Poly(dimethylsiloxane)-poly(ethylene oxide)-heparin block copolymers. I. Synthesis and characterization

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Amphiphilic block copolymers containing poly(dimethylsiloxane), poly(ethylene oxide), and heparin (PDMS-PEO-Hep) have been prepared via a series of coupling reactions using functionalized prepolymers, diisocyanates, and derivatized heparins. All intermediate steps of the synthesis yield quantifiable products with reactive end-groups, while the final products demonstrate bioactive, covalently bound heparin moieties. Due to the solvent systems required, commercial sodium heparin was

converted to its benzyltrimethyl ammonium salt to enhance its solubility. The same procedure was applied to heparin degraded by nitrous acid in order to covalently couple it in solutions with the semitelechelic copolymers. As might be expected, this derivatization reduces the apparent bioactivity of the heparin. However, preliminary findings suggest that the bioactivity can be restored by reforming the heparin sodium salt.

INTRODUCTION

Since their commercialization in 1943, silicone polymers have shown significant and widespread success as biomaterials in implant and pharmaceutical applications. Historically, silicone rubbers were thought to exhibit promising, acceptable nonthrombogenic behavior because of their hydrophobic character. These materials were applied early in contact lens development because of their high oxygen permeabilities. Later, improved biomedical properties were sought by modifying silicones, either by *in situ* surface modification to increase hydrophilicity,¹⁻³ or more recently, by modifying chemical constituents of the silicone polymer by incorporating polar groups into monomers and prepolymers.⁴⁻⁶

New research literature describing the synthesis⁷⁻⁹ and commercial availability¹⁰ of derivatized, functionalized siloxanes now offers ready access to a versatile array of reactive silicone prepolymers to modify and prepare new biomaterials.

Amphiphilic copolymers composed of hydrophilic and hydrophobic blocks have demonstrated unique properties and behaviors in biomaterials

applications.¹¹⁻¹⁴ Amphiphilic copolymers containing a third, pharmaceutically active block of heparin have also been synthesized and characterized.¹⁵ The aim of the work was to provide a blood-compatible, heparinized coating to improve the surface properties of existing polymer substrates without changing the bulk properties. The triblock coating idea, as idealized in possible morphologies in Figure 1, incorporates hydrophobic poly-(dimethylsiloxane) together with the promising hemocompatible advantages of poly(ethylene oxide)¹⁶ and the anticoagulant properties of heparin to yield a coatable system where the hydrophobic block may be anchored into a suitable, existing hydrophobic substrate and the hydrophilic block acts as solubilizable tether to the covalently bound heparin moiety (Figure 1, middle model). However, coating may bury PEO chains or promote phase separation of the hydrophobic and hydrophilic blocks, creating a heterogeneous surface containing heparin but without the effect of a solubilized hydrophilic spacer (Figure 1, left and right models). In any case, such a system of immobilized anticoagulant provides a localized, permanent nonthrombogenic environment and avoids the problems associated with systemically administered heparin. It has been demonstrated, however, that heparinized coatings lose various degrees of bioactivity unless the heparin group is immobilized away from the surface by a spacer group. Systems employing directly immobilized heparins¹⁷ have shown that while these heparinized surfaces bind thrombin and Factor Xa, the immobilized heparin was not able to inhibit their activity, suggesting a sensitivity to sites and modes of attachment. More specifically, immobilized heparin interactions with coagulation factors increase as bound heparin is moved away from the surface into a more bulklike plasma environment and appear to be dependent upon tether arm length.¹⁸ Spacer groups may permit increased exposure of heparin's binding sites for ATIII and thrombin to plasma.

This paper presents the initial results of synthesis and characterization of a block copolymer consisting of hydrophobic poly(dimethylsiloxane), hydrophilic poly(ethylene oxide), and pharmacologically active heparin. A detailed evaluation of the bioactivity of this material as a coating *in vitro* is presented in a following paper.¹⁹

Figure 1. Possible surface morphologies for block copolymers of poly-(dimethylsiloxane), poly(ethylene oxide), and heparin.

EXPERIMENTAL

Materials

Poly(dimethylsiloxanes) (PDMS): Functionalized prepolymers of PDMS were used as supplied by Petrarch Systems Inc. (Bristol, PA, USA). Mono-functionalized amino-PDMS ($\bar{M}_n = 9500$ (ref. 20)) and methyl-hydro PDMS containing 0.5%–1.0% reactive Si-H groups ($\bar{M}_n = 63,000$) were stored at -20°C under nitrogen.

Poly(ethylene oxides) (PEO): Diamino-telechelic poly(ethylene oxides) under the trade name Jeffamines were a gift from Texaco Chemical Company (Houston, TX, USA). Jeffamine 6000 ($\bar{M}_n = 6000$) was purified by dissolving in THF, precipitating in a 10-fold excess of diethyl ether two separate times, drying under vacuum over P_2O_5 , and finally drying *in vacuo* at 70°C for 2 hr. Diamino-telechelic PEO ($\bar{M}_n = 4000$) was a generous gift from Nippon Oil & Fats Ltd. (Tokyo, Japan) and was used as supplied.

Heparin: Commercial heparin (Diosynth, Oss, The Netherlands) with an activity of 165.4 IU/mg as indicated by the manufacturer was subject to chemical modifications, assayed for bioactivity, and utilized. Tritium-labeled heparin (6.3 mg, New England Nuclear, Boston, MA, USA) with a specific activity to $158\ \mu\text{Ci/mg}$ was diluted with 10 mL water, purified by chromatography and utilized.

Toluene 2,4-diisocyanate (TDI, Fluka Chemika, Buchs, Switzerland) was purified by distillation at reduced pressure under nitrogen and the fraction at $126^\circ\text{C}/11\ \text{mmHg}$ collected and stored under nitrogen at 4°C .

Methylene diphenyl diisocyanate (MDI) (Merck, Darmstadt, FRG) was purified by distillation at 3 mmHg. A yellow-white solid (m.p. = $38\text{--}40^\circ\text{C}$) was stored under nitrogen at -20°C .

Toluene and tetrahydrofuran (THF) were dried over CaCl_2 and distilled by conventional means.

Chloroform was purified by elution through a column of activated aluminum oxide (100 g/L, basic, Janssen Chimica, Beerse, Belgium) and the fraction from 50–800 mL stored in an amber bottle under nitrogen at -20°C .

Chloroplatinic acid (H_2PtCl_6 , solid, Janssen Chimica, Beerse, Belgium) was dissolved in freshly distilled *N,N*-dimethylformamide (DMF) under a nitrogen blanket and stored at reduced temperature (1.0% w/v).

Benzyltrimethylammonium bromide (Triton B, Fluka Chemika, Buchs, Switzerland) was used as received.

Sodium cyanoborohydride, allyl alcohol, and allyl isocyanate (Aldrich Chemicals, Brussels, Belgium) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Merck) were used as received and stored under nitrogen at reduced temperature.

Diphenyl guanidine (DPG, Janssen Chimica, Beerse, Belgium) was purified according to the technique of Carlton and coworkers.²¹

Synthesis

Amino-semitelechelic poly(dimethylsiloxane)-poly(ethylene oxide) block copolymers (PDMS—PEO—NH₂)

PDMS—PEO—NH₂ block copolymers [5] were synthesized by a procedure analogous to that used to prepare polystyrene-HEMA^{12,23} and polystyrene-poly(ethylene oxide)¹⁵ block copolymers. The overall scheme involves a coupling reaction between isocyanate-semitelechelic PDMS [PDMS—N=C=O, 3] and amino-telechelic PEO [H₂N—PEO—NH₂, 4]. PDMS—N=C=O [3] was prepared by the following procedure: PDMS—NH₂ ([1], 15% w/v in toluene) was added dropwise to a solution of TDI [2a] or MDI [2b] (2% w/v) in toluene over a period of 24 hr until a final ratio of [NH₂]/[NCO] = 0.5 was obtained. The reaction mixture was stirred constantly under nitrogen at room temperature. Unpurified aliquots of the reactions were vacuum dried and analyzed by IR spectroscopy and the products were compared to unreacted mixtures of the two species.

PDMS—PEO—NH₂ block copolymers [5] were synthesized by adding PDMS—N=C=O [3] solutions (15% w/v) in toluene dropwise over a period of 48 hr to a solution of H₂N—PEO—NH₂ [4] (15% w/v) in toluene until the final ratio of [NH₂]/[NCO] was 2.4. This reaction was performed under nitrogen at room temperature. Reactions were terminated by utilizing a dual precipitation technique after evaporating toluene to 50% of its original volume. Unreacted PDMS—NH₂ [1] was removed by precipitation of the reaction contents into a 10-fold excess (v/v) of diethyl ether, leaving a white powder consisting of a mixture of [4] and [5]. Unreacted PEO [4] was then removed by chopping the powder to small granules and stirring gently in a 50-fold excess (w/v) of water for 3 hr. Filtration, rinsing, and freeze-drying yielded a spongy, white block copolymer solid. The polymer was used immediately or stored at -20°C.

The entire reaction sequence was attempted unsuccessfully at both 0°C and 60°C in toluene. The PDMS phase precipitated as a cross-linked gel in the diisocyanate solution at the higher temperature while the PEO phase or PEO-PDMS complex precipitated out of solution at lower temperatures. Chloroform was also attempted as a solvent system at various temperatures but no products were identified. It is thought that residual ethanol present as a stabilizer in chloroform was not adequately removed by aluminum purification and is contaminating the diisocyanate.

In addition, other functionalized PDMS prepolymers were utilized in attempts to couple PEO. Methyl-hydro-PDMS reported to contain 0.5–1.0% reactive Si-H groups¹⁰ was reacted with allyl alcohol and allyl isocyanate in separate reactions in an attempt to produce the functionalized hydrosilation products via the addition reaction in the presence of a catalyst.^{9,23} Reactions catalyzed by chloroplatinic acid in various solvents and temperatures failed to produce quantifiable reaction products of PDMS—OH or PDMS—NCO as indicated by proton NMR. Other researchers have noted the inconsistent

reactivity of certain allyl derivatives in hydrosilation additions (allyl amine, allyl bromide) while others (allyl alcohol, *N*-dimethyl allyl amine) appear very reactive.⁹ In the supplied PDMS prepolymer, multiplet signals at 4.77 ppm, characteristic of the Si-H group, were not present, indicating that the functionality may have been contaminated (or too weak to detect). In addition, the triplet expected at 3.64 ppm⁹ for the hydroxymethylene product from allyl alcohol was not detected. Finally, quantifiable coupling reactions with functionalized PEO failed to occur.

Poly(dimethylsiloxane)-poly(ethylene oxide)-heparin block copolymers
(PDMS — PEO — Hep)

PDMS — PEO — Hep block copolymers [7, 9] were synthesized via a coupling reaction of PDMS — PEO — NH₂ block copolymers [5] with derivatized heparins [6, 8]. Commercial heparin was subject to cation exchange with benzyltrimethyl ammonium bromide to yield the Triton-B form [6]. The cation exchange procedure involves replacement of Na⁺ cations in heparin with the more hydrophobic quarternary ammonium cation.²⁴ The process entails addition of 3.5 g of Triton-B to an aqueous solution of heparin (2.3 g in 150 mL water) at pH = 7.5. Refrigeration for 16 hr is followed by dialysis against distilled water for 24 hr to remove low-molecular-weight salt and heparin fragments less than 5000 daltons. Repeating this entire operation three times followed by lyophilization provides a heparin salt with enhanced solubility in organic solvents but showing lower bioactivity.

Derivatized heparin [8] was prepared by nitrous acid degradation²⁵ followed by Triton-B treatment to yield an organically-soluble, bioactive heparin containing one aldehyde group per molecule.²⁶ This heparin form was coupled to PDMS — PEO — NH₂ block copolymers [5b] by reductive amination. Freshly prepared copolymer (2.0 g) was first dissolved in THF (45 mL) and water (11 mL) was then added slowly with brief sonication until a clear solution was obtained. Heparin [6] was dissolved separately in water (1.5 g in 12 mL) and THF (50 mL) dropped slowly to the solution with stirring at 37°C until a clear solution was obtained. The two solutions were mixed and brought to pH = 6.5 with 1N HCl or 1N NaOH. Sodium cyanoborohydride (NaCNBH₃), was dissolved in water (150 mg in 1mL) and THF (5 mL) was added. This solution was then added to the copolymer-heparin solution. The reaction was stirred at room temperature for 7 days with 100 mg NaCNBH₃ added on day 3 and day 5 and the pH maintained at 6.5. Reaction products were recovered by removing the THF by evaporation, lyophilization, and subsequent thorough rinsing of the white solid with water to remove unreacted heparin and reducing agent.

Heparin [8] was coupled to the block copolymer system [5b] using EDC at room temperature^{27–29} in the same THF:water solvent system as mentioned above. Fresh block copolymer (700 mg in 31 mL THF:water) was mixed with heparin [8] (350 mg. in 62 mL THF:water) at pH 5 for 24 hr with the EDC

added in 8 equal portions at 30-min intervals. The reaction was then adjusted to pH 7.5 for 24 hours with 1N NaOH. Finally, the reaction was terminated by removing the solvent by evaporation, lyophilization, and thorough rinsing of the resulting solid with water to yield the block copolymer [9].

Tritium-labeled heparin was incorporated into the block copolymers using the following procedure: disposable Sephadex G-25M PD-10 desalting columns (Pharmacia) were characterized for void volumes and dilution factors using Dextran Blue in water. Labeled heparin in water (3 mL, 300 μ Ci) was applied in 0.5-mL aliquots to the column and the eluent collected in 10 drop (approximately 0.5-mL) fractions. Fractions 7–10 corresponding to the highest molecular weight heparin molecules were pooled and assayed for radioactivity. The same procedure was utilized to fractionate commercial heparin (600 mg in 6 mL water). Fractions 7–10 of the commercial heparin were lyophilized, weighed, and added directly to 5 mL of the radioactive pool and 5 mL of distilled water to give a solution concentration of 17.0 mg/mL with a specific activity of 1.0×10^6 dpm/mg. THF (25 mL) was added dropwise to the heparin solution at 37°C and this solution was mixed with a THF:water solution containing 500 mg of fresh block copolymer. A coupling reaction was then undertaken using the EDC method described above. After washing with solvent, recovered product was assayed for radioactivity by dissolution in THF:water and scintillation counting with an LKB 1219 Rackbeta instrument.

Characterization

Spectroscopy

Infrared (IR) spectra of the prepolymers and block copolymers were obtained from films cast on sodium chloride crystals using a Perkin-Elmer 1310 spectrophotometer. Proton NMR spectra were obtained from samples in chloroform- d_1 using a Varian EM360A instrument. Tetramethylsilane used for referencing peak shift locations downfield was found to interfere with proton integration of PDMS signals and therefore was used only occasionally to determine downfield shifts.

Molecular weight

Molecular weights ($\overline{M}_n$) of the prepolymers and block copolymers were determined by vapor pressure osmometry (VPO) (Hewlett Packard 301A) in toluene at 37°C. These results were compared with data from gel permeation chromatography (GPC) (Waters Model 600A HPLC and Waters Differential Refractometer R401), using ultraStyragel columns with exclusion limits of 10^5 , 10^4 , 10^3 , and 500 Å in series. THF was used as a solvent at a flow rate of 2.02 mL/min at 21°C.

End-group analysis

Amino group functionalization for the PDMS and PEO prepolymers as well as the PDMS-PEO copolymers was analyzed with potentiometric titration. Polymer solutions in chloroform or DMF were titrated with 0.015N perchloric acid in dioxane. The titrant was standardized by titrating DPG in solutions of chloroform or DMF.

Swelling measurements

Solutions of PDMS—PEO—NH₂ in chloroform (5% w/v) were cast into silanized petri dishes and air-dried for 24 hr followed by vacuum drying for 24 hr. Films were removed, measured for thickness with a micrometer, and cut into pieces of identical geometry and size using a grid pattern. Film pieces were then weighed, immersed completely into solutions of water and monitored for swelling after 2 hr. Films were removed carefully from water, gently wiped dry with a dust-free towel and weighed.

Heparins

Prior to coupling reactions, derivatized heparins were compared to commercially supplied, unaltered heparin for bioactivity using activated-partial thromboplastin time (APTT) assays. Heparin solutions (0.05 mL) in water were incubated for 5 min at 37°C with 0.1 mL citrated fresh-frozen human plasma pooled from healthy donors. Cephalin/Kaolin activating reagent was added and incubated an additional 3 min before CaCl₂ was added to coagulate the samples. Clot formation was detected optically and automatically with a Lode Coagulometer (Model LC-6, Lode, Groningen, The Netherlands).

RESULTS AND DISCUSSION

The synthetic outline for the block copolymer chemistry is shown in Figure 2. Semitelechelic-isocyanated PDMS was prepared using both MDI and TDI in solution coupling reactions. IR spectroscopy, shown in Figure 3, demonstrates evidence for urea bond formation in this reaction by the presence of N—H stretch at 3320 cm⁻¹, Amide I at 1645 cm⁻¹ and Amide II at 1540 cm⁻¹. These peaks appeared for both the MDI and TDI functionalizations and were not present in simple mixtures of the PDMS and diisocyanates. A strong adsorption at 2270 cm⁻¹ is characteristic of the free isocyanate group. When these isocyanated prepolymers were coupled to amino-telechelic PEO, IR scans, shown in Figure 4, demonstrate new N—H stretching absorbance shifts at 3520 and 3380 cm⁻¹ as well as Amide I and II

Figure 2. Synthetic scheme for PDMS-PEO-heparin block copolymers.

Figure 3. Overlaid infrared spectra for PDMS-NH₂ (A), PDMS-TDI (B), and PDMS-MDI (C).

Figure 4. Overlaid infrared spectra for $\text{H}_2\text{N-PEO-NH}_2$ (A), PDMS-TDI-PEO-NH₂ (B), and PDMS-MDI-PEO-NH₂ (C).

peaks at 1670 and 1540 cm^{-1} , respectively. The prominent isocyanate peak disappears as well. This information indicates the formation of urea linkages between PDMS and PEO into two different chemical environments.

Proton NMR analysis shows the presence of both PDMS and PEO components as displayed in Figure 5 (siloxane backbone: 0.1 ppm and methylene-ether backbone: 3.4–3.8 ppm). Integration of the signals confirms the proper ratio of protons in the block copolymer constituents (mole% PDMS: calculated 67%, found 67%).

Molecular weight distributions of the synthesized block copolymers and prepolymers were determined using GPC. The most widely accepted parameter defining elution volume in GPC is the theoretical quantity, hydrodynamic volume. Under certain specified conditions, the relationship between hydrodynamic volume and elution volume has been shown to be independent of polymer type and structure over a wide range of polymer types with some exceptions. Thus, unknown species are typically calibrated against well-characterized polystyrene standards for molecular weight distribution determinations.³⁰ However, polystyrene standards used to make a calibration reference for GPC measurements in general, and for the PDMS-PEO-Hep system in particular, may not accurately relate to retention volumes of the PDMS for direct molecular weight determinations.¹⁰ Thus, GPC information for the pre- and heparinized block polymers containing PDMS was used only qualitatively to assess polydispersity. GPC plots for both the

Figure 5. Proton-NMR spectra for PDMS-PEO-NH₂ Copolymer (a = polyether backbone, 3.6 ppm, b = siloxane backbone, 0.1 ppm).

prepolymers and the block copolymers are shown in Figure 6 and accompanying molecular weight data are displayed in Table I. PEO prepolymers show quite a narrow distribution of molecular weights while PDMS shows a broad, plateau-like distribution. The copolymer products show a molecular weight distribution expected for the reaction of the two characterized prepolymers—broadly distributed over a range of values much like its

Figure 6. GPC profiles for molecular weight distributions for H₂N-PEO-NH₂ (A) PDMS-NH₂ (B), PDMS-PEO-(4000)-NH₂ (C), and PDMS-PEO(6000)-NH₂ (D).

TABLE I
Polymer Molecular Weights and End-Group Concentrations

Polymer	Commercial Suppliers' Data		Experimentally Determined Data			
	M. W.	Amino Groups (meQ/g)	VPO $\overline{M}_n$	$\overline{M}_{n, app}$	GPC $\overline{M}_{n, app}$	$\overline{M}_w/\overline{M}_n$ Amino Groups (meQ/g)
PEO-4000-(NH ₂)	4030	0.49	4200	5400	4000	1.35 0.495
Jeffamine 4000	4000	0.45	3920	3200	2100	1.52 0.390
Jeffamine 6000	6000	0.26	6150	—	—	— 0.240
PDMS	9500	0.55	4100	24700	8800	2.81 0.484
PDMS-PEO (4000)	—	—	12360	33400	12900	2.59 0.125
PDMS-PEO (6000)	—	—	13350	37600	15600	2.41 0.073

PDMS precursors. Copolymers, in general, have a distribution of chemical compositions associated with their molecular weight distribution. Since GPC separates molecules based on their size in solution, not according to molecular weight directly, exact molecular weight correlations and distributions for copolymers are difficult to assign between polystyrene standards and PDMS.³¹ The fact that retention volumes between polystyrene and PDMS cannot be compared directly (or even with a derived Q value¹⁰) makes it appropriate to use GPC data reported here for PDMS-containing compounds only for distribution information.

Osmometry data from solutions of polymers in toluene is shown in Table I for comparison. In general, agreement between VPO and commercial claims is relatively good except for the anomalies with the PDMS prepolymer. Given the GPC evidence for low molecular weight contaminants present in the sample, it is reasoned the VPO results could easily indicate lower molecular weights than expected due to contaminant effects on colligative properties. GPC data, corrected using calculated Q values, demonstrates a few discrepancies as well, perhaps due to the problems mentioned. Overall, however, molecular weight characterization agrees well with expected copolymer results and demonstrates successful reaction products, even if broadly dispersed. These data, together with NMR integral ratios, indicates that coupling reactions in the desired stoichiometry has been accomplished. In addition, end-group analysis, shown in Table I, demonstrates the existence of viable functional amino groups in all steps of the synthesis procedure. This adds strength to the assertion that the desired stoichiometric products were recovered.

For swelling determinations to assess the copolymer's behavior in aqueous conditions, films of the copolymers were cast from chloroform solutions onto silanized glass plates. The unheparinized block copolymer containing the PEO 4000 yielded a rubbery, flexible, translucent film lacking tear resistance but otherwise tough. Copolymers containing PEO 6000 yielded pasty, white films lacking mechanical strength and were not used in swelling measurements. The strengths of both types of films appeared to improve with increased exposure time to air, indicating some siloxane vulcanization may be occurring. Thickness of the films, averaged from random measurements was approximately $13.5\ \mu\text{m}$ in the dry state. Pieces measuring $2 \times 2\ \text{cm}$ turned to a gray color and lost their rigidity immediately on contact with water. After 2 hr of hydration, the films demonstrated nearly 350% ($\pm 40\%$) absorption of water by weight. Measurements after 4 hr were not possible due to loss of mechanical integrity. It appears as though the PEO phase maintains a dominant influence on the physical properties of the copolymer. This has various implications for coating behaviors. Both visual observation and clotting time results demonstrated that the coating on glass and PDMS remained intact in aqueous media. Nevertheless, swelling of the coatings probably did occur, possibly allowing the hydrophilic PEO and heparin phases increased exposure to an aqueous environment.

Other effects upon polymer mechanical strengths were qualitatively noted. Generally, copolymers containing TDI as a coupling agent exhibited poorer mechanical strength in films than the same copolymers containing MDI as a coupling agent. Lyman and coworkers³² have noted significant differences in physical properties of aromatic polyurethanes compared to their aliphatic analogues, purportedly due to chain stiffening from the presence of aromatic rings. It is proposed that, for the PDMS-PEO system, MDI permits increased opportunity to form associations of orbital complexes between phenyl rings in adjacent chains. Increased chain mobilities from rotations around the methylene group between MDI's phenyl rings may permit reorganization to maximize hydrogen bond effects. TDI formulations of PDMS-PEO may be sterically hindered from forming π -orbital complexes because of the methyl group pendant from TDI's aromatic ring.

Derivatized heparins [6, 8] were assayed for bioactivity using APTT and compared to commercial heparin before coupling reactions with the PDMS-PEO block copolymers. Figure 7 shows APTT results as standard curves for the different heparin preparations. Commercial heparin converted to its Triton B salt [6] shows an apparent activity comparable to 40% of its commercial sodium salt. Heparin degraded by nitrous acid displayed 36% of the bioactivity of its commercial precursor while heparin subject to both acid cleavage and Triton B exchange [8] retained only 15% of its original activity. Other researchers²⁶ have reported that nitrous acid treatment had similar deleterious effects on heparin activity. It is significant to note that since Triton B bioactivity assays are performed by dilutions of stock heparin solutions prepared as weight concentrations, the Triton B salt would necessarily contain a higher weight percentage of salt per weight of heparin (Triton B cation M.W. = 150.25 compared to Na cation M.W. = 22.99). Thus, any weight of Triton B heparin would contain less heparin molecules (hence, less detected bioactivity) than an equivalent weight of the natural, commercial form. Unpublished evidence²⁴ suggests that a large majority of Na cations in heparin are replaced by the Triton B exchange. These preliminary results also suggest that enough Triton B may be substituted for sodium in heparin to nearly double the molecular weight of the resulting heparin salt as compared to the commercial form. It is our assertion therefore, that assuming some intermediate degree of cation substitution is achieved, a significant weight correction factor must be introduced when comparing the bioactivity of this salt with other heparin forms. In addition, Triton B itself is an inherent physiological toxin and, in practical application, Triton B heparin would require retransformation back to its sodium salt prior to implantation or blood exposure. This transformation occurs readily and reversibly in aqueous media. As shown in Figure 7, the Triton B salt of commercial heparin regained the majority of its original activity when transformed back to its sodium salt form by exchange with NaCl. Nitrous acid-degraded heparin regained 50% of its original derivatized potency after reexchange with sodium. A final consideration in analyzing the reduced Triton B heparin

Figure 7. APTT standard curves comparing bioactivities for commercial and derivatized heparins in sodium and Triton B salt forms (▲ = commercial sodium salt, ● = commercial sodium salt regenerated from its Triton B salt, ■ = Triton B salt of commercial sodium salt, ○ = nitrous acid-degraded commercial sodium salt, △ = sodium salt of nitrous acid-degraded derivative regenerated from its Triton B salt, □ = Triton B salt of nitrous acid-degraded commercial sodium salt).

bioactivity is that dialysis purification removes the low-molecular-weight fragments of heparin which have been implicated in potentiating heparin activity³³ and have been shown to have higher affinity for binding coagulation factors.³⁴

Heparin was coupled directly to block copolymers of PDMS—PEO—NH₂ via two synthetic routes. In the first, EDC was used to activate carboxylic groups present on heparin. Since heparin contains a negligible amount of amine groups (1 glucosamine residue per 4–6 heparin molecules as determined by 2,3,6-trinitrobenzenesulfonic acid,³⁵ the activated carboxyl groups are expected to react exclusively with the amines present on the block copolymer. This method is based upon procedures described by Danishefsky

and coworkers²⁹ for coupling aminoethyl agarose to heparin as well as work by Hennink and coworkers²⁷ coupling albumin to heparin.

Carbodiimide chemistry and applications have been reviewed by Rich and Singh³⁶ and Kurzer and Douraghi-Zadeh.³⁷ Carbodiimides are very reactive species known to add to nucleophiles rapidly under acidic conditions as shown in Figure 8. In the case of heparin, EDC reacts first with the carboxylic group to form the *O*-acylisourea, a highly-activated acylating intermediate. Reactions of carbodiimides with carboxylic acids can also be catalyzed by acid in organic solvents which makes the THF : water system used here viable. The disadvantage of the carbodiimide method is the many accompanying side reactions leading to undesired products, chiefly *N*-acylureas. Reaction conditions favoring intermolecular nucleophilic attack on the *O*-acylisourea lead to fewer side reactions. Unfortunately, reactions in solvents of low dielectric constants suppress side reactions to *N*-acylureas more than reactants in high dielectric solvents.³⁶ The THF : water system used to couple PDMS-PEO to heparin is therefore not the optimum solvent system for reducing side reactions with EDC.

Carbodiimides can also react with aliphatic amines such as those present on amino-telechelic PEO, directly to form guanidines by additions at high temperatures or via acidic catalysis. The question remains whether these two side reactions have been avoided by various researchers as well as these authors in preparing covalently bound heparin using the EDC method. Improvements in yields and anticoagulant potencies might be achieved by investigation into minimizing side reactions in this synthesis system.

The anticoagulant activity of heparin in the block copolymer system is decreased from that of natural, unbound heparin. Work by various researchers³⁸⁻⁴⁰ has shown that heparin's bioactivity is sensitive to modification of its carboxylic groups, leading to a decrease in anticoagulant activity. Thus, the EDC reaction utilizing carboxylic moieties is expected to reduce heparin activity. In addition, it is possible that in the casting of PDMS-PEO-Hep onto substrates, the hydrophilic spacer of PEO and its attached heparin moiety may be partially or completely caught within the coating matrix. Since the system is designed as a coatable material, the effects of coating on bioactivity are a primary concern and are reported in a following paper.¹⁹ Reactions with radiolabeled heparin demonstrate incorporation of only 3 mole% heparin into the copolymers using EDC. Side reactions described above could have reduced heparin coupling. Yields could also have been affected by the sensitivity heparin displays to the THF : water solvent system. Very small amounts of excess THF or water caused minor precipitation of the heparin or copolymer species respectively. Lastly, the fact that the radiolabeled hepa-

Figure 8. Carbodiimide reactions with carboxylic groups on heparin.

rin was not a Triton B salt may have resulted in its precipitation from solution and failure to couple to the copolymer. Because of the small amounts of radiolabeled species used, this could easily have escaped detection.

In the second synthetic route, heparin was degraded by nitrous acid treatment to convert the 2-amino-2-deoxy-D-glucopyranosyl residues into 2,5-anhydro-D-mannose residues with release of the aglycon.²⁵ The resulting aldehyde groups were reacted with primary amines onto PDMS-PEO copolymers to give intermediate Schiff-bases convertible to the stable secondary amine by reduction.^{26,41,42} Specific reactions of aldehydes with primary amines such as those present on PDMS-PEO at pH 6–8 can be achieved in the presence of the BH_3CN^- anion leading to stable secondary amines via reductive amination of the carbonyl group.^{43,44} Thus, heparin-containing terminal aldehyde group (1 per molecule) by specific cleavage with nitrous acid was reacted with the amino-semitelechelic PDMS—PEO— NH_2 block copolymers using NaCNBH_3 as a selective reducing agent. Optimum conditions for this reaction, investigated by Solomon and coworkers,²⁶ showed highest yields with 5% w/w NaCNBH_3 at 50°C and unspecified pH. We believe these conditions to be deleterious to heparin bioactivity and are presently investigating optimum reaction conditions which maximize yield and minimize bioactivity losses.

CONCLUSIONS

Amino-semitelechelic poly(dimethylsiloxane) (PDMS, [1]), was coupled to diamino-telechelic poly(ethylene oxide) (PEO, [4]) using two different diisocyanate species yielding block copolymers of PDMS—PEO— NH_2 [5]. The terminal amino groups on this copolymer were then used to covalently bind heparin in two different procedures in THF:water solutions. In the first procedure, carboxylic acid groups on heparin were activated with EDC and subsequently coupled with the amino-semitelechelic PDMS—PEO— NH_2 [5] to give the heparinized product [7]. In the second experiment, heparin was first derivatized by acid hydrolysis to give heparin with aldehyde groups [8]. This heparin form was then coupled to the copolymer [5] via a Schiff's base complex (reductive amination) using NaCNBH_3 as a selective reducing agent. Both reactions yielded block copolymers in quantitative yields having bioactive heparin moieties. Incorporation of fractionated, radiolabeled heparin into the block copolymers using the EDC method indicated 3 mole% heparin content in the copolymer system.

These results demonstrate the successful development of a new technique to improve the surface properties of biomaterials using a coatable amphiphilic copolymer system containing bioactive, covalently bound heparin.

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