Heparin release from thermosensitive polymer coatings: *In vivo* studies

Anna Gutowska,[1] You Han Bae,[1] Harvey Jacobs,[1] Fazal Mohammad,[1] Donald Mix,[1] Jan Feijen,[2] and Sung Wan Kim[1,*]

[1]Department of Pharmaceutics and Pharmaceutical Chemistry and Center for Controlled Chemical Delivery, University of Utah, Salt Lake City, Utah 84108; [2]Department of Chemical Technology, University of Twente, Enschede, The Netherlands

Biomer/poly(*N*-isopropylacrylamide)/[poly(NiPAAm)] thermosensitive polymer blends were prepared and their application as heparin-releasing polymer coatings for the prevention of surface-induced thrombosis was examined. The advantage of using poly(NiPAAm)-based coatings as heparin-releasing polymers is based on the unique temperature-dependent swelling of these materials. At room temperature, i.e., below the lower critical solution temperature (LCST) of poly(NiPAAm), the Biomer/(poly(NiPAAm) coatings are highly swollen. The high swelling enables fast loading of hydrophilic macromolecules (e.g., heparin) into the coating by a solution sorption technique. At a body temperature, i.e., above the LCST of poly(NiPAAm) the coatings are in a deswollen state and the absorbed macromolecules may be slowly released from a dense coating via a diffusion controlled mechanism. Biomer/poly(NiPAAm) coatings were obtained by blending and coprecipitation of the two linear polymers, Biomer and (poly(NiPAAm). The structure and water-swelling properties of the coatings were examined. Significant differences in water swelling at room temperature (RT) and 37°C were observed as a result of the thermosensitivity of poly(NiPAAm). The surface structure of the coatings in dry and swollen states at RT and 37°C was examined by scanning electron microscopy. Heparin was loaded into the coatings via a solution sorption at room temperature. Kinetic studies of heparin loading demonstrated that maximum loading was obtained within 1 h. The *in vitro* (37°C) release profiles were characterized by a rapid initial release due to the squeezing effect of the collapsing polymer network, followed by a slower release phase controlled by heparin diffusion through the dense coating. The short-term antithrombogenicity of intravenous polyurethane catheters coated with heparin-releasing Biomer/poly(NiPAAm) thermosensitive coating was evaluated in a canine animal model. The results show that the heparin release from Biomer/poly(NiPAAm)-coated surfaces resulted in a significant reduction of thrombus formation on test surfaces in contact with venous blood as compared to control surfaces.

INTRODUCTION

Recently, pharmaceutic approaches have been suggested as the most effective methods to obtain blood-compatible materials.[1] Such pharmaceutic approaches include the use of materials in which anticoagulant and/or antiplatelet drugs are incorporated along with the polymer. Drugs which have been studied include heparin,[2–4] prostaglandins,[5] urokinase,[6] heparin–prostaglandin,[7] and heparin–albumin conjugates.[8] Therapeutically, high concentrations of anticoagulant and/or antiplatelet agents present at the blood–polymer interface may be advantageous over systemic anticoagulation for patients using cardiovascular devices. With localized drug action, concentrations exceeding those tolerable at the systemic level could be applied, and side-effects such as hemorrhage and thrombocytopenia associated with systemic administration can be minimized.[9]

Heparinization of polymers for the design of blood-compatible surfaces has developed as heparin-releasing and heparin-immobilized systems[10]; the focus of this article is a heparin-releasing system. To date, two main approaches to heparin-releasing systems have been pursued: heparin ionically bound onto positively charged surfaces,[4,11–15] and heparin dispersed within hydrophobic polymeric matrices.[16–19] Ionically bound heparin is released into the blood by an ion-exchange mechanism, leaving a positively charged surface which may adversely interact with platelets.

*To whom correspondence should be addressed at Department for Pharmaceutics and Pharmaceutical Chemistry and Center for Controlled Chemical Delivery, University of Utah, 421 Wakara Way #318, Salt Lake City, UT 84108.

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Heparin could be dispersed within the hydrophobic polymeric matrix and either cast to form a bulk material[16] or applied as a coating on the surface of another polymer substrate.[17,18] Dispersed heparin is released from the polymeric matrix by a diffusion mechanism. Arterial grafts, fabricated using silicone rubber or silicone rubber–graphite containing dispersed heparin, demonstrated diminished thrombus formation for 2 h.[16] Heymann et al.[18] described heparin-releasing polyurethane coatings prepared using a dip-coating technique. Polyurethane catheters were coated with a polyurethane solution containing a heparin dispersion in dimethyl acetamide. Application of this method of coating resulted in heparin-releasing polyurethane catheters that exhibited a significant reduction in thrombus formation after 1 h of exposure to canine blood. Using polyester–polyurethanes (PEO–SPU), Lin et al.[17] obtained polyurethane coatings containing dispersed heparin by coating the inner lumens of PEO–SPU tubes with a PEO–SPU–heparin solution in dimethyl formamide. This coating technique involved an initial dissolution of heparin in formamide. A heparin release rate of 0.24 $\mu g/cm^2/h$ from the coating was needed to maintain the patency of a rabbit arterio-arterial shunt for 5 h. A controlled-release heparin surface was also fabricated from poly(hydroxyethylmethacrylate), with heparin release from the monolithic device lasting 10 h.[19] More recent studies demonstrated the possibility of heparin release from an electroerodible polyelectrolyte complex for a pulsatile heparin release system. An insoluble polyelectrolyte complex was formed by combining two water-soluble polymers, poly(allylamine) and heparin. Upon the application of an electric current, a rapid structural change of the complex caused the dissolution of polymer matrix and subsequent heparin release in the amount proportional to the applied current.[20]

In this report we describe a novel thermosensitive heparin-releasing polymer coating for the prevention of surface-induced thrombosis. The thermosensitive coating was fabricated by blending and coprecipitation of two linear polymers: Biomer and poly(NiPAAm). Biomer is a multiple block copolyether–urethane–urea which has been used for biomaterial application by numerous investigators, and demonstrated to have acceptable mechanical and biologic properties.[21] As a hydrophobic polymer, Biomer is expected to enhance the mechanical properties of the coating and also to improve binding between the gel coating and a hydrophobic polyurethane substrate.

Poly(NiPAAm) is a thermosensitive polymer with a lower critical solution temperature (LCST) in aqueous solutions of around 32°C.[22] LCST results from temperature dependence of polymer–water and polymer–polymer interactions leading to a coil–globule transition of polymer chains. As a result of this transition, gels composed of crosslinked poly(NiPAAm) demonstrate decreased swelling levels with increasing temperatures, and deswell dramatically at temperatures close to the LCST. This so-called negative thermosensitivity implies many possible applications of poly(NiPAAm)-based thermosensitive hydrogels in controlled drug delivery[23,24] and molecular separation processes.[25,26]

The rationale of using Biomer/poly(NiPAAm) thermosensitive coatings as heparin-releasing polymers is the following: When immersed into a heparin loading solution at low temperature a thermosensitive coating exhibiting negative thermosensitivity would swell and absorb the drug. At higher (body) temperature, the swollen coating would collapse and release heparin (Fig. 1). An initial rapid release of heparin due to the squeezing effect of the collapsing polymer would be expected to be followed by a slow release of drug controlled by solute diffusion within the collapsed network.

A significant advantage of the thermosensitive hydrogel coating is the possibility of solution sorption loading of relatively large amounts of drugs, such as proteins and heparin, which demonstrate limited solubility in organic solvents. This simple solution sorption loading procedure, using the thermosensitivity of Biomer/poly(NiPAAm) coatings, is advantageous over procedures that involve the use of organic solvents which may affect the bioactivity of the loaded drugs. Also, compared to other heparin-releasing polymers, this system offers flexibility as to the amount and type of anticoagulant drug(s) which can be loaded into the thermosensitive coating. Different anticoagulant and antiplatelet agents, including drugs with short-term stability (e.g., prostacycline), could be loaded from the same loading solution into the coated device (e.g., catheter) directly before use.

This article describes the preparation and characterization of Biomer/poly(NiPAAm) thermosensitive coatings as heparin-releasing polymers for the prevention of surface-induced thrombosis. Surface morphology of coated catheters was studied using scan-

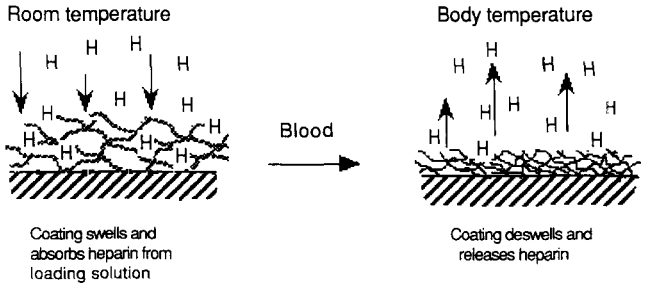

Figure 1. Biomer/poly(NiPAAm) thermosensitive coatings as heparin-releasing polymers.

ning electron microscopy (SEM). Loading and *in vitro* release kinetics of heparin from coated catheters was also investigated. The effect of such coatings on blood–material interactions was examined *in vivo* in a canine model.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NiPAAm) and 2,2′-azobisizobutyronitrile (AIBN) obtained from Eastman Kodak Company (Rochester, NY), were recrystallized from hexane and methanol, respectively. Benzene, HPLC grade, and *N,N*-dimethylacetamide (DMAc), glass-distilled spectral grade were obtained from Aldrich Chemical Company (Milwaukee, WI) and used as received. Solution-grade Biomer (copolyether–urethane–urea) was purchased from Ethicon (Summerville, NJ) as a 25% solution in DMAc and precipitated twice in methanol, vacuum dried, and stored at 20°C. Biomer solutions were prepared as 10 wt % of polymer in DMAc prior to coating. Heparin sodium USP from porcine intestinal mucosa, 201.4 USP U/mg, was purchased from Diosynth (Chicago, IL). Polyurethane intravenous catheters (Vialon®; 1.3 mm OD) were obtained from Becton Dickinson Vascular Access (Salt Lake City, UT). Freshly prepared Karnovsky solution[27] was used as a fixative. Phosphate-buffered saline solution (PBS), containing 0.01M phosphate, pH = 7.4, was used as a medium in all *in vitro* studies.

METHODS

Synthesis of poly(NiPAAm)

Poly(NiPAAm) was synthesized using free-radical polymerization. The reaction was carried out in benzene using AIBN as an initiator.[28] A solution of NiPAAm (10 g) and AIBN (1 mol %) in benzene (80 mL) was degassed under vacuum and stirred for 20 h at 50°C under a nitrogen atmosphere. After polymerization the solvent was evaporated and the remaining solute was dissolved in acetone (100 mL) and precipitated into a 10-fold excess of hexane. After filtering and drying, 8.7 g of polymer was obtained (87% yield).

Synthesized poly(NiPAAm) was characterized using IR spectroscopy and gel permeation chromatography (GPC). The IR spectrum of the polymer did not contain the following bands usually observed in the spectrum of monomer: 1620 cm (C=C), 1401 cm (CH), and C–H vinyl out of plane bending vibrations.[28] This suggested that the unreacted monomer content was below the detection limit of the IR spectroscopy (i.e., below 1%). The molecular weight of the polymer estimated by GPC, based on polystyrene standards,[28] was in the range of 280 K_d with a polydispersity index, M_w/M_N = 1.75. GPC analysis also confirmed the high purity of the polymer sample.

LCST of the polymer in PBS solution was determined by cloud point measurements using UV spectroscopy (450 nm).[28] An abrupt increase in absorbance (cloud point) corresponding to the LCST was observed at 32°C.

Coating procedure

Polyurethane intravenous catheters (Vialon®) were coated with a 10 wt % Biomer/poly(NiPAAm) solution in DMAc, using a dip-coating technique followed by the coprecipitation of Biomer and poly(NiPAAm) in hot water (the nonsolvent for both polymers). To obtain reproducible and even coatings, polyurethane catheters were immersed in and removed from the coating solution at the same rate. Catheters were kept in a vertical position throughout the coating procedure. Compositions of the coating solutions are summarized in Table I. Each Biomer/poly(NiPAAm) solution layer was precipitated in water at 50°C for 1 h and air dried for 0.5 h. Coating and precipitation pro-

TABLE I
Composition and Properties of Biomer/Poly(NiPAAm) Coating Solutions

Coating Solution	Solution Composition (wt %)			Solubility of Components	Phase Separation
	Biomer	Poly(NiPAAm)	DMAc		
B	10.0		90	Soluble	No phase separation
B/N (1:1)	5.0	5.0	90	Soluble	Phase sep. in 5 min
B/2N (1:2)	3.3	6.6	90	Soluble	Phase sep. in 10 h
B/3N (1:3)	2.5	7.5	90	Soluble	Phase sep. in 3 h
N		10.0	90	Soluble	No phase separation

B, Biomer; N, poly(NiPAAm).

cedures were repeated four times to obtain a 0.3-mm-thick coating.

Water-swelling properties

Water-swelling properties of Biomer/poly(NiPAAm) thermosensitive coatings were studied at room temperature and 37°C. The room temperature water content was determined after swelling of coated catheters in PBS for 1 h. At longer times (after 2 h) slow dissolution of poly(NiPAAm) from the blend matrix was observed. The dissolution of poly(NiPAAm) was monitored by measuring the absorbance of the swelling medium at 450 nm. A small sample of the swelling medium was incubated at 37°C (above the cloud point), and its absorbance was compared to the absorbance of pure water.

The equilibrium water content at 37°C was determined after swelling in PBS for 3 days. Water percent in the coating was calculated as

$$W\% = (W_s - W_d)/(W_s - W_n) \times 100\%$$

where W_s denotes the weight of catheter swollen in PBS, W_d denotes the weight of a dry-coated catheter, and W_n denotes the weight of the catheter before coating.

Heparin loading

Directly before use, catheters with Biomer/poly(NiPAAm) thermosensitive coatings in a dry state were immersed in 5 wt % heparin loading solution (PBS) at RT. After loading for 1 h, catheters were rinsed briefly in PBS, air dried for 20 min, and used immediately. This loading procedure was applied for both *in vitro* and *in vivo* studies. The total amount of loaded drug was determined after extensive extraction in buffer solution at RT using the Azure II colorimetric assay.[29] In this assay the absorption of heparin–Azure II complex was measured at 500 nm. Samples (0.5 ml) of the release medium were added to 4.5 mL of 0.01 mg/mL Azure II solution in water and vigorously mixed on the Vortex mixer. Absorption of the solution was measured after 1 min, with water used as a reference.

Loading percent in the coated polymer layer was calculated as

$$\text{Load}\% = \{W_{\text{drug}}/(W_{\text{polymer}} + W_{\text{drug}})\} \times 100$$

where W_{drug} denotes the weight of heparin and W_{polymer} denotes the weight of the dry polymer coating.

In vitro heparin release

The *in vitro* heparin release from coated catheters was conducted at 37°C in PBS at pH 7.4. To maintain sink conditions, catheters were transferred at predetermined times into 10 mL of fresh PBS preequilibrated at 37°C. The amount of released heparin was measured by the Azure II colorimetric assay.[29] Calculations of release rates, expressed in $\mu g/cm^2$ per h, were based on the dimensions of catheters at 37°C.

In vivo experiments

Surgical protocol

Mongrel dogs (20–30 kg) were used for the *in vivo* experiments. NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23, Rev. 1985) were observed, and the procedure was approved by the Institutional Animal Care and Use Committee, University of Utah. Dogs were preanesthetized with Biotol, anesthetized with Halothane, and mechanically ventilated. Intravenous catheters with a thermosensitive Biomer/poly(NiPAAm) coating and control polyurethane catheters (18 GA, 2 in.) were inserted percutaneously into front and hind contralateral saphenous veins and kept in place for 1.5 h. The vessels were then exposed and ligated both proximal and distal to the catheter site; all branches were tied off. The entire vein and catheter was removed and placed in fresh saline. The anesthetized animals were then euthanized intravenously with Beuthanasia. During the experiment, blood samples were withdrawn to measure activated partial thromboplastin times (APTT)[30] to evaluate the systemic effect of the released heparin.

Examination of catheters surface after *in vivo* experiments

Macroscopic evaluation

Vessels with the catheters in place were opened longitudinally for gross examination and photographed. Catheters were then placed in the fixative solution for subsequent processing and evaluation under the scanning electron microscope (SEM).

Scanning electron microscopic evaluation

Following 3 days of exposure to fresh fixative solution at 37°C, catheters were rinsed and transferred

to a saline solution containing 0.02% sodium azide. Representative catheter sections were selected for SEM examination. Samples were rinsed in distilled water to remove the saline solution, freeze dried, coated with gold (Technics Sputter Coaster; Hummer III, Alexandria, VA), and examined with an SEM (JEOL JSM-35; JEOL USA, Peabody, MA) at 15–25 KV accelerating voltage. Surfaces with and without adherent thrombus were examined and photographed at magnifications ranging from ×10 to ×1200. Coated catheters that were not exposed to blood were also examined in the same way.

RESULTS AND DISCUSSION

Properties of Biomer/poly(NiPAAm) thermosensitive coatings

The blending of hydrophilic and hydrophobic polymers produces a phase-separated composite hydrogel.[31] A solvent-casting technique is the most common method used to blend polymers. In this process, the polymers to be blended are dissolved in a common solvent and a desired form is cast. The polymer blend is obtained after the removal of solvent. The resulting materials are often opaque as a result of the phase-separated structure.

This blending technique was used in this study to obtain Biomer/poly(NiPAAm) thermosensitive coatings (Fig. 2). Biomer is a hydrophobic, multiple-block copolyether–urethane–urea with acceptable biocompatibility and good mechanical properties. Poly(NiPAAm) is a thermosensitive polymer demonstrating a lower critical solution temperature in aqueous solutions. Hence, the resulting polymer blend will likely have the properties enhanced by the qualities of both polymers, the thermosensitivity of poly(NiPAAm) and the mechanical strength of Biomer.

Figure 2 illustrates the schematics of blending procedure and the chemical structure of components: the repeating monomer unit in poly(NiPAAm) polymer and the Biomer structure. Solution-grade Biomer, as determined by Lelah et al.[21] consists of poly(tetramethylene oxide) soft segments and hard segments identified as 4,4′-diphenylmethanediisocyanate, chain extended with diamines. Biomer as a hydrophobic polymer precipitates in water. At temperatures above the LCST (i.e., 32°C) the poly(NiPAAm) undergoes a coil–globule transition and also precipitates in aqueous solutions.[32]

Biomer/poly(NiPAAm)-coating solutions were prepared as a 10 wt % polymer solution in DMAC, a com-

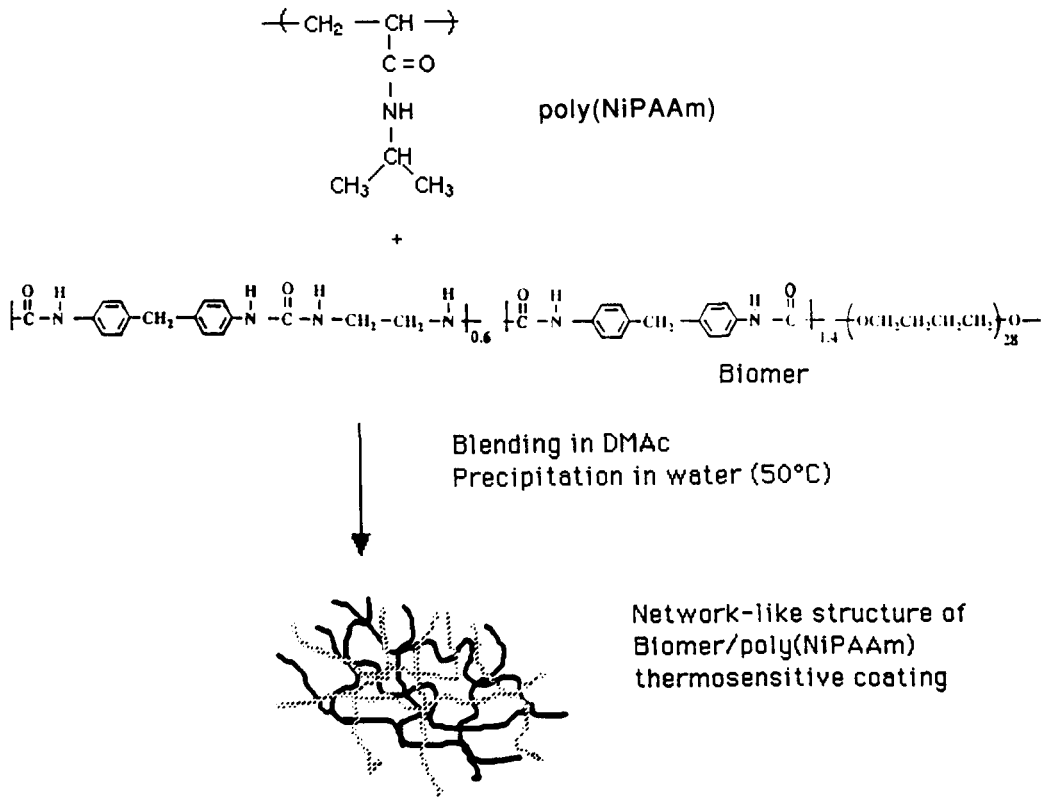

Figure 2. Schematic diagram showing components used for the synthesis and the resulting networklike structure of Biomer/poly(NiPAAm) polymer blend.

mon solvent for both polymers. Compositions of the coating solutions are listed in Table I. Polymers were readily soluble at the concentrations used; however, some phase separation was observed. Compatibility of the components on a molecular level is often a problem in the preparation of stable polymer solutions. The phase separation, often observed in solutions containing two different polymers, is due to long-chain polymer–polymer incompatibility, resulting from the negligible gain in the entropy of mixing.[33] A solution containing equal amounts of Biomer and poly(NiPAAm) exhibited almost immediate and extensive phase separation, and thus was not suitable for coating. In the case of B/2N and B/3N solutions, containing 1:2 and 1:3 ratios of Biomer and poly(NiPAAm), respectively, the phase separation was observed only after 10 and 3 h, respectively (Table I). Hence, freshly prepared B/2N and B/3N solutions exhibited sufficient stability to be used for the coating procedure.

Intravenous polyurethane catheters (Vialon®) were coated with Biomer, poly(NiPAAm), and Biomer/poly(NiPAAm) thermosensitive coatings, B/2N and B/3N. The dip-coating of catheters was followed by precipitation of the coated layer in hot water (50°C), a nonsolvent for both polymers. Simultaneous precipitation of Biomer and poly(NiPAAm) resulted in entanglement of polymer chains; therefore, Biomer prevented immediate dissolution of poly(NiPAAm) at temperatures below the LCST. Biomer/poly(NiPAAm) coatings exhibited a networklike structure and did not readily disintegrate in aqueous solutions at room temperature. In contrast, the coating containing only poly(NiPAAm) starts to dissolve within minutes of the contact with water at room temperature. All coatings were very stable at 37°C; at this temperature, water is a nonsolvent for both polymers.

Table II summarizes the properties of Biomer/poly(NiPAAm) thermosensitive coatings. The room temperature water content was determined after swelling in PBS for 1 h. After this time, slow leaching of poly(NiPAAm) from B/3N coating into the buffer was observed. Poly(NiPAAm) is soluble in aqueous solutions at temperatures below the LCST (i.e., 32°C). The B/2N coating, containing less poly(NiPAAm), was more stable in PBS at RT, and leaching was observed after 3 h of swelling. A slow dissolution of poly(NiPAAm) was observed only at temperatures below the LCST. Since the coatings were stable at 37°C [i.e., above the LCST of poly(NiPAAm], it was possible to determine the equilibrium water content at this raised temperature after 3 days of swelling.

Surface morphology of the bare and coated catheters was evaluated using SEM. The surface of the control polyurethane catheter shown in Figure 3A was relatively smooth, with some extrusion marks clearly visible at this magnification. Figure 3B illustrates the surface of the B/2N coating in a dry state, at the same magnification as Figure 3A. Although the surface of the dry coating was rougher, the difference in the fabrication procedure should be taken into account: the coating was obtained by coagulation and the polyurethane catheter was extruded.

Figure 3C shows the freeze-dried surface of the B/2N coating swollen at RT, while Figure 3D shows the same material deswollen at 37°C. The B/2N coating swollen at RT contained about 50% water, and the surface of the coating appeared porous after freeze drying. In a deswollen state at 37°C, the coating contained about 15% water and exhibited a dense, rough surface.

Heparin loading and release *in vitro*

Coated, dried catheters were immersed in the heparin loading solution at RT and the kinetics of heparin loading was examined. The results presented in Figure 4 indicate that maximum loading was attained after 1 h. The amount of heparin in the coatings was determined after an extensive RT extraction of loaded catheters with the loading percent calculated from the weight of dry coating. The maximum heparin loading in B/2N was 1.5 ± 0.3% and in B/3N coating, 2.0 ± 0.4% (n = 4) (Table II). The higher heparin loading percent in B/3N coating was related to the increased

TABLE II
Properties of Biomer/Poly(NiPAAm) Thermosensitive Coatings

Coating	Water % at Room Temp.	Water % at 37°C	Thickness (mm)			Heparin Load %
			Dry	Swollen, Room Temp.	Swollen, 37°C	
B	0.25 ± 0.05	0.65 ± 0.08	0.25 ± 0.03	0.25 ± 0.03	0.25 ± 0.03	0.0
B/2N (1:2)	50.7 ± 2.3	15.4 ± 1.4	0.25 ± 0.03	0.55 ± 0.03*	0.35 ± 0.03	1.5 ± 0.3
B/3N (1:3)	71.3 ± 4.5	16.3 ± 1.2	0.25 ± 0.03	0.65 ± 0.03*	0.35 ± 0.03	2.0 ± 0.4
N	Dissolves	15.9 ± 1.7	0.21 ± 0.03	Dissolves	0.30 ± 0.03	Not loaded

B, Biomer; N, poly(NiPAAm).
*Measured after 1 h (at longer times, slow leaching of poly(NIPAAm) from coatings was observed at room temperature).

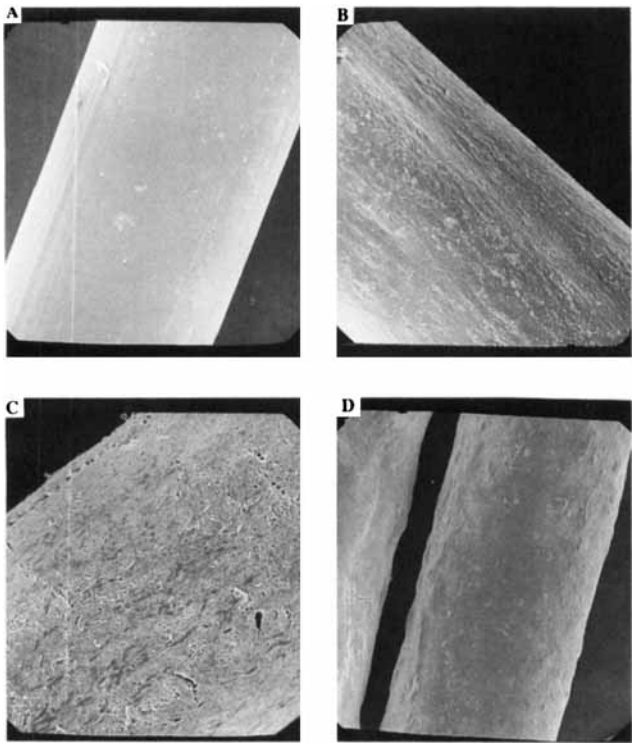

Figure 3. Scanning electron micrographs of the polyurethane catheter and Biomer/poly(NiPAAm) thermosensitive material coated on the polyurethane catheter. (A) Control polyurethane catheter (×47 magnification); (B) B/2N coating in a dry state (×47); (C) B/2N coating swollen at room temperature (×50); (D) B/2N coating swollen at 37°C (×33).

swelling resulting from the higher poly(NiPAAm) content in the B/3N polymer blend.

For *in vitro* heparin release studies, catheters with B/2N and B/3N coatings were loaded with heparin at RT for 1 h, air dried for 20 min, and immersed in PBS at 37°C. Heparin release profiles presented in Figure 5 indicate that 70% of the total heparin content was released within the first 15 min and the remaining amount was released with a slower rate for 6 h. The initial rapid heparin release was related to the squeezing action of the collapsing polymer network, and the following slow release was controlled by heparin diffusion through the collapsed matrix. Considering the thickness of the Biomer/poly(NiPAAm) coatings in a collapsed state at 37°C (Table II), the total heparin content could diffuse out in a relatively short time. However, solute diffusion was decreased within the collapsed network.

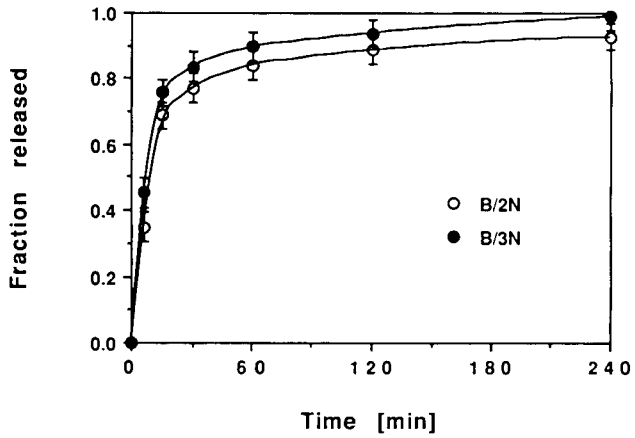

Figure 5. *In vitro* heparin release from Biomer/poly(NiPAAm) thermosensitive coatings.

Heparin release rates, presented in Figure 6, were calculated for the B/2N coating, based on catheter dimensions at 37°C. The initial squeezing of heparin from the collapsing thermosensitive coating resulted in an initial rate above 100 μg/h per cm^2. The subsequent slower release, controlled by heparin diffusion within the collapsed matrix, resulted in a release rate above 1.0 μg/h per cm^2 for about 6 h. This release rate was above the minimum heparin release rate required to maintain the nonthrombogenicity of a polyurethane surface, as determined by Lin et al.[17] In their studies, a minimum critical heparin release rate of 0.24 μg/h per cm^2 was needed to maintain a thrombus-free polymer–blood interface in a rabbit arterio-arterial shunt model.

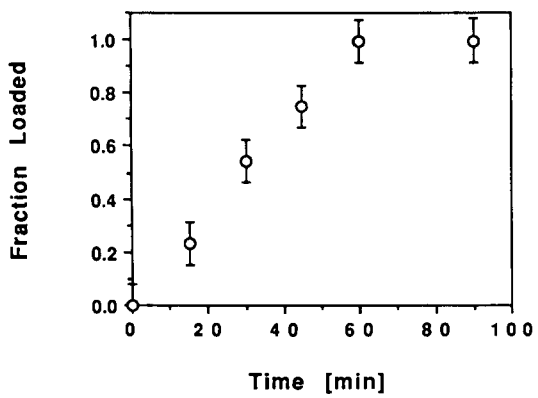

Figure 4. The kinetics of heparin loading into Biomer/poly(NiPAAm) thermosensitive coatings.

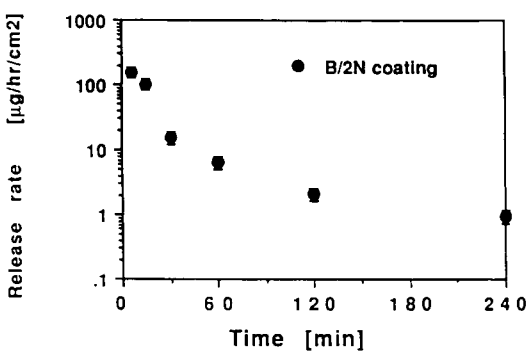

Figure 6. Heparin release rate from Biomer/poly(NiPAAm) thermosensitive coating (B/2N); calculated based on the catheter dimensions at 37°C.

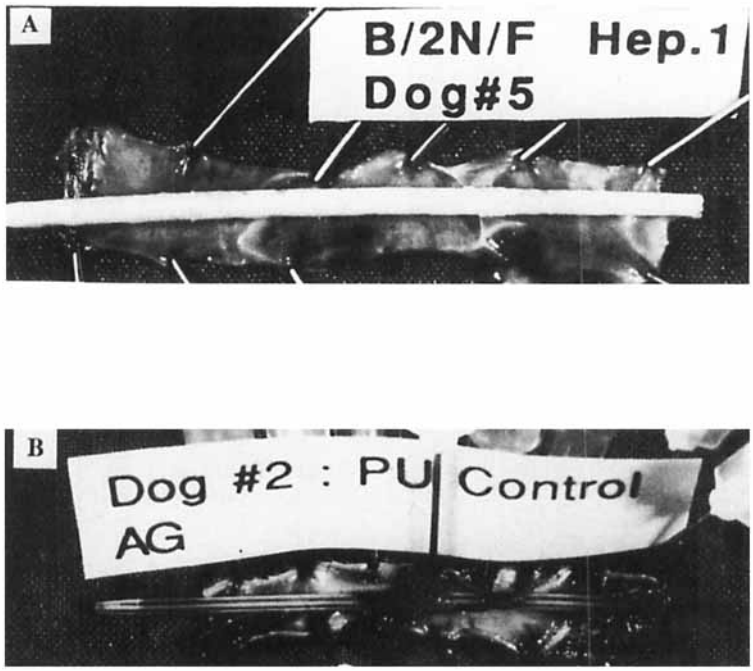

Figure 7. Gross view of tested and control catheters after *in vivo* contact with dog venous blood for 1.5 h. (A) Catheter with heparin-releasing Biomer/poly(NiPAAm) thermosensitive coating, surface free of adherent blood elements; (B) control polyurethane with associated thrombus.

The B/2N and B/3N coatings demonstrated comparable heparin release rates; however, the superior mechanical properties of B/2N coating in swollen state suggested the use of this coating for *in vivo* studies.

IN VIVO STUDIES

The initial events occurring on the blood-contacting surface are important in controlling surface-induced thrombosis. These events include the immediate adsorption of a protein layer and the subsequent activation of the intrinsic clotting pathway. If not prevented, thrombosis occurs within minutes upon the contact of blood with a polymer surface. Initially high local concentrations of heparin released from Biomer/poly(NiPAAm) at the blood-contacting surface would prevent clotting, and the subsequent slow release of heparin would further deter the surface-induced thrombosis.

The short-term *in vivo* blood compatibility of a heparin releasing Biomer/poly(NiPAAm) thermosensitive coating was evaluated in a canine model. Control polyurethane catheters and catheters with heparin-releasing B/2N thermosensitive coating were inserted in to the saphenous veins of dog for 1.5 h. Saphenous veins were selected because of their size and the fact that slower blood flow and reduced pulsatility creates a relatively more thrombogenic environment.

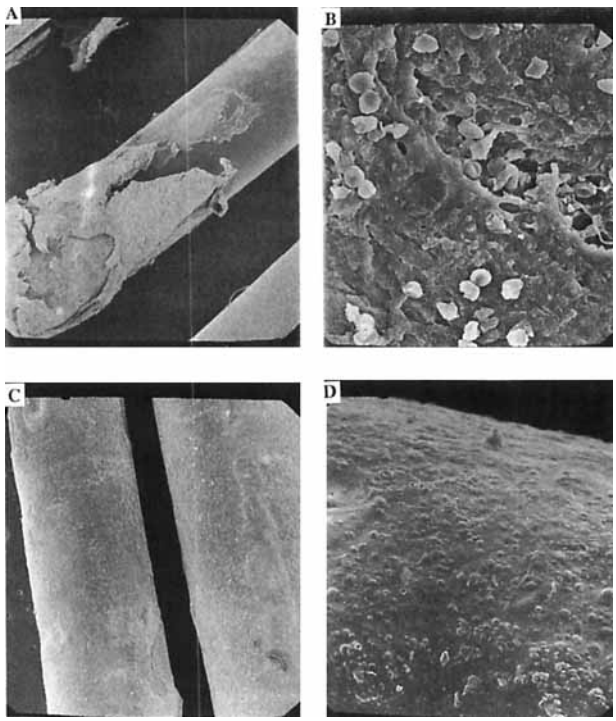

Figure 8. Scanning electron micrographs of catheters from Figure 7: the control polyurethane catheter and the catheter with heparin-releasing Biomer/poly(NiPAAm) thermosensitive coating. (A) Control polyurethane catheter with the adherent thrombus (same catheter as in Fig. 7B) (×34); (B) Higher magnification of the thrombus, with the fibrin network and cellular blood elements clearly visible (×1100); (C) Surface of B/2N coating, fibrin network and blood elements not visible (same catheter as in Fig. 7A) (×22); (D) Higher magnification of the clean surface of B/2N coating (×100).

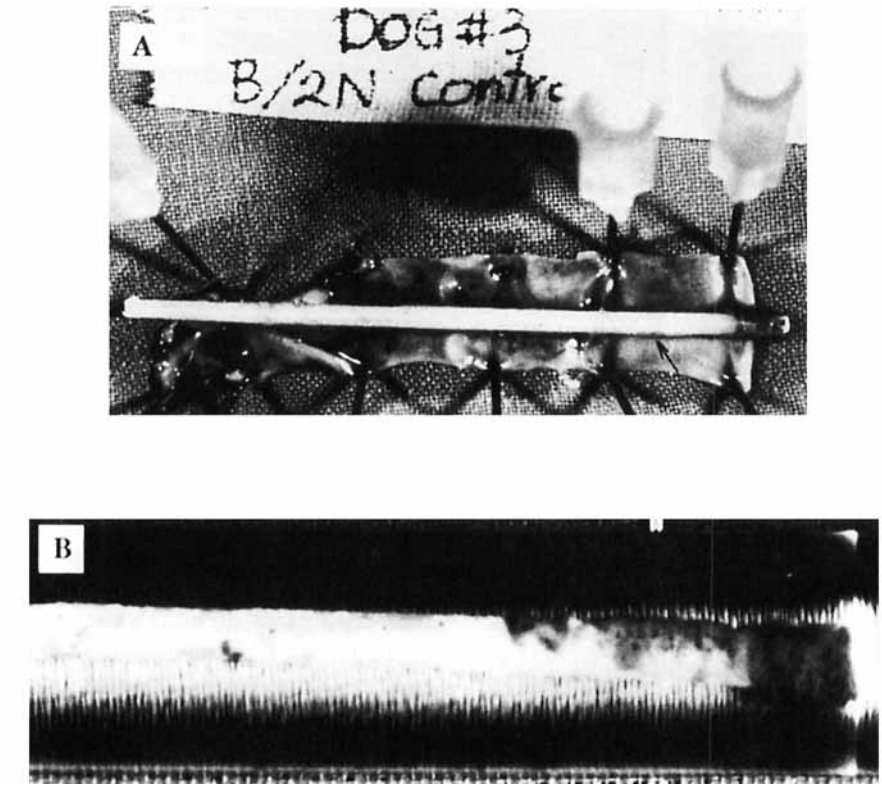

Figure 9. Gross view of the catheter with control Biomer/poly(NiPAAm) thermosensitive coating containing no heparin, after *in vivo* contact with dog venous blood for 1.5 h. (A) Catheter with thrombus visible on the distal end; (B) the underside of the catheter from (A).

Following a 1.5-h exposure to venous blood, vessels and catheters were removed and placed in fresh saline. Vessels were then opened longitudinally for gross examination, and photographs of exposed catheters with associated thrombus were taken. A representative picture of the catheter with heparin releasing Biomer/poly(NiPAAm) thermosensitive coating (B/2N coating) after exposure to dog venous blood is presented in Figure 7A. After 1.5 h exposure to venous blood, catheters with heparin-releasing B/2N coating showed mostly clean surfaces, with some fibrin network formation but no massive thrombi. Two of five examined catheters exhibited a small thrombus adherent to the surface, but the vein surface remained thrombus free. It was suggested that, as a result of the relatively weak mechanical properties of the coating in a swollen state, some damage of the coating may have occurred during the percutaneous insertion of the coated catheter. As shown in Figure 7B, the control polyurethane catheter also demonstrated thrombus formation during *in vivo* contact with dog venous blood.

Scanning electron micrographs of catheters from Figure 7 are presented in Figure 8. A control polyurethane catheter with adherent thrombi is shown in Figure 8A. At higher magnification (×1100), fibrin networks and cellular blood elements (red blood cells and platelets) are clearly visible (Figure 8B). The clean surface of the heparin-releasing B/2N coating, with no blood elements visible, is shown in Figures 8C and 8D, suggesting that catheters with heparin releasing Biomer/poly(NiPAAm) thermosensitive coating demonstrated reduced thrombus formation as compared to the control polyurethane catheters.

The blood compatibility of a catheter with Biomer/poly(NiPAAm) thermosensitive coating containing no heparin was also examined. Figure 9 shows the control B/2N catheter (no heparin) after exposure to dog venous blood *in vivo*. In Figure 9A, the thrombus formation on the distal end of the catheter is clearly visible. On the underside of the same catheter (hidden from sight in Fig. 9A) thrombus formation appears to have started from the middle of the catheter (Fig. 9B). SEMs of the above control B/2N catheter (no heparin) are presented in Figure 10. Higher magnification of the distal end with the thick layer of adherent thrombus is shown in Figure 10B. The structure of the thrombus with a dense fibrin network, platelets, and red blood cells is presented at high magnification (×1200) in Figure 10C. The results presented in Figures 9 and 10 suggested that the Biomer/poly(NiPAAm) coating itself was thrombogenic, and a local high concentration of released heparin was needed to create the nonthrombogenic surface.

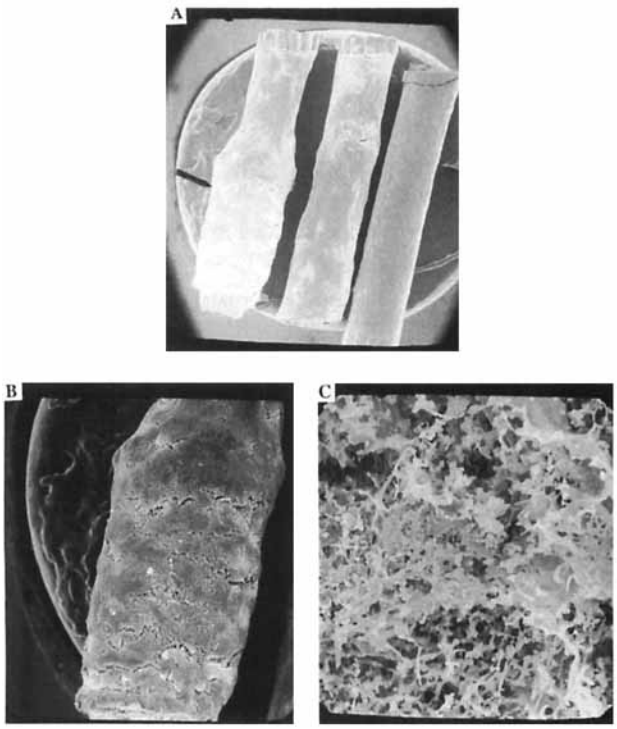

Figure 10. Scanning electron micrographs of the catheter with control Biomer/poly(NiPAAm) thermosensitive coating containing no heparin, after *in vivo* contact with dog venous blood for 1.5 h. (A) Gross view of the control B/2N catheter (same catheter as in Fig. 8) (×10); (B) distal end with adherent thrombus (×20); (C) high magnification of thrombus with fibrin network and cellular blood elements clearly visible (×1200).

To ensure that the releasing heparin did not affect the systemic coagulation parameters, blood samples were taken for activated partial thromboplastin time (APTT) tests. Since no changes in APTT were observed throughout the experiment, it can be concluded that all heparin effects were due only to a local concentration. It is also important to note that within the experimental error, the APTT times for all tested dogs were very similar. Hence, it can be concluded that heparin released from the Biomer/poly(NiPAAm) thermosensitive coating prevented surface-induced thrombosis.

CONCLUSIONS

Biomer/poly(NiPAAm) thermosensitive coatings were obtained by blending and coprecipitation of the two linear polymers. The simultaneous precipitation of Biomer and poly(NiPAAm) resulted in a network-like structure of the coating with extensive entanglement of the two polymers. The entangled structure deterred dissolution of poly(NiPAAm) in water solutions at temperatures below the LCST and allowed for room temperature solution sorption loading of heparin.

Commercial polyurethane intravenous catheters were coated with Biomer/poly(NiPAAm) thermosensitive coatings. The coatings displayed significant difference in water content at room temperature (~15%) and 37°C (~50%), due to the thermosensitivity of poly(NiPAAm). Furthermore, studies of heparin loading kinetics into Biomer/poly(NiPAAm) thermosensitive coatings showed that maximum loading was attained within 1 h. Studies of *in vitro* heparin-release kinetics demonstrated release rates of 1 $\mu g/cm^2$ per h for up to 6 h.

Antithrombogenic properties of heparin-releasing coated catheters were evaluated following 1.5 h of *in vivo* contact with venous blood. Catheters with heparin-releasing Biomer/poly(NiPAAm) thermosensitive coating were compared with the control uncoated polyurethane catheters. Heparin-releasing coated surfaces demonstrated a significant reduction of thrombus formation in contact with venous blood as compared to the control polyurethane surface. Since no changes in APTT times were observed throughout the experiment, it can be concluded that heparin released from the thermosensitive coating prevented surface-induced thrombosis without systemic effects.

These results demonstrate that heparin-releasing Biomer/poly(NiPAAm) thermosensitive coatings have a potential in short-term prevention of surface-induced thrombosis on blood-contacting prosthetic devices.

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