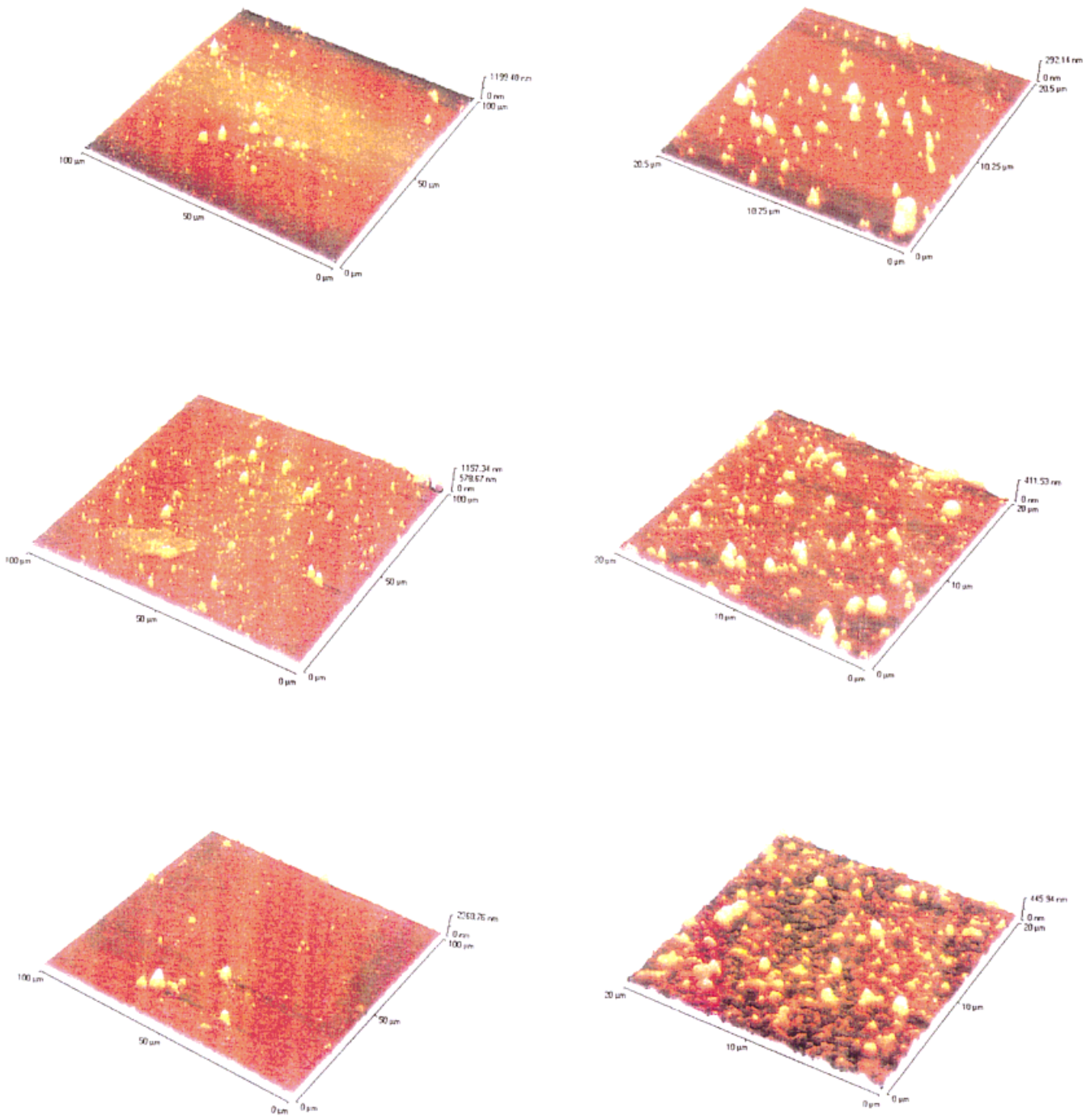

Figure 2 Atomic force microscopy (AFM) images showing surface morphology of (a) untreated; (b) argon treated; (c) oxygen-treated membranes. The AFM images have been shaded by computer reprocessing to enhance the surface structure. The area of the images is 100 μm $\times$ 100 μm for (i) and 20.5 μm $\times$ 20.5 μm for (ii).

the adsorption capacities of BSA and fibrinogen decreased with an increase in the discharge power, which is a result of the increase in the number of active groups on the surface of polyurethane membranes with the applied discharge power.

The rate of adsorption of albumin and fibrinogen onto the untreated membranes was 202.6 and 115 μg/cm^2, respectively. In both cases, the adsorption of albumin is almost 2 times higher than that of fibrinogen. It was observed that the adsorption drop of BSA from 202.6 to 46.5 μg/cm^2

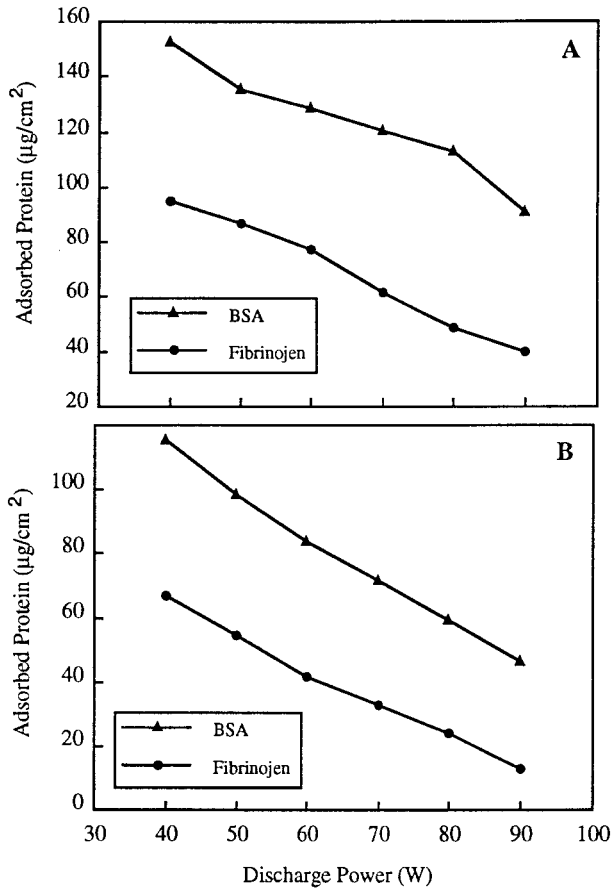

Figure 3 Effect of glow-discharge power on protein adsorption: (a) argon plasma; (b) oxygen plasma (plasma treatment time: 5 min; initial protein concentration: 1 mg/mL; pH: 7.4; *T*: 25°C). Each data point is an average of three parallel studies.

and the adsorption drop of fibrinogen from 115 to 12.9 $\mu g/cm^2$ were the lowest values for the samples on which oxygen plasma was applied with 90 W for 5 min. The present investigation showed that the order in which the interaction of proteins (i.e., adsorption capacity) with the polymers was weakened: untreated polyurethane > argon-plasma-treated polyurethane > oxygen-plasma-treated polyurethane.

Effects of Glow-Discharge Exposure Time

The adsorption capacities of both the argon- and the oxygen-glow-discharge-treated membranes for BSA and fibrinogen decreased significantly with glow-discharge exposure time. The lowest values obtained were 69.1 and 27.3 $\mu g/cm^2$ for albumin and fibrinogen, respectively, for samples treated by oxygen plasma with 50 W for 40 min. These effects can be attributed to an increase in the number of oxidative groups on the surfaces of the membranes (Fig. 4).

Competitive Adsorption of Blood Proteins from Human Plasma

Adsorption Time

Blood plasma and argon-treated polyurethane samples were incubated together for periods ranging between 5 min and 60 min, and the competitive adsorption behavior of the blood proteins was obtained as a function of time. As seen from the results, as shown in Figure 5, the initial rate of adsorption is high. Plateau values (i.e., adsorption equilibrium) are achieved within 20 min for all proteins. When the adsorption rates of blood proteins are compared, it can be seen that the adsorption of albumin (HSA) is faster than

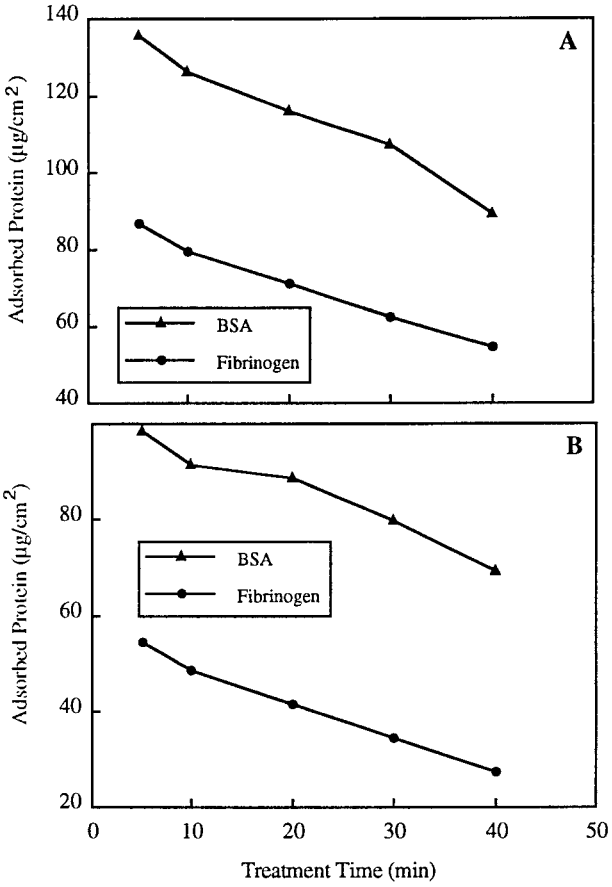

Figure 4 Effect of treatment time on protein adsorption: (a) argon plasma; (b) oxygen plasma (glow-discharge power: 50 W; protein concentration: 1 mg/mL; pH: 7.4; *T*: 25°C). Each data point is an average of three parallel studies.

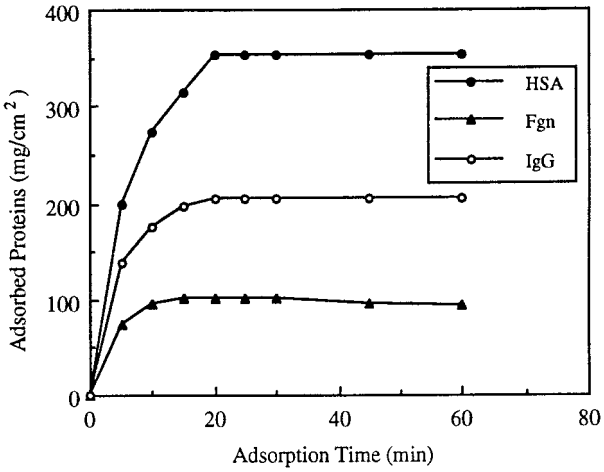

Figure 5 Adsorption behavior of blood proteins on argon-treated polyurethane membranes as function of time at 20°C (glow-discharge power: 40 W; treatment time: 5 min; initial concentrations of HSA: 38.5 mg/mL; IgG: 18.4 mg/mL and fibrinogen: 2.43 mg/mL). Each data point is an average of three parallel studies.

gamma globulin (IgG) and fibrinogen (Fgn). This shows the strong affinity between albumin and the surface. From these results the order of the adsorption rate can be given as: albumin > gamma globulin > fibrinogen

The results of several experimental studies on the adsorption kinetics of proteins by various biomaterials have demonstrated a wide range of adsorption rates. For example, Lassen and Malmsten recently investigated competitive protein adsorption from a ternary mixture of human serum albumin (HSA), human IgG, and human fibrinogen onto plasma polymerized hexamethyldisiloxane. They considered the optimum adsorption time to be 2 h.[19] Lemm investigated the competitive adsorption of blood proteins on different biomaterial surfaces including biomer, avcothane, and plathuran.[20] He reported that the surface concentrations of proteins are rather unstable during the first 60 min; albumin seems to replace recently adsorbed fibrinogen and gamma globulin. Han et al. investigated the adsorption of blood proteins onto sulfonated poly(ethylene oxide)–grafted polyurethane surfaces and found that the adsorption rates were very high (equilibrium adsorption time: 60 min).[21] Takahara et al. investigated fibrinogen adsorption onto segmented polyurethanes with various polyol soft segments and reported 30–40 min as the equilibrium adsorption time.[22] Okkema et al. investigated the blood-contacting properties of polyurethanes based on sulfonic acid–containing diol chain extenders such as poly(tetramethylene oxide) and poly(ethylene oxide), and they obtained 60 min as the equilibrium time for fibrinogen deposition.[23] Note that in such an adsorption process, there are several parameters that determine the adsorption rate, such as agitation (or flow) rate in the aqueous phase, sorbent structural properties (e.g., size, porosity, surface area), amount of sorbent, initial concentrations of proteins, and of course existence of other biomolecules that may compete with the protein of interest for possible adsorption sites. All individual experimental study published in the literature has been performed under different conditions; therefore, it is not easy to compare the reported adsorption rates and equilibrium times. In our case the optimum equilibrium time was taken as 30 min.

Adsorption Isotherms

The relationship between the amounts of adsorbed protein and the initial concentrations of the proteins is given in Figure 6. Note that this figure was constructed by using the plateau values (with the equilibrium time considered as 30 min) given in the previous section. The adsorbed amounts increased with an increase in the initial concentrations of blood proteins and reached saturation for the initial concentrations of 4.8, 2.3 and 0.6 mg/mL for HSA, IgG, and fibrinogen, respectively. The order of adsorption capacity obtained was: HSA > gamma globulin > fibrinogen.

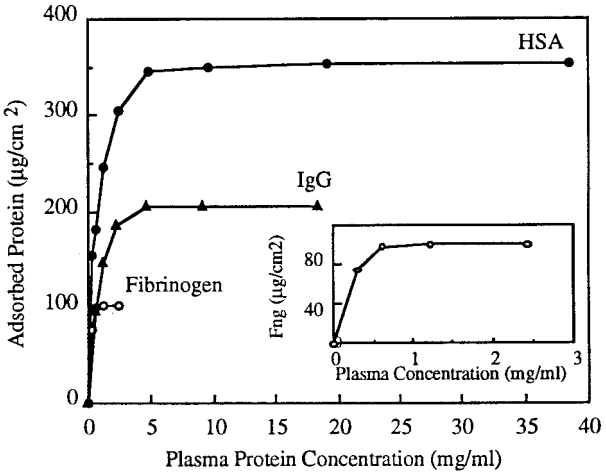

Figure 6 Adsorption isotherms for blood proteins on argon-treated PU surfaces (glow-discharge power: 40 W; treatment time: 5 min). Each data point is an average of three parallel studies.

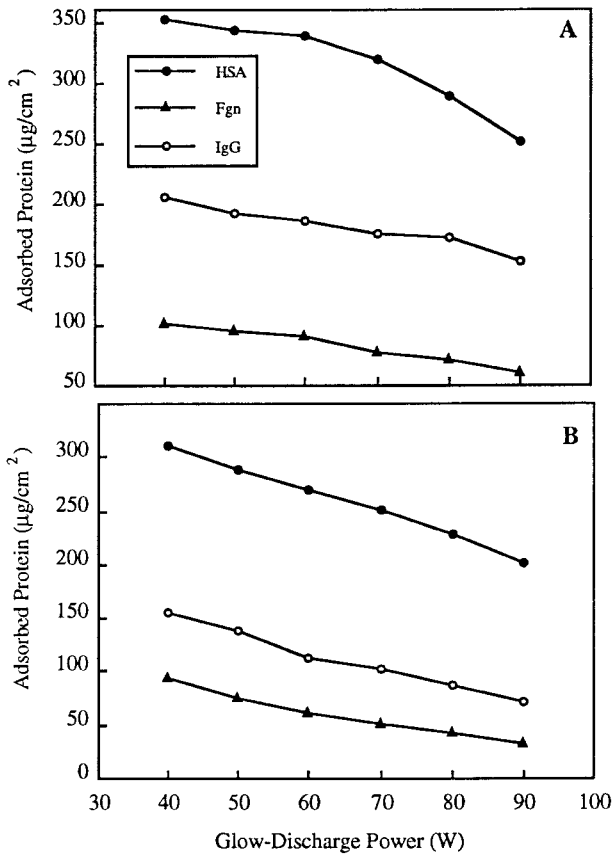

Figure 7 Effects of glow-discharge power on competitive adsorption of blood proteins onto polyurethane membranes: (a) argon treated; (b) oxygen treated (glow-discharge treatment time: 5 min; HSA: 38.5 mg/mL; IgG: 18.4 mg/mL; fibrinogen: 2.43 mg/mL). Each data point is an average of three parallel studies.

Effect of Glow Discharge Power and Exposure Time

When blood is placed in contact with any foreign surface, a spontaneous competitive adsorption of proteins and glycoproteins occurs at the surface and forms a complex protein coating on the surface. These adsorptions depend greatly on the surface characteristics of polymers, which affect their blood compatibility. Some of these adsorption processes are partially or completely reversible.[24]

Interesting results were obtained in the competitive protein adsorption studies. Figures 7 and 8 show that albumin adsorption on both untreated and glow-discharge-treated polyurethane membranes were higher than the adsorption of the other two proteins. The lowest adsorption was observed for fibrinogen. Ito et al. investigated the adsorption of plasma proteins to the poly(ether urethane)–urea derivatives carrying tertiary amino groups in the side chains, and they observed the order of protein adsorption as fibrinogen < gamma globulin < albumin.[24] This order agrees with the physiological concentrations of the respective proteins.

The protein adsorption values onto the untreated polyurethane membranes were high (387.5 $\mu g/cm^2$ for HSA, 236.5 $\mu g/cm^2$ for gamma globulin, and 115.5 $\mu g/cm^2$ for fibrinogen). Glow-discharge treatment significantly decreased the protein adsorption capacities of the membranes for oxygen treatment (201.3 $\mu g/cm^2$ for HSA, 68.7 $\mu g/cm^2$ for gamma globulin, and 32.5 $\mu g/cm^2$ for fibrinogen) and for argon treatment (251.6 $\mu g/cm^2$ for HSA, 150.7 $\mu g/cm^2$ for gamma globulin, and 55.8 $\mu g/cm^2$ for fibrinogen), possibly because of the hydrophilization of polymeric surfaces (Fig. 9). It is well known that an increase in surface

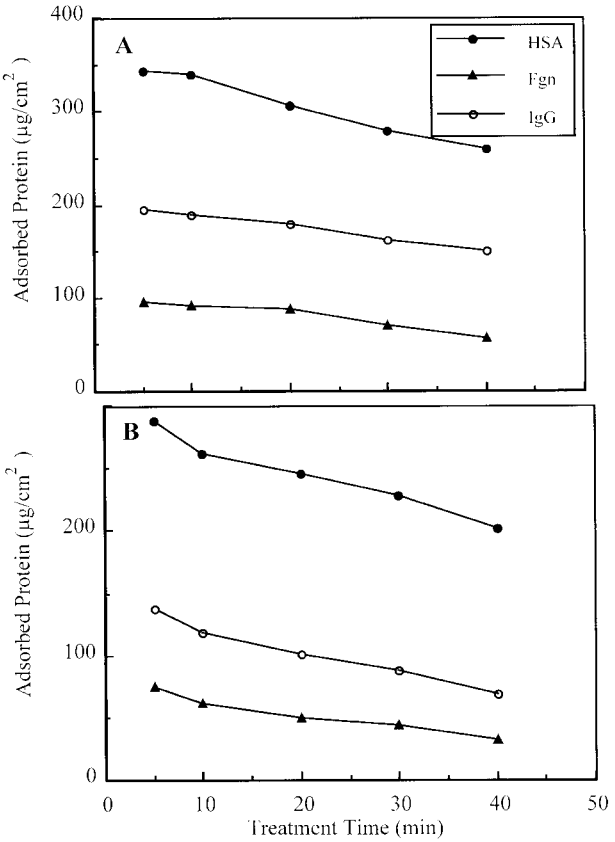

Figure 8 Effects of glow-discharge treatment time on competitive adsorption of blood proteins onto polyurethane membranes: (a) argon treated; (b) oxygen treated (glow-discharge power: 50 W; HSA: 38.5 mg/mL; IgG: 18.4 mg/mL; and Fibrinogen: 2.43 mg/mL). Each data point is an average of three parallel studies.

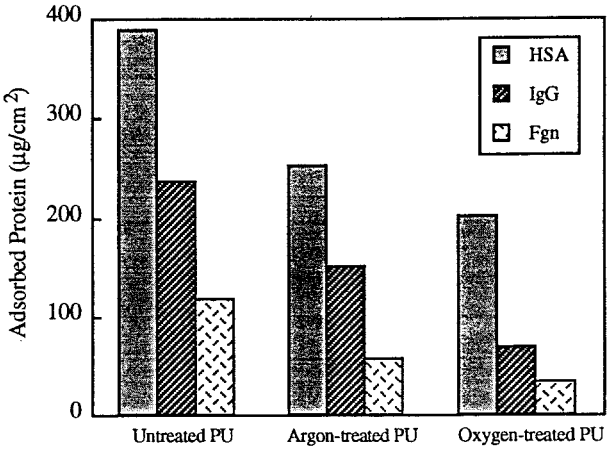

Figure 9 Comparison of protein adsorption capacities.

hydrophilicity leads to a diminished interaction between the surface and the blood components.[25]

Conformational Changes of Adsorbed Proteins

The conformational changes of the proteins on adsorption were examined with fluorescence spectrophotometry. The fluorescence spectra of native, heat-denatured, desorbed albumin (HSA reference materials) were obtained. The results showed that a clear difference existed between the fluorescence spectra of native albumin and heat-denatured albumin. On the other hand, the fluorescence spectra of the desorbed samples were very close to those of native albumin, and no significant shift of maximum wavelength was detected (Fig. 10). It can be concluded that adsorption on the polyurethane surfaces did not cause any denaturation of human serum albumin molecules.

CONCLUSIONS

It was observed that glow-discharge treatment of polyurethane membranes reduced the water contact angles for both oxygen- and argon-plasma-treated surfaces. The contact-angle value of untreated membrane was 63.2°. There was a significant decrease in this value after glow-discharge treatment because of the oxidation of radicals that were created by the plasma process. The determined minimum contact-angle values were 52.5° for argon-plasma-treated and 45.7° for oxygen-plasma treated samples. The equilibrium water uptake was about 8.9% (w/w) for untreated samples. But after glow-discharge treatment, the equilibrium water-uptake values increased to 12.6% (w/w) for argon- and to 14.8% (w/w) for oxygen-treated samples because of the hydrophilization of the structures. These results demonstrate that oxygen-glow-discharge treatment provides more hydrophilic surfaces compared to argon treatment. Atomic force microscopy demonstrated high smoothness and homogeneity for untreated surfaces. It is apparent that the glow-discharge-treated membranes have rougher surfaces than the untreated membranes. The mean surface roughness of untreated membrane was 25.08 nm. The values obtained for the argon- and oxygen-glow-discharge-treated membranes imaged in contact mode were 38.74 nm and 50.31 nm, respectively. Albumin and fibrinogen adsorptions onto the untreated membranes were 202.6 $\mu g/cm^2$ and 115 $\mu g/cm^2$, respectively. The present investigation showed that the interaction of proteins (i.e., adsorption capacity) with polymers was weakened according to the properties of polymers in the order of: untreated membrane > argon-plasma-treated membrane > oxygen-plasma-treated membrane. High initial adsorption rates were observed for blood proteins, and then plateau values (i.e., adsorption equilibrium) were gradually reached within 30 min for all proteins (i.e., albumin, fibrinogen, and gamma globulin) for argon-treated membranes. The order of adsorption rates of proteins obtained was as follows: albumin > gamma globulin > fibrinogen. Parallel to the initial concentrations of blood proteins, the

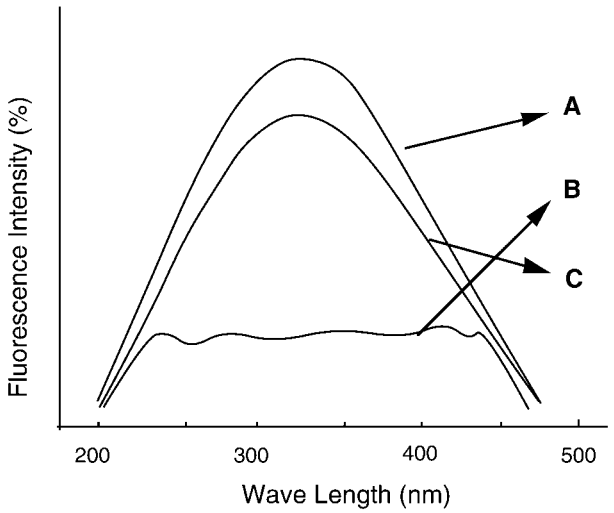

Figure 10 Fluorescence spectra of adsorbed albumin molecules: (a) native albumin; (b) heat-denatured albumin; (c) plasma-interacted albumin.

adsorption capacities changed. They reached to a saturation level at 4.8, 2.3, and 0.61 mg/mL of the initial protein concentrations of HSA, IgG, and fibrinogen, respectively. The order of adsorption capacity obtained was: HSA > gamma globulin > fibrinogen for argon-treated membranes. The protein adsorption values onto the untreated membranes were high (387.5 $\mu g/cm^2$ for HSA, 236.5 $\mu g/cm^2$ for gamma globulin, and 115.5 $\mu g/cm^2$ for fibrinogen). Glow-discharge treatment significantly decreased the protein adsorption capacities of the membranes. Lower values were obtained for oxygen-treated samples (201.3 $\mu g/cm^2$ for HSA, 68.7 $\mu g/cm^2$ for gamma globulin, and 32.5 $\mu g/cm^2$ for fibrinogen) than for argon-treated samples (251.6 $\mu g/cm^2$ for HSA, 150.7 $\mu g/cm^2$ for gamma globulin, and 55.8 $\mu g/cm^2$ for fibrinogen), possibly because of the hydrophilization of polymeric surfaces. The fluorescence spectra of native, desorbed, and heat-denatured albumin (HSA reference materials) demonstrated no significant difference between the desorbed and native albumin. Thus it may be concluded that polyurethane surfaces did not cause any denaturation of human serum albumin molecules on adsorption.

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