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Synthesis of anionic poly(ethylene glycol) derivative for chitosan surface modification in blood-contacting applications

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To improve blood compatibility, chitosan surface was modified by the complexation–interpenetration method using an anionic derivative of poly(ethylene glycol) (PEG). Methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate)-modified chitosan was prepared by allowing the base polymer to swell in an acidic medium, followed by polyelectrolyte complexation and interpenetration of MPEG sulfonate with the chitosan matrix. Addition of a strong base collapsed the base polymer to permanently immobilize the modifying agent on the surface. Electron spectroscopy for chemical analysis (ESCA) confirmed the presence of MPEG sulfonate on chitosan and the high resolution C1s peak showed an increase in -*C*—O- which is indicative of the ethylene oxide residues. The number of adherent platelets and the extent of platelet activation was significantly reduced on MPEG sulfonate-modified chitosan. Compared to an average of more than 66 fully activated platelets on unmodified chitosan surface, only 3.0 contact-adherent platelets were present on MPEG sulfonate-modified chitosan. Plasma recalcification time, a measure of the intrinsic coagulation reaction, was about 11.5 min in contact with modified chitosan. The results of this study show that chitosan surface can be modified by the complexation–interpenetration method with anionic PEG derivative. Surface-immobilized MPEG sulfonate was effective in preventing plasma protein adsorption and platelet adhesion and activation by the steric repulsion mechanism. © 1997 Elsevier Science Ltd

INTRODUCTION

Chitosan, a random copolymer of $\beta(1\rightarrow4)$-D-glucosamine and acetyl-$\beta(1\rightarrow4)$-D-glucosamine, is obtained by alkaline N-deacetylation of chitin. The major source of commercial chitin is the exoskeleton of marine crustaceans such as crabs, lobsters, krill, and shrimps (Li *et al.*, 1992). As such, chitin and chitosan are abundant natural polymers available from a renewable resource. To increase the utilization of chitosan, one potential area of significant interest is in medical and pharmaceutical applications. Chitosan has been proposed for the development of membranes and fibers for hemodialysis and blood oxygenators, skin substitute and wound dressing material, as a matrix for immobilization of enzymes and cells, for binding with bile and fatty acids, and as a vehicle for drug and gene delivery (Amiji, 1995; Chandy & Sharma, 1990; Hirano *et al.*, 1987; Murata *et al.*, 1996; Patel & Amiji, 1996).

Although chitosan does possess suitable physical properties for medical and pharmaceutical applications, for those applications that involve blood-contact such as hemodialysis membranes, chitosan promotes surface-induced thrombosis and embolization (Amiji, 1995; Hirano *et al.*, 1987). When blood comes in contact with biomaterial surfaces like chitosan, there is the initial adsorption of plasma proteins, followed by adhesion and activation of platelets (Baier, 1978; Hoffman, 1982). Upon activation, platelets secrete their granular contents including adenosine diphosphate, serotonin, and thrombin to activate other resting platelets as well as the coagulation cascade reaction (Anderson & Kottke-Marchant, 1985). The overall outcome of platelet activation is the formation of thrombus on the biomaterial surface. The loose thrombus can be easily dislodged from the surface by the shear stresses of blood leading to embolization of the clot in the systemic circulation.

Since the interactions that lead to surface-induced thrombosis occur at the blood–biomaterial interface, surface modification with water-soluble polymers, such as poly(ethylene glycol) (PEG) or poly(ethylene oxide)

(PEO), can prevent plasma protein adsorption, platelet adhesion, and thrombus formation by the steric repulsion mechanism (Amiji & Park, 1995; Hoffman, 1987; Ikada, 1984). Steric repulsion by surface-bound water-soluble polymer chains occurs as a result of overlapping polymer layers which could lead to loss in configurational entropy because of volume restriction and/or osmotic repulsion between interdigitated polymer chains (Amiji & Park, 1995). For effective steric repulsion, the surface modifying agent must satisfy the following three requirements (Amiji & Park, 1994). First, the polymer chains must be tightly bound to the surface by covalent or non-covalent interactions. Second, some part of the surface-bound polymer must extend into the bulk medium. The dominance of steric repulsion over van der Waals attractive forces occur only when the polymer chains have extended into the bulk medium. Finally, the surface must be fully covered with the modifying agent. Steric repulsion is not effective if a significant portion of the surface is unmodified. PEO-modified surfaces have been prepared by physical adsorption of PEO homopolymer and block copolymer (Amiji & Park, 1992; Amiji & Park, 1994), by entrapment of PEO (Desai & Hubell, 1991), and by covalent grafting of PEO to the biomaterial surface (Akizawa, 1989).

In the present study, the chitosan surface was modified by the complexation–interpenetration method using an anionic PEG derivative. The complexation–interpenetration method allows for non-covalent surface modification of chitosan surface in an aqueous system. Methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate)-modified chitosan was prepared by allowing the base polymer film to first swell in dilute acidic solution, followed by polyelectrolyte complexation and interpenetration of the anionic modifying agent into the swollen chitosan matrix. Upon addition of a strong base, the chitosan film collapsed and the modifying agent was permanently immobilized on the surface. In an aqueous environment, surface-bound MPEG chains will extend into the bulk medium to prevent plasma protein adsorption and platelet adhesion by the steric repulsion mechanism. Blood compatibility of MPEG sulfonate-modified chitosan was tested by measuring the extent of platelet adhesion and activation and the time required for fibrin clot formation.

MATERIALS AND METHODS

Materials

Chitosan with a degree of deacetylation of 87% and an average molecular weight of 750 000 daltons was obtained from Pronova Biopolymers (Raymond, WA). Methoxypoly(ethylene glycol) (MPEG) with an average molecular weight of 5000 daltons was purchased from Fluka Chemika-Biochemika (Ronkonkoma, NY). Chlorosulfonic acid was purchased from Aldrich Chemicals (Milwaukee, WI). Deionized distilled water (DDW, NANOpure II, Barnsted/Thermolyne, Dubuque, IO) was used exclusively to prepare all aqueous solutions. All other reagents and chemicals were of analytical grade or better.

Synthesis of methoxypoly(ethylene glycol) sulfonate

The terminal hydroxyl group of MPEG was reacted with chlorosulfonic acid to form MPEG sulfonate. The reaction scheme is illustrated in Fig. 1. Typically, 5.0 g (1.0 mmol) of MPEG was added to 100 ml of dry dimethylformamide containing 1.0% (w/v) triethanolamine. After complete dissolution, 0.58 g (5.0 mmol) of chlorosulfonic acid was added dropwise to the MPEG solution. With continuous stirring, the reaction for conversion of the terminal hydroxyl group of MPEG into sulfonate proceeded for 10 hours under reflux conditions. MPEG sulfonate was precipitated in diethyl ether and washed extensively with diethyl ether. The polymer was dried in vacuum. The reaction yield was approximately 90%. Elemental analysis of MPEG sulfonate was performed at the Schwarzkopf Microanalytical Laboratory (Woodside, NY).

Chitosan surface modification by complexation–interpenetration method

Glass microscope slides (25×75 mm), purchased from Fisher Scientific (Pittsburgh, PA), were washed with 2.0% (w/v) Isoclean® (Isolab, Akron, OH) solution at 50°C for 3 h. After rinsing with DDW, clean glass slides were dried at 60°C. Glass slides were coated with chitosan from a 1.0% (w/v) solution of the polymer in 0.1 M acetic acid as previously described (Amiji, in press). Chitosan acetate film, with an approximate thickness of

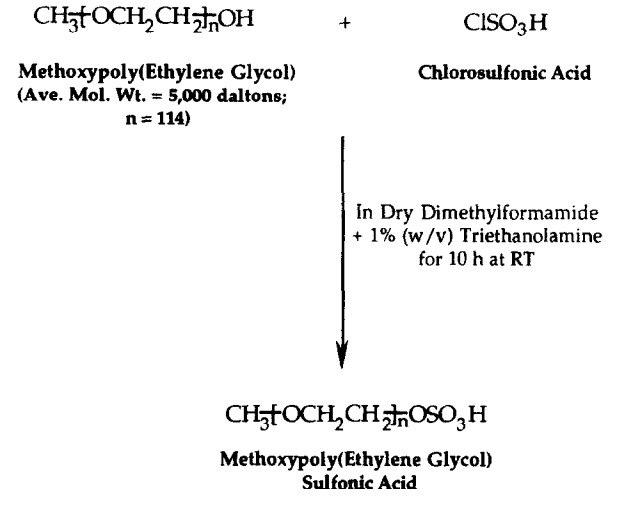

Fig. 1. Reaction scheme for synthesis of methoxypoly(ethylene glycol) sulfonate.

5.0 μm, casted on the glass slide, was neutralized in 0.1 M sodium hydroxide. After extensive washing with DDW, chitosan-coated slide was placed in 10 mM acetic acid solution for exactly 10 min to induce ionization and swelling in the polymer film. Being a cationic polymer, chitosan swells at low pH due to electrostatic repulsion between like charges and the osmotic effect of bound counterions (Patel & Amiji, 1996). MPEG sulfonate was dissolved in DDW to prepare a solution of 10 mg/ml concentration. Chitosan-coated slide was transferred into the MPEG sulfonate solution. Following a brief period (~30 s) of polyelectrolyte complexation and interpenetration of the MPEG sulfonate into the rubbery chitosan matrix, sodium hydroxide was added to collapse the chitosan film and permanently immobilize the anionic PEG derivative onto the chitosan surface. MPEG sulfonate-modified chitosan slide was washed with DDW and stored in phosphate-buffered saline (PBS, pH 7.4) containing 0.02% (w/v) sodium azide as a preservative at 4°C.

Characterization of MPEG sulfonate-modified chitosan

Electron spectroscopy for chemical analysis (ESCA) is a surface analytical technique that measures the elemental composition and identifies the chemical functional groups on the surface at up to 100Å thick layer. Clean glass slide, chitosan-coated glass, and MPEG sulfonate-modified chitosan surfaces were analyzed by ESCA to measure the surface elemental composition and the identity of the chemical functional groups. The ESCA experiments were carried out at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) at the University of Washington, (Seattle, WA). Analysis was performed using an X-Probe ESCA instrument (Surface Science Instruments, Mountain View, CA) equipped with an 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 (Scofield, 1976). The identity of chemical functional groups was obtained by high resolution peak analysis of carbon-1s (C1s), oxygen-1s (O1s), nitrogen-1s (N1s) and sulfur-2p (S2p) envelopes.

Platelet adhesion and activation on MPEG sulfonate-modified chitosan

An observation chamber for adherent platelets was assembled consisting of clean glass slide, chitosan-coated slide, or MPEG sulfonate-modified chitosan slide, two polyethylene spacers, and a glass coverslip. Human blood, obtained from healthy volunteers after informed consent, was collected in heparin-containing evacuated containers (Vacutainers®, Becton-Dickinson, Rutherford, NJ). Heparinized blood was centrifuged at 100 *g* for 10 min to obtain platelet-rich plasma (PRP). Two hundred microliters of PRP was instilled into the platelet observation chamber. Platelets in PRP were allowed to adhere and activate on the control and surface-modified chitosan slides for 1 h at room temperature. Non-adherent 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. Images of activated platelets were obtained from the Sony Trinitron® video display screen using a Polaroid ScreenShooter® camera (Cambridge, MA).

Plasma recalcification time measurements

Plasma recalcification time measurement, an indicator of the intrinsic coagulation reaction, is a useful marker of the interactions of blood on biomaterials surfaces. For recalcification time measurements, 16×100 mm clean glass test tubes (Fisher Scientific) were coated with chitosan and the surface was modified with MPEG sulfonate as described above. Human blood was collected in sodium citrate-containing evacuated containers. Citrated blood was centrifuged at 2000 *g* for 20 min to obtain platelet-poor plasma. Plasma recalcification time of citrated plasma in contact with control and surface-modified chitosan was measured according to the procedure described by Brown (1993). Briefly, 1.0 ml of citrated plasma was mixed with 0.5 ml of 0.05 M calcium chloride and incubated in glass tube, chitosan-coated glass, or MPEG sulfonate-modified chitosan in a water-bath at 37°C. The test tubes were occasionally removed from the water-bath and gently stirred. The time, in minutes, required for fibrin clot formation was recorded.

RESULTS AND DISCUSSION

Synthesis of MPEG sulfonate

MPEG sulfonate, an anionic derivative of PEG, was synthesized for surface modification of chitosan by complexation–interpenetration method. The reaction for conversion of the terminal hydroxyl group of

Table 1. Elemental analysis of methoxypoly(ethylene glycol) (MPEG) and methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate)[a]

Element	MPEG	MPEG sulfonate
Carbon	54.60	49.73
Hydrogen	9.37	9.06
Oxygen	36.25	36.98
Sulfur	—	2.04

[a]The average molecular weight of methoxypoly(ethylene glycol) was 5000 daltons.

MPEG into sulfonate is shown in Fig. 1. Elemental analysis of MPEG, as presented in Table 1, showed 54.6% carbon (C), 9.37% hydrogen (H), and 36.5% oxygen (O). These values were consistent with the theoretical values of 54.5% C, 9.10% H, and 36.4% O for the ethylene oxide residue. Elemental analysis of the product after 10 h of reaction showed the presence of sulfur (S) at 2.04%.

Characterization of modified surface

Since the interactions between blood and polymer surface are dictated by the surface properties, ESCA was used as an essential analytical technique to determine the composition and chemistry of the modified chitosan surface. The surface elemental composition of clean glass showed a characteristic C, O, sodium (Na), and silicone (Si) peaks as shown in Table 2. The atomic composition of 26.6% C, 44.2% O, 8.50% Na, and 16.8% Si on clean glass is consistent with the values that have been reported earlier (Park *et al.*, 1991). The chitosan-coated glass slide showed a higher percentage of C (66.4%) and lower percentage of O (24.4%) and nitrogen (N, 3.60%) than the theoretical values of D-glucosamine residue of 55.0% C, 36.0% O, and 9.0% N. The difference in the elemental composition could be due to the predominant presence of acetyl-D-glucosamine residues on the surface in the dry state or due to environmental contamination. MPEG sulfonate-modified chitosan showed a decrease in the composition of C to 63.0% and a corresponding increase in O to 30.3%. In addition, the MPEG sulfonate-modified chitosan had 1.90% S or a S/C ratio of 0.03 which confirms the modification of chitosan surface with the anionic agent.

ESCA data is also rich with the information on the surface chemistry of the control and modified chitosan. Table 3 shows the high resolution C1s, O1s, N1s, and S2p peaks which were used to determine the chemical identity of the functional groups that were associated with these elements. The peak at the binding energy of 285.0 eV, for instance, is associated with the -*C*—H- (or hydrocarbon) component of the C1s envelope on the surface. On clean glass, 71.0% of the carbon was associated with -*C*—H-, 10.3% with -*C*—O- (or ether), and 18.8% with -*C*=O- (or carboxyl) functional groups. The presence of the -*C*=O- peak of carbon and the -*O*=C- peak of oxygen are typically due to the unavoidable environmental contamination as a result of adsorption of carbon dioxide from air (Amiji, 1995). A

Table 2. Surface elemental composition of control and methoxy(polyethylene glycol) sulfonate-modified chitosan surfaces[a]

	Percent atomic composition					
Surface type	C	O	N	S	Na	Si
Clean glass	26.6	44.2	—	—	8.50	16.8
Chitosan-coated glass	66.4	24.4	3.60	—	—	—
MPEG sulfonate-modified chitosan[b]	63.0	30.3	4.80	1.90	—	—

[a]Surface elemental composition was obtained by electron spectroscopy for chemical analysis (ESCA) survey scans. ESCA was carried out at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington (Seattle, WA).
[b]Chitosan surface was modified by complexation–interpenetration with methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate, Ave. mol. wt 5000 daltons) at a bulk concentration of 10 mg/ml.

Table 3. High-resolution peaks of ESCA on control and methoxy(polyethylene glycol) sulfonate-modified chitosan surfaces[a]

	Relative peak intensity						
Surface type	-*C*—H- (285.0 eV)	-*C*—O- (286.5 eV)	-*C*=O- (288.0 eV)	-*O*=C- (531.5 eV)	-*O*—C- (533.0 eV)	-*N*—C- (399.6 eV)	-*S*—O- (168.3 eV)
Clean glass	71.0	10.3	18.8	44.2	53.7	—	—
Chitosan-coated glass	59.5	27.1	10.2	35.5	64.5	100	—
MPEG sulfonate-modified chitosan	42.0	45.9	10.0	20.0	80.0	100	100

[a]High-resolution peak analysis was conducted at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington (Seattle, WA).

high resolution scan of the carbon envelope on chitosan-coated glass resolved the hydrocarbon peak (59.5%), ether (27.1%) and the carboxyl (10.2%) peaks. The presence of the carboxyl peak suggests the presence of acetyl-D-glucosamine residues on the surface. The O1s envelope was associated with two peaks corresponding to -*O*=C- and -*O*—C- species at 35.5 and 64.5%, respectively. The N1s envelope was fitted to only one peak at 399.6 eV binding energy which corresponds with the -*N*—C- functionality. In MPEG sulfonate-modified chitosan, the high resolution C1s envelope showed a decrease in the hydrocarbon peak (42.0%) and an increase in the ether peak (45.9%) as compared to the C1s signal on unmodified chitosan. The increase in the ether peak is consistent with the presence of ethylene oxide residues of MPEG on the modified surface. In addition, in MPEG sulfonated-modified chitosan, the S2p envelope was entirely due to the -*S*—O- functionality which is indicative of sulfate or sulfonate groups.

Platelet adhesion and activation

The extent of platelet adhesion and surface-induced activation is considered an early indicator of the thrombogenicity of blood-contacting biomaterials (Baier *et al.*, 1985). Clean glass, as shown in previous studies (Park *et al.*, 1990), does promote platelet adhesion and activation. Approximately 148 fully activated platelets were present per 25 000 μm^2 surface area on glass as shown in Table 4. Chitosan is also highly thrombogenic as more than 66 fully activated platelets per 25 000 μm^2 were present on the surface (Fig. 2). The number of adherent platelets and the extent of platelet activation was significantly reduced on MPEG sulfonate-modified chitosan. On average, only 3.0 contact-adherent platelets per 25 000 μm^2 were found on the modified surface. Surface modification with MPEG sulfonate was very effective in preventing platelet adhesion and activation. The extension and flexibility of surface-bound MPEG prevents platelet adhesion on chitosan by the steric repulsion mechanism.

Table 4. Number of adherent platelets per 25 000 μm^2 on control and methoxypoly(ethylene glycol) sulfonate-modified chitosan surfaces[a]

Surface type	Number of platelets/ 25 000 μm^2
Clean glass	147.8±35.2[b]
Chitosan-coated glass	66.8±12.1
MPEG sulfonate-modified chitosan	3.00±1.65

[a]Platelets in platelet-rich plasma (PRP) were allowed to adhere and activate on the control and methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate)-modified chitosan surfaces for 1 h at room temperature.
[b]Mean±S.D. (n = 12).

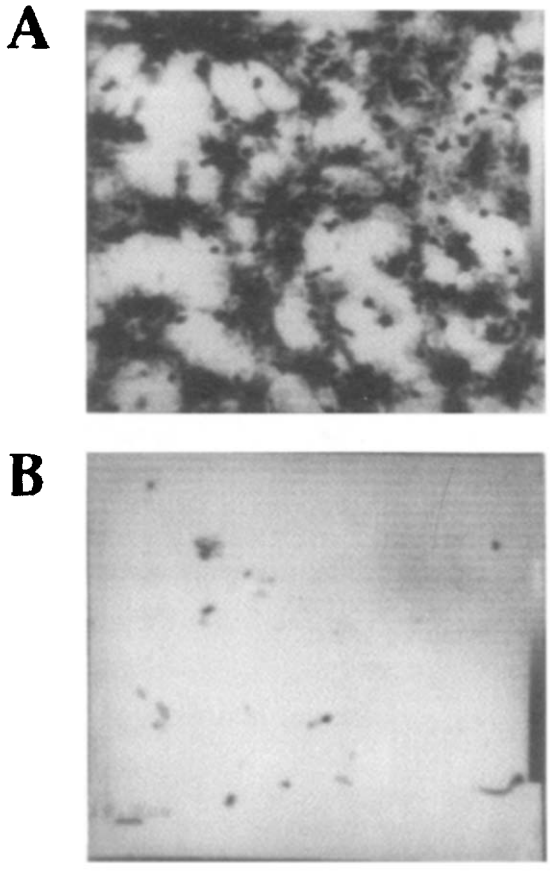

Fig. 2. Light micrographs of stained platelets on chitosan (A) and methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate)-modified chitosan (B). The bulk concentration of MPEG sulfonate used for surface modification was 10 mg/ml. Platelets in platelet-rich plasma were allowed to adhere and activate on the surface for 1 h at room temperature. The images were obtained using a 40× objective lens and the scale bar is equal to 10 μm.

Plasma recalcification time

Plasma recalcification time, a measure of the intrinsic coagulation mechanism, indicates the time required for fibrin clot formation in calcium-containing citrated plasma (Renaud, 1969). Since the time required for contact activation of plasma varies with the type of surface, the plasma recalcification time is also a useful indicator of blood–biomaterial interactions (Rhodes & Williams, 1994). Plasma recalcification time on glass surface, as shown in Table 5, was about 5.67 min. Glass, like other negatively charged surfaces, is a potent activator of the intrinsic coagulation reaction probably due to the surface-induced activation of factor XI or factor XII (Naito & Fujikawa, 1991; Silverberg & Diehl, 1987). Chitosan-coated glass increased the plasma recalcification time to about 11.0 min. The significant increase in the time required for fibrin clot formation on chitosan suggest that this surface does not readily activate the intrinsic coagulation mechanism. Surface modification of chitosan with MPEG sulfonate also did

Table 5. Plasma recalcification time on control and methoxypoly(ethylene glycol) sulfonate-modified chitosan surfaces[a]

Surface type	Time (min)
Clean glass	5.67±0.79[b]
Chitosan-coated glass	11.0±0.24
MPEG sulfonate-modified chitosan	11.5±0.32

[a]Glass test tube was coated with chitosan and modified with methoxypoly(ethylene glycol) sulfonate (MPEG sulfonate). Calcium-containing citrated human plasma was placed in the test tube and incubated at 37°C. The time, in minutes, required for fibrin clot formation was determined.
[b]Mean±S.D. ($n=5$).

not readily activate the intrinsic coagulation reaction. The plasma recalcification time on MPEG sulfonate-modified chitosan was 11.5 min. Prevention of the interactions between plasma proteins and the surface by the MPEG chains increases the time required for fibrin clot formation.

CONCLUSIONS

Although chitosan has the physical properties that makes the polymer useful for various biomedical and pharmaceutical applications, the negative consequences of interactions with blood may limit the use of chitosan in blood-contacting applications. In this study, we have modified the surface of chitosan with an anionic PEG derivative to improve blood compatibility of chitosan. Chitosan surface was modified with MEPG sulfonate by the complexation–interpenetration method. The surface modification method is based on the ionization and swelling of chitosan in acidic medium. After complexation and interpenetration of the anionic surface modifier, the polymer film was collapsed by addition of a strong base to permanently immobilize MPEG sulfonate on the surface. ESCA analysis showed that the modifying agent was present on chitosan. MPEG sulfonate-modified chitosan had an S signal at 1.9% and a S/C ratio of 0.03. High resolution analysis showed that the modified surface had an increase in the -*C*—O- signal of C1s envelope which is indicative of the ethylene oxide residues. Chitosan being highly thrombogenic had more than 66 fully activated platelet per 25 000 μm^2 surface area. On MPEG-modified chitosan, on the other hand, only 3.0 contact-adherent platelets were present per 25 000 μm^2. The number of adherent platelets and the extent of activation clearly shows that surface modification with MPEG sulfonate was effective in preventing cell adhesion on chitosan surface. Although chitosan does not readily activate the clotting reaction, surface modification did not change the plasma recalcification drastically.

The results of this study shows that chitosan surface can be permanently modified by complexation–interpenetration of anionic PEG derivative to improve blood compatibility of chitosan. When in contact with blood, the modified surface can resist plasma protein adsorption and cell adhesion by the steric repulsion mechanism.

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REFERENCES

Akizawa, T., Kino, K., Koshikawa, S., Ikada, Y., Kishida, A., Yamashita, M. and Imamura, K. (1989) Efficiency and biocompatibility of a polyethylene glycol grafted cellulosic membrane during hemodialysis. *Transactions of the American Society of Artificial Internal Organs* **35**, 333–335.

Amiji, M. (1995) Permeability and blood compatibility properties of chitosan-poly(ethylene oxide) blend membranes for hemodialysis. *Biomaterials* **16**, 593–599.

Amiji, M. (In press) Surface modification of chitosan membranes by complexation–interpenetration of anionic polysaccharides for improved blood compatibility in hemodialysis. *Journal of Biomaterial Science, Polymer Edition.*

Amiji, M. and Park, K. (1992) Prevention of protein adsorption and platelet adhesion on surfaces by PEO/PPO/PEO triblock copolymers. *Biomaterials* **13**, 682–692.

Amiji, M. and Park, K. (1994) Surface modification of polymeric biomaterials with poly(ethylene oxide): A steric repulsion approach, in *Polymers of Biological and Biomedical Significance*, ed. S. W. Shalaby, Y. Ikada, R. Langer and J. Williams. ACS Symposium Series Publication, Vol. 540. American Chemical Society, Washington, DC (1994) pp. 135–146.

Amiji, M. and Park, K. (1995) Surface modification of polymeric biomaterials with poly(ethylene oxide), albumin, and heparin for reduced thrombogenicity, in *Polymer Biomaterials: In Solution, as Interfaces, and as Solids*, eds S. L. Cooper, C. H. Bamford and T. Tsuruta. VSP, The Netherlands, pp. 535–552.

Anderson, J. M. and Kottke-Merchant, K. (1985) Platelet interaction with biomaterials and artificial devices. *CRC Critical Reviews of Biocompatibility* **1**, 111–204.

Baier, R. E. (1978) Key events in blood interactions. *Artifiual Organs* **2**, 422–426.

Baier, R. E., DePalma, V. A., Goupil, D. W. and Cohen, E. (1985) Human platelet spreading on substrata of known surface chemistry. *Journal of Biomedical Materials Research* **19**, 1157–1167.

Brown, B. A. (1993) *Hematology: Principles and Procedures*, 6th Edn. Lea and Febiger, Philadelphia, PA, p. 220.

Chandy, T. and Sharma, C. P. (1990) Chitosan as a biomaterial. *Biomaterials, Artificial Cells and Artificial Organs* **18**, 1–24.

Desai, N. P. and Hubbell, J. A. (1991) Solution technique to incorporate polyethylene oxide and other water soluble polymers into the surfaces of polymeric biomaterials. *Biomaterials* **12**, 144–153.

Hirano, S., Noishiki, Y., Kinugawa, J., Higashima, H. and Hayashi, T. (1987) Chitin and chitosan for use as a novel biomedical material, in *Advances in Biomedical Polymers*, ed. G. C. Gebelien. Plenum Press, New York, pp. 285–297.

Hoffman, A. S. (1982) Blood–biomaterial interactions: An overview, in *Biomaterials: Interfacial Phenomena and Applications*, Vol. 199, ed. S.L. Copper and N. A. Peppas. American Chemical Society, Washington, DC, pp. 3–8.

Hoffman, A. S. (1987) Modification of material surfaces to affect how they interact with blood. *Annals of the New York Academy of Sciences* **516**, 96–101.

Ikada, Y. (1984) Blood-compatible surfaces. *Advances in Polymer Science* **57**, 103–140.

Li, Q., Dunn, E. T., Grandmaison, E. W. and Goosen, M. F.A. (1992) Applications and properties of chitosan. *Journal of Bioactive and Compatible Polymers* **7**, 370–397.

Murata, J.-I., Ohya, Y. and Ouchi, T. (1996) Possibility of application of quaternary chitosan having pendant galactose residues as gene delivery tool. *Carbohydrate Polymers* **29**, 69–74.

Naito, K. and Fujikawa, K. (1991) Activation of the human blood coagulation factor XI independent of factor XII. Factor XI is activated by thrombin and factor XIa in the presence of negatively charged surfaces. *Journal of Biological Chemistry* **266**, 7353–7358.

Park, K., Mao, F. W. and Park, H. (1990) Morphological characterization of surface induced platelet activation. *Biomaterials* **11**, 24–31.

Park, K., Mao, F. W. and Park, H. (1991) The minimum surface fibrinogen concentration necessary for platelet activation on dimethyldichlorosilane-coated glass. *Journal of Biomedical Materials Research* **25**, 407–420.

Patel, V. R. and Amiji, M. (1996) pH-sensitive swelling and drug release properties of chitosan–poly(ethylene oxide) semi-interpenetrating polymer network, in *Hydrogels and Biodegradable Polymers for Bioapplications*, ed. R. Ottenbrite, S. Huang and K. Park. ACS Symposium Series Publication. Volume 627. American Chemical Society, Washington, DC, pp. 209–220.

Renaud, S. (1969) The recalcification plasma clotting time. A valuable general clotting test in man and rats. *Canadian Journal of Physiology and Pharmacology* **47**, 689–693.

Rhodes, N. P. and Williams, D. F. (1994) Plasma recalcification time as a measure of the contact phase activation and heparinization efficiency after contact with biomaterials. *Biomaterials* **15**, 35–37.

Scofield, J. Hartree–Slater (1976) subshell photoionization cross-sections at 1254 and 1487 eV. *Journal of Electron Spectroscopy and Related Phenomenom* **8**, 129–134.

Silverberg, M. and Diehl, S. V. (1987) The activation of the contact system of human plasma by polysaccharide sulfates. *Annals of the New York Academy of Sciences* **516**, 268–279.