Evaluation of the Permeability and Blood-Compatibility Properties of Membranes Formed by Physical Interpenetration of Chitosan with PEO/PPO/PEO Triblock Copolymers

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ABSTRACT: In order to develop blood compatible membranes with controlled porosity, we have fabricated and examined the properties of physical interpenetrating network (PIN) of chitosan and poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO) triblock copolymers (Pluronics®). Degree of equilibrium swelling, scanning electron microscopy, and electron spectroscopy for chemical analysis (ESCA) were used to characterize the bulk and surface properties. Vitamin B_{12} and human serum albumin were used as permeability markers. Platelet adhesion and activation were used to determine the blood-interaction properties of the PIN membranes. Unlike chitosan membranes that were nonporous, the chitosan-Pluronic PIN membranes were highly porous with the pore size, depending on the type of incorporated Pluronic polyol. ESCA results showed a significant increase in the —C—O— signal of C1s spectra on the PIN membranes that correlates with the presence of PEO chains on the surface. The permeability coefficients of vitamin B_{12} and albumin were higher in the chitosan-Pluronic PIN membranes than in the control. The number of adherent platelets and the extent of activation were significantly reduced on the chitosan-Pluronic PIN membranes. The decrease in platelet adhesion and activation correlated positively with the PEO chain length of the incorporated Pluronic polyols. The results of this study show that chitosan-Pluronic PIN membranes offer a blood-compatible alternative with a higher-molecular-weight cutoff for use in hemodialysis and related applications. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 1274–1284, 2001

Key words: chitosan; PEO/PPO/PEO triblock copolymers; physical interpenetrating network; porous membranes; blood compatibility

INTRODUCTION

Since the development of the first rotating drum artificial kidney by Wilhelm Kolff in 1942, hemodialysis has become an important medical procedure for the treatment of end-stage renal disease (ESRD) and severe poisoning.[1] Current estimate suggests that more than 16 million dialyzers are used annually in the United States.[2] The etiologic factors of ESRD include diabetes mellitus, chronic hypertension, glomerulonephritis, and other urological diseases. In ESRD, dialysis treatment is initiated to remove uremic toxins from the body through a positive concentration gradient between blood and the dialysate fluid. Although a number of advances have been made, the morbid-

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ity and mortality rates associated with dialysis therapy remain very high. The mortality rate of 24% per year in dialysis patients in the United States is the highest in developed countries. It has been shown that the average life expectancy of an ESRD patient is one-fourth to one-fifth that of the general population.[3,4]

The central element of the dialysis instrument is a semipermeable membrane that selectively removes low-molecular-weight uremic toxins from blood. The most commonly used dialysis membranes are made from cellulose or cellulose derivatives.[5] These cellulosic membranes are known to cause significant thrombosis and complement activation upon blood contact.[6,7] Surface-induced thrombosis on polymeric membrane surface is initiated by the adsorption of plasma proteins followed by adhesion and activation of platelets.[8] Secretion of adenosine diphosphate, thrombin, and other granular contents from activating platelets interact with resting platelets to cause aggregates. In addition, platelet-activating factors trigger the intrinsic coagulation reaction leading to the formation of cell-entrapped fibrin clot on the membrane surface.[9] Currently, surface-induced thrombosis on the membranes is minimized by the systemic infusion of anticoagulants, heparin, or sodium citrate, before dialysis therapy, which could sometimes lead to fatal outcome.[10] The complement system is designed as a first line of defense against foreign particulate matter such as bacterial infection.[11] On cellulosic membranes, complement activation occurs by the alternative pathway within a half-hour of initiation of hemodialysis therapy.[12] Complement activation leads to a marked rise in the plasma C3a concentration associated with a corresponding sharp decline in the white blood cell count.[13,14]

Furthermore, the cellulosic membranes remove only an insignificant fraction of solutes at $>$ 2000 daltons. By contrast, normal kidney can excrete substances, particularly proteins, at $\leq$ 60,000 daltons. Newer high-flux membranes made from hydrophobic synthetic polymers, such as poly(methylmethacrylate), can nonspecifically adsorb high-molecular-weight proteins on their surface.[15] However, the available surface area and the large number of proteins competing for the sites limit the maximum adsorbed amount on the membrane. One the major complication of chronic hemodialysis leading to significant morbidity is the accumulation of β2-microglobulin (β2M), a component of the amyloid fibrils.[16] β2M, a protein of 11,600 daltons, accumulates in dialysis patients resulting in amyloid formation. Amyloid deposition mainly involves bone and joint structures, presenting as carpal tunnel syndrome, destructive arthropathy, and bone erosions and cysts. Although the molecular pathogenesis of this complication remains largely unknown, various diagnostic imaging techniques have shown progressive amyloid deposition in the bone and joint tissues of dialysis patients.[17] Therapy for dialysis-related amyloidosis is limited to symptomatic and palliative approaches, as well as surgical removal of amyloid deposit.[18] Because of the severity and the destructive nature of the complication, it is very important to investigate alternative dialysis membrane materials through which β2M can permeate.

Over the past several years, our group has used chitosan, a natural polymer of D-glucosamine, for the fabrication of hemodialysis membranes (Figure 1).[19–21] Chitosan is derived from chitin, a waste product of marine crustaceans.[22] Like cellulose, the inter- and intramolecular hydrogen bonding in chitosan structure allows it to form membranes with excellent mechanical strength suitable for dialysis application. Chitosan, unfortunately is highly thrombogenic probably due to the cationic surface charge.[20,23,24] To improve blood compatibility of chitosan membranes, we have modified the surface by a novel surface interpenetration method using negatively charged modifiers such as heparin and poly(ethylene glycol) sulfonate.[20,21,24] The control and surface-modified chitosan membranes also were poorly permeable to compounds of $>$ 2000.[19,20]

To improve the blood compatibility and permeability properties of chitosan membranes, in the present study, we have fabricated physical interpenetrating network (PIN) membranes from chitosan and poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO) triblock copolymers (Pluronic®). Four different types of Pluronic polyols, with varying ethylene oxide (EO) and propylene oxide (PO) chain lengths, were used. The hydroxyl and amine functional groups of chitosan are known to interact with the ether oxygen of PEO or PPO by intermolecular hydrogen bonding to form macromolecular complexes.[25] Upon neutralization of the chitosan salt, the hydrogen-bonded structures result in the formation of physical interpenetrating network (PIN). The hydrophilic PEO chains of Pluronic polyols are expected to extend into the bulk aqueous medium and prevent plasma protein adsorption and cell adhesion by a steric repulsion mech-

Table I Properties of the Poly(ethylene Oxide)Poly(Propylene Oxide)/Poly(Ethylene Oxide) (Pluronic®) Triblock Copolymers

Copolymer	Abbreviation	Physical State	Ave. M_r	n(EO/PO/EO)
Pluronic P65	PP65	Paste	3,400	19/30/19
Pluronic F68	PF68	Solid	8,350	75/30/75
Pluronic P105	PP105	Paste	6,500	37/56/37
Pluronic F108	PF108	Solid	14,000	122/56/122

Adapted from Amiji and Park.[26]

anism.[26–28] In addition, the incorporation of hydrophilic polymers such as PEO with chitosan does tend to increase the permeability of higher-molecular-weight compounds.[19]

MATERIALS AND METHODS

Materials

Chitosan with a viscosity-average M_r of 750,000 and degree of deacetylation of 87.6% was obtained from Pronova Biopolymers (Raymond, WA). As shown in Table I, four different types of Pluronic® polyols (i.e., PP65, PF68, PP105, and PF108) were obtained from BASF (Parsipanny, NJ). The choice of the Pluronic polyols was based on the chain length of the EO and PO residues. The EO residues varied from 19 to 122, and the PO residues were 30 and 56. Vitamin B_{12} (cyanocobalamine, 1355 daltons) and human serum albumin (69,000 daltons) were purchased from Sigma Chemical Company (St. Louis, MO). Deionized distilled water (NanoPure II, Barnsted/Thermolyne, Dubuque, IO) was used to prepare all aqueous solutions. All other chemicals were of standard analytical grade and were used as received.

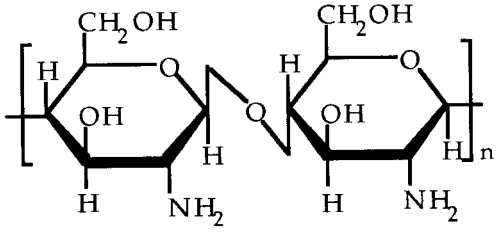
Membrane Fabrication

Chitosan was dissolved in 0.10*M* acetic acid to prepare a 1.0% (w/v) solution. The polymer was purified from proteins and inorganic impurities by precipitation in 0.10*M* sodium hydroxide, followed by washing with deionized distilled water, and freeze-drying; 100 mg of purified chitosan, dissolved in 10 mL of 0.10 *M* acetic acid, was allowed to spread evenly on a polystyrene Petri dish. In some instances, Pluronic polyols, dissolved in 0.10*M* acetic acid, were added to the chitosan solution, for a final concentration ranging from 10% (w/w) to 30% (w/w). After drying at room temperature for approximately 48 h, the acetate salt of chitosan was neutralized in 0.10*M* sodium hydroxide. After extensively washing the control and chitosan-Pluronic PIN membranes in deionized distilled water, they were stored at 4°C in deionized distilled water.

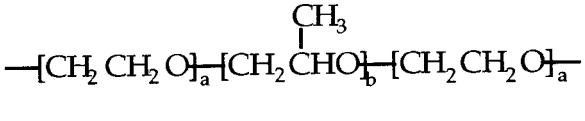
Membrane Characterization

Swelling Studies

The samples of control and chitosan-Pluronic PIN membranes were air-dried for up to 48 h. After



Chitosan



PEO/PPO/PEO Triblock Copolymer (Pluronic®)

Figure 1 The chemical structures of chitosan and poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymers (Pluronic®).

carefully weighing the membrane samples, each was placed in 100 mL of phosphate-buffered saline (PBS, pH 7.4) for 24 h at room temperature. Equilibrium swelling ratio (Q) of the membranes was calculated as follows:

$$Q = W_s/W_d$$

where W_s is the weight (in grams) of the swollen membrane and W_d is the weight of the dry membrane.

Scanning Electron Microscopy Analysis

Control and chitosan-Pluronic PIN membranes, containing 20% (w/w) of the Pluronic polyols, were dried by critical point drying using carbon dioxide as a transitional fluid. The samples were mounted on an aluminum sample mount and sputter-coated with gold-palladium. The coated samples were observed with an AMR 1000 (Amray Instruments, Bedford, MA) scanning electron microscope at 10-kV accelerating voltage. The original magnification of the surface scanning electron microscopy (SEM) images of the membranes was ×12,500.

Electron Spectroscopy for Chemical Analysis

Control and chitosan-Pluronic PIN membranes, containing 20% (w/w) of the Pluronic polyols, were sent to the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) at the University of Washington, (Seattle, WA). Analysis of the membranes sample at ≤ 100-Å-thick surface layer was performed using an X-Probe ESCA instrument (Surface Science Instruments, Mountain View, CA) equipped with aluminum $K_{\alpha 1,2}$ monochromatized X-ray source. An electron flood gun set at 5.0 eV was used to minimize surface charging. Surface elemental composition was determined using the standard Scofield photoemission cross sections.[29] The chemical functional groups identity was obtained from the high-resolution peak analysis of carbon-1s (C_{1s}), oxygen-1s (O_{1s}), and nitrogen-1s (N_{1s}) envelopes.

Permeability Studies

The permeability coefficients of vitamin B_{12} and human serum albumin were determined through the control and chitosan-Pluronic PIN membranes, containing 20% (w/w) of the Pluronic polyols, at 37°C using a dialysis apparatus as described previously.[19,20] In this study, 15 mL of vitamin B_{12} (0.1 mg/mL) or albumin (1.0 mg/mL) solution in PBS was placed in the donor compartment of the dialysis apparatus. The receptor compartment, separated by the membrane, was filled with 15 mL of PBS. Both sides of the dialysis apparatus were continuously stirred to ensure uniform analyte distribution during the experiment. At predetermined time intervals, 1 mL of the sample from the receptor compartment was removed and assayed for the diffused analyte. The absorbance of vitamin B_{12} at 361 nm and of albumin at 280 nm was measured with a Shimadzu 160U (Columbia, MD) UV/VIS spectrophotometer. The concentration of the diffused analyte in the receptor compartment was calculated from a calibration curve. The permeability coefficients of vitamin B_{12} and albumin through the control and chitosan-Pluronic PIN membranes were calculated according to the following equation:

$$\ln(C_0/C_t) = PSt/lV$$

where C_o is the initial concentration of the vitamin B_{12} and albumin in the donor compartment, C_t is the concentration at any time t, P is the permeability coefficient (in cm^2/min), S is the surface area of the membrane (1.77 cm^2), V is the volume of solution in the donor compartment (15 mL), and l is the wet thickness of the membrane (~25 μm). Plots of $\ln (C_o/C_t)$ versus (dialysis time/membrane thickness) were constructed and the slope of the line was used to calculate P. The results represent the average permeability coefficient (±SD) of vitamin B_{12} and albumin from three independent experiments.

Platelet Adhesion and Activation

For platelet adhesion studies, control and chitosan-Pluronic PIN membranes (5–7 μm thick), containing 20% (w/w) of the Pluronic polyols, were casted on a 25 × 75-mm glass microscope slide (Fisher Scientific, Pittsburgh, PA) as previously described.[19–21] Briefly, glass slides, cleaned with 2.0% (v/v) Isoclean® (Isolab, Akron, OH) solution, were dip-coated with the chitosan or chitosan-Pluronic solutions in 0.1M acetic acid. The coated slide was neutralized with sodium hydroxide and washed with deionized distilled water as described above. An observation chamber for adherent platelets was assembled consisting of clean glass slide, chitosan-coated slide, or chi-

tosan-Pluronic PIN slide, two polyethylene spacers, and a glass coverslip.

Human blood, obtained from healthy volunteers after informed consent, was collected with heparin in evacuated glass tubes (Vacutainers, Becton-Dickinson, Rutherford, NJ). Heparinized blood was centrifuged at 100*g* for 10 min to obtain platelet-rich plasma (PRP); 200 μL of PRP was instilled into the platelet observation chamber. Platelets in PRP were allowed to adhere on the control and PIN membrane surfaces for 1 h at room temperature. Nonadherent platelets and plasma proteins were removed by washing the chamber with PBS. Adherent platelets were fixed with 2.0% (w/v) glutaraldehyde solution in PBS for 1 h. After washing with PBS, the platelets were stained with 0.1% (w/v) Coomassie Brilliant Blue (Bio-Rad) dye solution for 1.5 h. Stained platelets were observed using a Nikon Labophot® II (Melville, NY) light microscope at ×40 magnification. The image of adherent platelets was transferred to a Sony Trinitron® video display using a Hamamatsu CCD® camera (Hamamatsu City, Japan). The Hamamatsu Argus-10® image processor was used to calculate the number of platelets per 25,000-μm^2 surface area in every field of observation. The extent of platelet activation was determined qualitatively from the spreading behavior of adherent platelets, using a scale of 1–5, where 5 = fully activated platelets, as previously described.[24]

RESULTS AND DISCUSSION

Swelling Studies

The degree of hydration of polymeric materials is an indirect measure of the mechanical strength.[19] As shown in Table II, the equilibrium–swelling ratio of control chitosan membranes was 2.38. The ratio increased to 3.66 and 4.88 upon incorporation of 20% (w/w) PF68 and PF108, respectively. An increase in the equilibrium swelling ratio in chitosan-Pluronic PIN membranes was due to increased hydrophilicity and porosity which allows for more aqueous medium to imbibe in the matrix by capillary action. The mechanical strength of the membranes, however, was compromised by the incorporation of Pluronic polyols at concentrations exceeding 30% (w/w).

Table II Equilibrium Swelling of the Control and Chitosan-Pluronic® Physical Interpenetrating Network Membranes

Membrane Type	Swelling Ratio
Chitosan	2.38 ± 0.22[a]
Chitosan-PP65	3.45 ± 0.89
Chitosan-PF68	3.66 ± 0.50
Chitosan-PP105	4.21 ± 0.72
Chitosan-PF108	4.88 ± 0.34

The equilibrium swelling studies were carried out in phosphate-buffered saline (pH 7.4) for 24 h at room temperature with the control and chitosan-Pluronic membranes containing 20% (w/w) Pluronic polyols.

[a] Mean ± SD (n = 4).

Scanning Electron Microscopy Analysis

As shown in Figure 2, SEM observations showed that the surface of chitosan membrane was completely nonporous. Because of inter- and intramolecular hydrogen bonding in the structure, chitosan is expected to yield highly crystalline matrix upon drying. The addition of Pluronic polyols to chitosan, on the other hand, resulted in membranes with varying degree of porosity. Chitosan-Pluronic PIN membranes, containing 20% (w/w) PF68 [Fig. 2(C)], for instance, were highly porous, with an approximate pore diameter of ~ 100–200 nm. Increasing the number EO residues to 122 and PO residues to 56 in PF108-containing PIN membranes, however, the pore diameter decreased to ~ 50 nm. From a previous study with chitosan–PEO blend membranes,[19] we have found that the pore size and distribution does not correlate with the molecular weight of the incorporated polyol. The mechanism of pore formation in the membrane could be because of leaching or self-association of the Pluronics into micelle-type structures within the membrane. Depending on the molecular weight, approximately 10–20% of the original weight of the incorporated Pluronic polyols was leached out of the membrane during the initial washing steps. Subsequently, however, we did not observe any changes in the net weight of the membranes when they were kept hydrated for up to 1 month, suggesting that there was no additional loss of Pluronic polyols from the membranes.

Surface Chemical Analysis

Because the interactions between blood and polymer surfaces are dictated by the solid–liquid interfacial properties, ESCA was used as an important analytical technique to determine the surface composition and identify the chemical

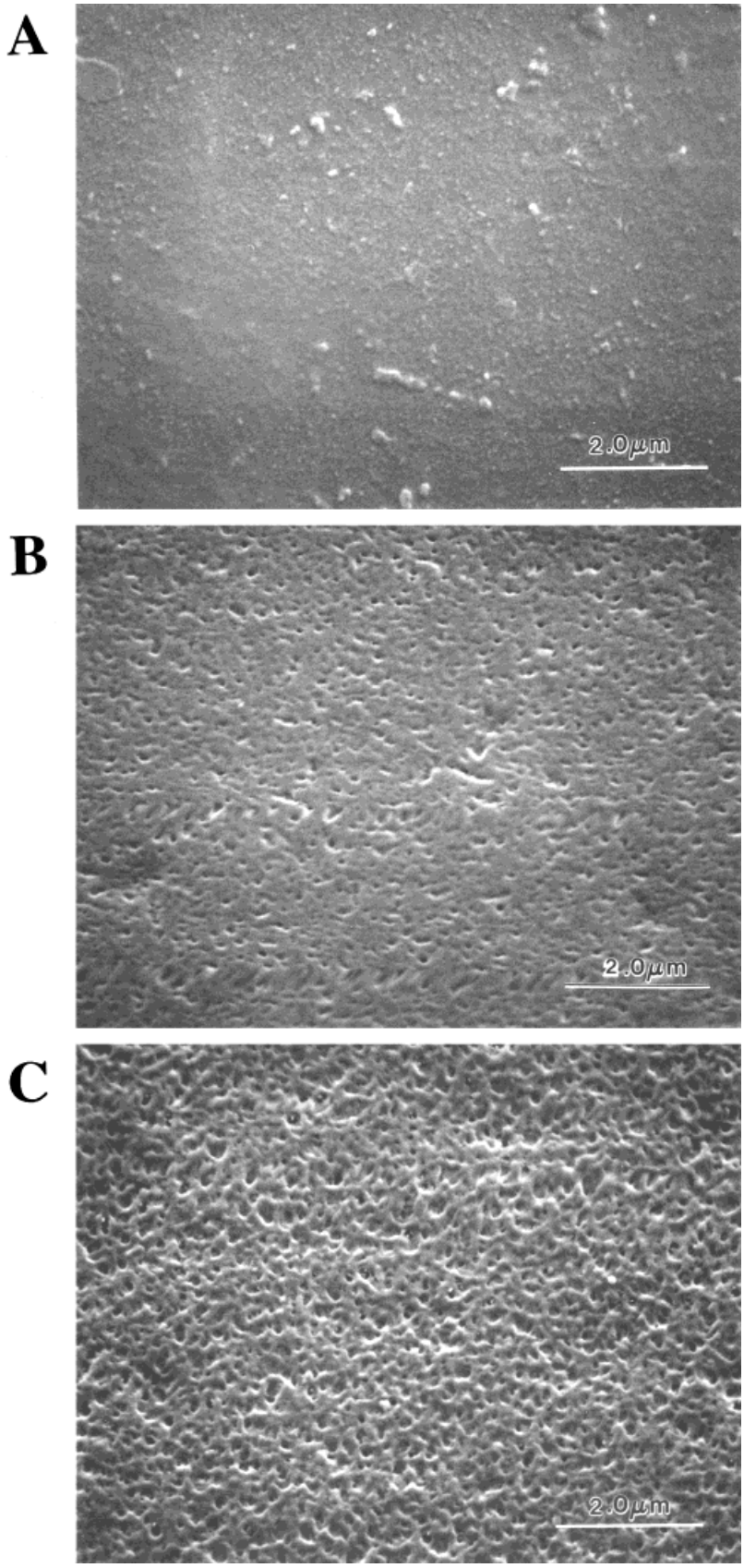


Figure 2 Scanning electron micrographs of the control chitosan memrane (A) and those formed by physical interpenetration of chitosan with poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymers (Pluronic®). The final concentration of Pluronic P-65 (B), F-68 (C), P-105 (D), and F-108 (E) in the membrane was 20% (w/w). Original magnification was 12,500X and the scale bar is equal to a distance of 2.0 μm.

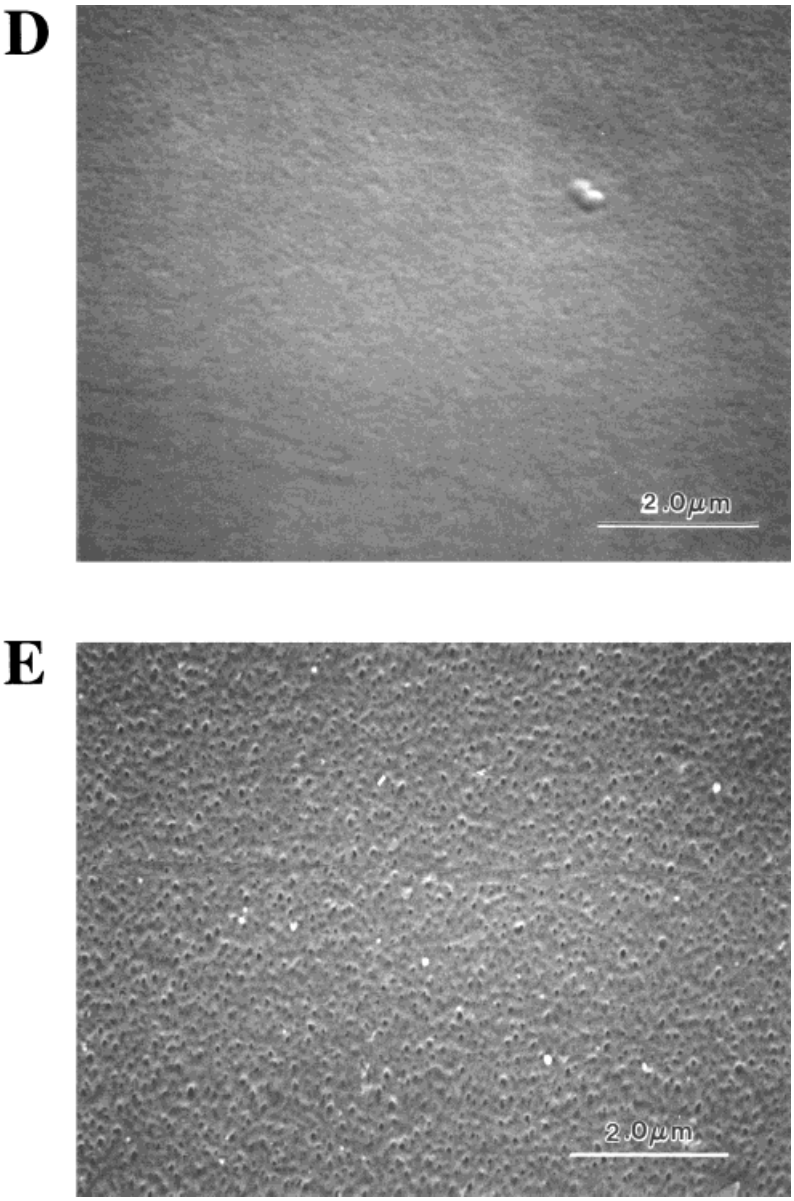


Figure 2 (*Continued from the previous page*)

functional groups on the surface of control and chitosan-Pluronic PIN membranes. The results of ESCA (Table III) show that the elemental composition of carbon (C), oxygen (O), and nitrogen (N) of unmodified chitosan membrane was 65.6% C, 22.0% O, and 4.5% N. This composition is significantly different from the predicted values of 55.0% C, 36.0% O, and 9.0% N for pure chitosan sample. The difference in the elemental composition could be due to the predominant presence of acetyl-D-glucosamine residues on the membrane surface in the dry state or due to environmental contamination. For chitosan-Pluronic PIN membranes, the composition of O on the surface increased as the number of EO residues of Pluronics increased. For chitosan-PF68 PIN membrane, for instance, the O composition was 26.6%. An increase in the O composition of the chitosan-Pluronic PIN membranes as compared with the control could be attributable to the presence of the EO residues of PEO on the membrane surface.

As shown in Table IV, about 57.0% of the high-resolution $C1_s$ spectra at 285.0 eV of chitosan was assigned to the hydrocarbon (—C—C— or —C—H—) functional groups and 33.0% was assigned to the —C—O— groups at 286.5 eV. The

Table III Surface Elemental Analysis on the Control and Chitosan-Pluronic® Physical Interpenetrating Network Membranes

	Surface Elemental Composition (%)			
Membrane Type	C	O	N	Na
Chitosan	65.6	22.0	4.5	2.4
Chitosan-PP65	68.1	24.3	6.0	—
Chitosan-PF68	65.0	26.6	5.4	—
Chitosan-PP105	65.2	26.9	5.1	—
Chitosan-PF108	63.6	26.3	5.7	—

Electron spectroscopy for chemical analysis (ESCA) was performed at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington, Seattle, WA, with the control and chitosan-Pluronic membranes containing 20% (w/w) Pluronic polyols.

remaining 11% was assigned to the —C═O— functional groups at 288.0 eV. The —C═O— functionalities on the surface were probably due to the presence of acetyl-D-glucosamine residues or adsorption of carbon dioxide from the atmosphere. The O_{1s} envelope on chitosan associated with two peaks corresponding to —O—C— and —O═C— species at 73.0% and 27.0%, respectively. The N_{1s} envelope was fitted to only one peak at 399.6-eV binding energy, which corresponds to the — N—C— functionality. On chitosan-Pluronic PIN membrane surfaces, there was a marked reduction in the —C—H— peak intensity and a corresponding increase in the —C—O— signal as compared with unmodified chitosan. On chitosan-PF68 PIN membrane, for instance, the —C—H— peak was 45.0% and the —C—O— peak was 43.0%. The increase in the ether peak on chitosan-Pluronic PIN membranes is consistent with the presence of EO residues of the PEO segment on the membrane surface. Surface-accessible PEO chains will be able to prevent protein adsorption and cell adhesion from blood-contacting membranes by the steric repulsion mechanism.[28]

Permeability Studies

From previous studies.[19,20] we have observed that for relatively low-molecular-weight compounds, such as urea and creatinine, chitosan membranes had similar permeability profile as clinically used cellulose (Cuprophan®) membranes. When the solute molecular weight was increased, as in the case with vitamin B_{12}, for instance, however, the permeability coefficient in chitosan membranes was significantly lower than that in Cuprophan® membranes. The average permeability coefficient of vitamin B_{12} was 4.84×10^{-6} cm^2/min in Cuprophan® membranes and only 1.09×10^{-6} cm^2/min in chitosan membranes.[20]

Incorporation of Pluronic polyols with chitosan to for PIN membranes induces porosity and thus will increase the permeability of higher-molecular-weight compounds such as vitamin B_{12}. The permeability coefficients of vitamin B_{12} and albumin through control and chitosan-Pluronic PIN membranes are shown in Table V. The permeability coefficient of vitamin B_{12} in chitosan-PF68 PIN membranes, for instance, was 3.45×10^{-6} cm^2/min. Increasing the PEO and PPO chain

Table IV High-Resolution Peak Analysis of the Control and Chitosan-Pluronic® Physical Interpenetrating Network Membranes

	Area Under the Peak (%)					
Membrane Type	—C—H— (285.0 eV)	—C—O— (286.5 eV)	—C═O— (288.0 eV)	—O—C— (533.0 eV)	—O═C— (531.5 eV)	—N—C— (399.6 eV)
Chitosan	57.0	33.0	11.0	73.0	27.0	100.0
Chitosan-PP65	53.0	36.0	11.0	75.0	25.0	100.0
Chitosan-PF68	45.0	43.0	12.0	75.0	25.0	100.0
Chitosan-PP105	37.0	51.0	12.0	74.0	26.0	91.0
Chitosan-PF108	40.0	45.0	14.0	75.0	25.0	91.0

High-resolution analysis was performed at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington, Seattle, WA, with control and chitosan-Pluronic membranes containing 20% (w/w) Pluronic polyols.

Table V Permeability Coefficients of Vitamin B_{12} and Human Serum Albumin Through the Control and Chitosan-Pluronic® Physical Interpenetrating Network Membranes

	Permeability Coefficient ($cm^2/min \times 10^6$)	
Membrane Type	Vitamin B_{12}	Albumin
Chitosan	1.09 ± 0.12[a]	0.03 ± 0.00
Chitosan-PP65	2.16 ± 0.45	0.06 ± 0.01
Chitosan-PF68	3.45 ± 0.65	0.10 ± 0.05
Chitosan-PP105	2.68 ± 0.91	0.21 ± 0.07
Chitosan-PF108	4.17 ± 0.59	0.55 ± 0.06

The permeability coefficients of vitamin B_{12} (1355 daltons) and human serum albumin (69,000 daltons) were measured at 37°C through the control and chitosan-Pluronic physical interpenetrating network membranes containing 20% (w/w) Pluronic polyols.

[a] Mean ± SD ($n = 3$).

lengths to 122 EO and 56 PO residues in PF108 increased vitamin B_{12} permeability coefficient to 4.17×10^{-6} cm^2/min. The permeability of albumin also increased with the incorporation of Pluronic polyols. The average permeability coefficients of albumin in control chitosan and chitosan-PF68 PIN membranes were 3.00×10^{-8} cm^2/min and 1.00×10^{-7} cm^2/min, respectively. When the PEO and PPO chain lengths were increased to 122 EO and 56 PO residues in PF108, the albumin permeability increased to 5.50×10^{-7} cm^2/min.

Results of the permeability studies clearly show that incorporation of Pluronic polyols with chitosan can significantly increase the molecular-weight cutoff of the membranes. By selecting the appropriate type of Pluronic polyol and the concentration, one can specifically fabricate the PIN membranes to allow higher-molecular-weight compounds, such as β2M, to be efficiently removed from plasma in hemodialysis.

Platelet Adhesion and Activation

The extent of platelet adhesion and surface-induced activation is considered an early indicator of the thrombogenic potential of blood-contacting biomaterials.[30] The extent of platelet adhesion was determined by counting the number of platelets per 25,000-μm^2 surface area. When in contact with polymeric surfaces, platelets initially retain their discoid shape present in the resting state and the spread area is typically 10–15 μm^2. Upon activation, platelets extend their pseudopods and initiate the release of granular contents. During the partial activation stage, the area of the spread platelet can increase to ~ 35 μm^2. When the platelets are fully activated, they retract the pseudopods to form circular or "pancake" shape and the spread area increases to 45 or 50 μm^2.[31] The spreading profiles of activated platelets were used to create five activation stages as described by Lin et al.[32]

Table VI shows the number of adherent platelets and the extent of activation on control and chitosan-Pluronic PIN membrane surfaces. Clean glass, as shown in previous studies,[21] does promote platelet adhesion and activation. An average of almost 157 adherent platelets were present per 25,000 μm^2 on the glass surface. The degree of activation of 4.75 was also very high, indicating that the platelets were fully activated. Chitosan also is highly thrombogenic and allowed on the average 73 platelets to adhere per 25,000 μm^2. The degree of platelet activation on chitosan-coated glass of 3.65 was significantly less than that on control glass. Because of the hydrophilic nature of the surface, chitosan does not promote complete platelet activation and a significant number of pseudopods remained. On the chitosan-Pluronic PIN membrane surface, the number of adherent platelets and the extent of activation was significantly reduced. On chitosan-PF68 membrane, for instance, there were on the average 27.5 platelets per 25,000 μm^2, and the extent

Table VI Number of Adherent Platelets per 25,000-μm^2 Surface Area and the Extent of Activation on the Control and Chitosan-Pluronic® Physical Interpenetrating Network Membranes

Membrane Type	No. of Platelets	Extent of Activation
Glass slide	156.5 ± 16.0[a]	4.75 ± 0.13
Chitosan-coated slide	73.4 ± 11.4	3.65 ± 0.46
Chitosan-PP65	52.0 ± 8.3	2.65 ± 0.54
Chitosan-PF68	27.5 ± 5.5	1.35 ± 0.19
Chitosan-PP105	41.2 ± 12.4	2.87 ± 0.78
Chitosan-PF108	12.8 ± 3.8	1.15 ± 0.25

Human platelets in platelet-rich plasma were allowed to adhere and activate for 1 h on the control and chitosan-Pluronic physical interpenetrating network membrane surface, containing 20% (w/w) of Pluronic polyols.

[a] Mean ± SD ($n = 12$).

of activation was only 1.35. Both the number of adherent platelets and the extent of activation were reduced further on chitosan-PF108 membranes. On the average, only 13 platelets were present on chitosan-PF108 membrane surface per 25,000 μm^2 and the extent of activation was 1.15 to indicate that the platelets had retained their contact-adherent state.

CONCLUSIONS

Chitosan-Pluronic PIN membranes were fabricated with controlled porosity to selectively remove higher-molecular-weight compounds, such as β2M from blood during hemodialysis. Inclusion of Pluronic polyols with chitosan in the PIN membranes leads to the development of porous morphology. The size and distribution of the pores are dependent on the type of Pluronic used. Increase in the —C—O— signal from high-resolution ESCA in the Pluronic-incorporated membranes correlates with the surface accessibility of PEO chains. We expect the PEO chains on the surface of the PIN membranes to prevent protein adsorption and cell adhesion form plasma, improving blood compatibility. The permeability of vitamin B_{12} and albumin also significantly increased with the inclusion of Pluronic polyols due to the porous morphology of PIN membranes. The permeability coefficient of vitamin B_{12} and albumin in chitosan-PF68 PIN membranes, for instance, were 3.45×10^{-6} cm^2/min and 3.00×10^{-8} cm^2/min, respectively. In sharp contrast to the control chitosan membranes, platelet adhesion and activation were also significantly reduced on the PIN membranes.

The results of this study clearly show that the incorporation of Pluronic polyols with different PEO and PPO chain length will allow for the development of "tailored" membranes with selective permeability profile. In addition, the PEO chains of Pluronic would be effective in preventing platelet adhesion and activation to improve blood interaction properties of the membranes. Further studies are in progress to determine the permeability profile of β2M through the control and chitosan-Pluronic PIN membranes.

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