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Surface modification of polymeric biomaterials with poly(ethylene oxide), albumin, and heparin for reduced thrombogenicity

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Abstract—Appropriate surface modification has significantly improved the blood compatibility of polymeric biomaterials. This article reviews methods of surface modification with water-soluble polymers, such as polyethylene oxide (PEO), albumin, and heparin. PEO is a synthetic, neutral, water-soluble polymer, while albumin and heparin are a natural globular protein and an anionic polysaccharide, respectively. When grafted onto the surface, all three macromolecules share a common feature to reduce thrombogenicity of biomaterials. The reduced thrombogenicity is due to the unique hydrodynamic properties of the grafted macromolecules. In aqueous medium, surface-bound water-soluble polymers are expected to be highly flexible and extend into the bulk solution. Biomaterials grafted with either PEO, albumin, or heparin are able to resist plasma porotein adsorption and platelet adhesion predominantly by a steric repulsion mechanism.

Key words: Poly(ethylene oxide); albumin; heparin; surface modification; blood compatibility; steric repulsion.

INTRODUCTION

Many different materials have been used for biomedical applications including polymers, ceramics, metals, carbon, and composites [1]. Of all these materials, polymers offer ease in processing and control over the physical properties necessary for appropriate biomedical applications [2]. Like other blood-contacting biomaterials, long-term use of polymers in blood is limited in part due to surface-induced thrombosis, initiated by the adsorption of plasma proteins and activation of platelets [3, 4]. The adsorption of plasma proteins is known to produce a 'conditioning film' which determines the outcome of other processes, such as cell adhesion and activation of the complement system [5].

Following protein adsorption, the adhesion of platelets onto biomaterials contributes to surface-induced thrombosis. In irreversible interactions with the surface, adherent platelets change their shape by extending pseudopods and spread on the surface [6, 7]. When fully activated, platelets release the biochemical contents of their granules, which causes further activation of other platelets and simultaneous initiation of the coagulation cascade reaction to form thrombi [6]. Thrombi formed on the biomaterial surface could be either red or white depending on their content. Red thrombi, for instance, consist of platelet aggregates and red blood cells all trapped in a fibrin network [8]. Interactions at the biomaterial-blood interface leading to surface-induced thrombosis are illustrated in Fig. 1.

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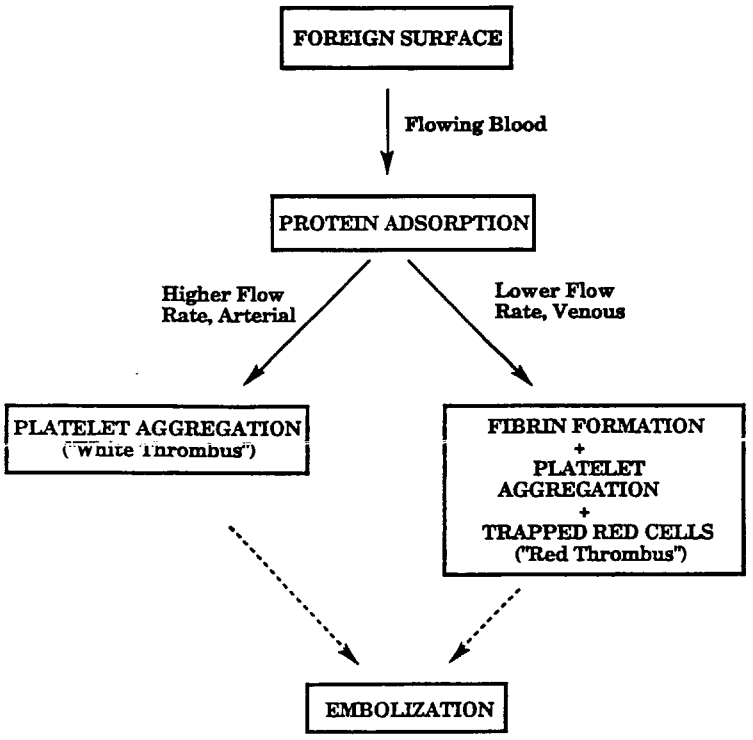


Figure 1. Schematic illustration of surface-induced thrombosis on synthetic biomaterial surface (from ref. 1).

Since the interactions that lead to thrombus formation occur at the biomaterial–blood interface, appropriate surface modification methods will be beneficial in improving the blood compatibility of biomaterials without altering the bulk properties of the material necessary for biomedical applications. Hoffman [9] reviewed the surface modification methods and grouped them into two general categories: physicochemical and biological methods. Examples of physicochemical methods are coating deposition, chemical modification, graft copolymerization, and plasma treatment. The biological methods include pre-adsorption of proteins, drug or enzyme immobilization, cell seeding, and preclotting. Ikada [10] proposed that a diffuse hydrophilic biomaterial surface would be blood compatible. To prepare diffuse hydrophilic surfaces, various types of water-soluble monomers have been graft-polymerized to biomaterial surfaces [11]. Water-soluble polymers have also been grafted to create a diffuse hydrophilic surface. The hydrophilic polymers in the diffuse layer exert steric repulsion to proteins and cells that reach the surface.

Steric repulsion is due to a loss of configurational entropy resulting from volume restriction and/or osmotic repulsion between the two overlapping polymer layers [12, 13]. Steric repulsion of plasma proteins and platelets from a biomaterial surface by water-soluble polymers is illustrated in Fig. 2. For effective steric repulsion, water-soluble polymers of the diffuse layer must satisfy the following three requirements. First, the polymer molecules should have high affinity to the surface so that they can anchor tightly to the surface. Second, part of the polymer chain should also

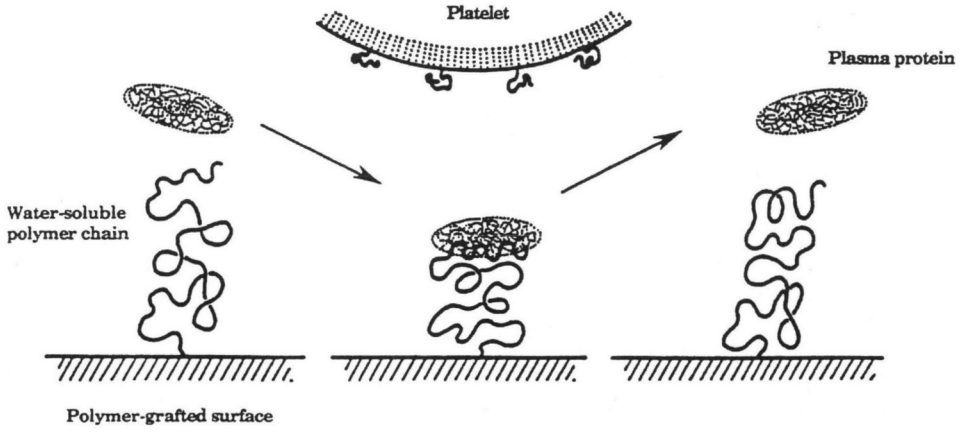


Figure 2. Steric repulsion of plasma proteins and platelets by surfaces grafted with water-soluble polymers.

extend into the bulk solution. The domination of steric repulsion over the van der Waals attractive forces is dependent on the extension and flexibility of the polymer chain in the bulk solution. Block copolymers containing both hydrophilic and hydrophobic segments are more effective in steric repulsion than homopolymers [13, 14]. The hydrophobic segment can anchor the copolymer to the hydrophobic surface, while the water-soluble segment can extend into the bulk solution. If covalently grafted, homopolymers will be as effective as amphiphatic block copolymers. Finally, the polymer molecules should completely cover the surface. If a significant portion of the surface is exposed, steric repulsion is not effective.

In this review, we describe grafting of three water-soluble polymers—PEO, albumin, and heparin—which have been used for surface modification of biomaterials. PEO is a synthetic neutral polymer; albumin and heparin are natural macromolecules. Albumin is a globular plasma protein, while heparin is an anionic polysaccharide. Biomaterial surfaces modified with these three water-soluble polymers have shown a remarkable improvement in blood compatibility.

PEO-TREATED SURFACES

PEO in aqueous medium

As shown below, PEO is a polyether type of the water-soluble synthetic polymer.

$$—[CH_2CH_2O]_n—$$

When the molecular weight is less than 10000 Daltons, the polymer is called poly(ethylene glycol) (PEG). The polymers with higher molecular weight are known as poly(ethylene oxide) (PEO) or polyoxyethylene [15]. Compared to other polyethers, such as poly(propylene oxide), PEO is highly water soluble [16]. Kjellander and Florin [17] explained the high water solubility of PEO in terms of a good structural fit between water molecules and the polymer, resulting in hydrogen bonding between the ether oxygen of PEO and water molecules.

With PEO, aqueous solubility decreases with increasing temperature. The decreased solubility at elevated temperatures is due to a decrease in hydrogen bonding and corresponding increase in hydrophobic interactions between polymer chains [18].

Using ^{13}C-NMR, Bjorling *et al.* [19] reported that PEO adapts to a gauche conformation in the polar solvent such as water and a trans conformation in nonpolar medium. The gauche conformation would be more suitable for hydrogen bonding.

Steric repulsion with terminally attached PEO

At the solid–liquid interface, PEO anchored through terminal end will interact with water molecules and extend into the bulk aqueous medium [20]. Surface-bound PEO molecules are very effective in preventing adsorption of other macromolecules by the steric repulsion mechanism [21]. Using the surface force technique, Luckam [22, 23] observed that steric repulsion with PEO is mainly due to osmotic repulsion between interdigitated PEO chains. Jeon *et al.* [24, 25] have theoretically modeled protein–surface interactions in the presence of PEO and found that steric repulsion by surface-bound PEO chains is mainly responsible for the prevention of protein adsorption on PEO-rich surface.

Interfacial properties of PEO-treated biomaterials

PEO-rich surfaces have been prepared by physical adsorption of PEO [26, 27]. Only high molecular weight PEO ($M_w > 100\,000$) can be effectively adsorbed on hydrophobic surfaces [28]. Chromatographic supports used for the separation of proteins, cells, and viruses have been treated by physical adsorption of high molecular weight PEO [26, 27]. Physically adsorbed PEO homopolymers, however, can be displaced by other macromolecules which have higher affinity for the surface. Many proteins and cells in the blood can easily displace physically adsorbed PEO from the surface.

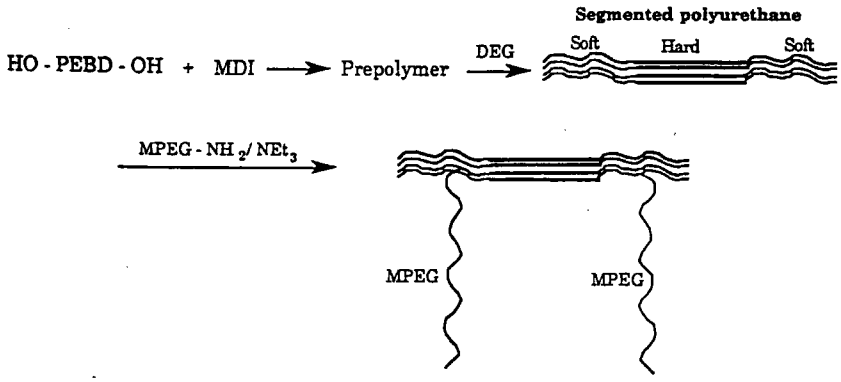
Adsorption of PEO-containing amphipathic block copolymers would be more stable than that of homopolymers as the hydrophobic segment can anchor the copolymer molecule on the surface, while the hydrophilic PEO chains can extend into the bulk aqueous medium. Adsorption of PEO/poly(propylene oxide)(PPO)/PEO triblock copolymers (Pluronics®) have been used for the prevention of protein adsorption and cell adhesion on hydrophobic surfaces. Lee *et al.* [29] did not observe a significant decrease in albumin adsorption on Pluronic-treated surfaces if Pluronics had 30 propylene oxide (PO) residues, since the copolymer was weakly bound to the surface. Albumin could easily displace weakly bound Pluronics and interact directly with the surface. To improve the stability of copolymers on the surface, Lee *et al.* [30] synthesized copolymers of PEO-methacrylates containing alkyl chains for tight binding to hydrophobic surface. Tight surface binding was achieved with Pluronics containing longer hydrophobic PPO segments. Pluronic F-108 which has 56 PO residues and 129 ethylene oxide (EO) residues, minimized protein adsorption on polystyrene latex particles [31]. The adhesion of cells onto octadecyldimethylsilane-treated glass was also inhibited, when the surface was coated with Pluronic F-108 [32]. Using ten different Pluronics with varying chain length of PEO and PPO, we have shown that Pluronics containing a minimum of 56 PO residues and 19 EO residues were sufficient to repel proteins and platelets [33, 34]. The adsorption of Pluronics onto hydrophobic surface presents the simplest method to modify the surface for prevention of protein adsorption and cell adhesion.

Desai and Hubbell [35] have entrapped PEO chains to the surface by partially dissolving the base polymer with a suitable solvent. Poly(ethylene terephthalate) (PET) with entrapped PEO (M_w 18 500) decreased albumin adsorption by 80% and platelet adhesion by more than 95% as compared to the control PET. Ruckenstein and coworkers [36, 37] have used a similar approach to entrap PEO-containing block copolymers to poly(methyl methacrylate), polystyrene, and poly(vinyl acetate) surfaces. The base polymer was swollen in an organic solution containing PEO block copolymer. This caused the hydrophobic segment of the copolymer to be entangled within the swollen surface and was secured by placing the system in a non-solvent (water). Physical entrapment of PEO homopolymers or block copolymers should consider the toxicity of the organic solvents used for swelling the base polymer and poor adaptability of the technique to other polymeric substrates.

Covalent grafting of PEO to surfaces is the most effective way of creating a permanent PEO layer. Merrill *et al.* [38] initially reported that PEO soft-segment polyurethanes are highly blood compatible. Ito and Imanishi [39] reviewed the work of many investigators who prepared polyurethanes with polyether soft-segment. In some cases, polyurethanes made of low molecular weight PEG ($M_w \sim 1000$) allow platelets to adhere and activate. The overall results, however, have proven that segmented polyurethanes containing high molecular weight PEO had improved blood compatibility as compared to other types of polyethers [39]. Yu *et al.* [40] developed PEO-containing polyurethaneurea hydrogels as coatings for biomedical products. Polyurethanes with PEO grafted to the side chains were found to be highly blood compatible [41, 42]. Figure 3 shows the reaction scheme for synthesis of polyurethanes with methoxy-PEG side chains. Chaikof *et al.* [43] recently developed interpenetrating polymer networks (IPN's) of PEO and polyether substituted polysiloxane. A significant decrease in platelet adhesion was observed when the PEO (M_w 8000) content in the IPN was increased up to 65%.

Allmer *et al.* [44] covalently coupled PEO chains to a glycidyl methacrylate-bound polyethylene surface. Akizawa *et al.* [45] coupled methoxy-PEG with a terminal carboxyl group to form an ester linkage with the hydroxyl groups of cellulose dialysis membranes. Improved dialysis efficiency and blood compatibility was observed with PEG grafting. Desai and Hubbell [46] grafted cyanuric chloride activated PEO to amine derivatized PET surfaces. Figure 4 illustrates the reaction for covalent coupling of cyanuric chloride activated PEO to amine-derivatized PET film. A 50% decrease in plasma protein adsorption and more than 90% decrease in platelet adhesion was observed using PEO (M_w 18 500 and 100 000)-grafted surfaces. Gombotz *et al.* [47] recently reported coupling of *bis*-amino PEO to cyanuric chloride activated PET films. The adsorption of albumin and fibrinogen was found to decrease with increasing molecular weight of immobilized PEO. Chemical coupling of PEO to polymeric surfaces, as described, is possible only if the surface has functional groups that can react with PEO derivatives. For inert polymers such as polyethylene, PEO coupling is possible only when the surface is pre-modified with reactive functional groups [44]. Grafting by use of UV or gamma irradiation, however, may not require premodification of the polymer surface.

Mori and Nagaoka [48] prepared PEO-rich surfaces by photoinduced grafting of methoxy poly(ethylene glycol) methacrylates to poly(vinyl chloride) surface in the presence of dithiocarbamate. With increasing PEO chain length up to 100 EO residues, plasma protein adsorption and platelet adhesion was significantly

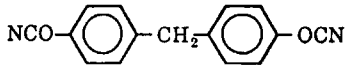


HO - PEBD - Epoxidised polybutadiene-diol, M_n = 3020; Epoxy groups = 14

MPEG: Methoxy polyethylene glycol-amine $CH_3-(OCH_2CH_2)_n-NH_2$; M_n = 100

DEG: Diethylene glycol $OH-CH_2CH_2-OH$

MDI: 4,4'-diphenylmethane di-isocyanate



NEt_3 : Triethylamine

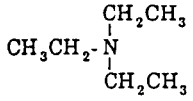


Figure 3. Covalent coupling reaction of methoxy-PEG (MPEG) to segmented polyurethane (from ref. 39).

decreased on PEO-grafted surfaces. Tseng and Park [49] synthesized PEG-phenylazide for photoinduced grafting to various polymeric surfaces. In the presence of UV light, azide groups are converted into highly reactive nitrine groups. The reaction scheme for synthesis of PEG-phenylazide and photoinduced grafting onto dimethyldichlorosilane-treated glass (DDS-glass) is shown in Fig. 5. The number of platelets decreased by 95% on PEG-grafted DDS-glass as compared to that on control DDS-glass. The spread area of individual platelets also decreased from 45 μm^2 on control DDS-glass to less than 20 μm^2 on PEG-grafted DDS-glass. If platelets are in the contact adherent state, their spread area is less than 20 μm^2.

Sheu *et al.* [50] recently introduced a method of grafting PEO-containing block copolymers (Brij®) by exposing the adsorbed copolymers to glow discharge treatment. High energy gamma or electron beam irradiation can also be used to graft PEO to various surfaces. We have grafted Pluronic F-68 (76/30/76) copolymers to DDS-glass by γ-irradiation. Effective grafting was achieved when Pluronic-adsorbed DDS-glass was exposed to γ-irradiation in the presence of an aqueous buffer [51]. The number of adherent platelets decreased by 85% on Pluronic-grafted DDS-glass as compared to that on control DDS-glass. In addition, the spread area of individual platelets decreased from 45 μm^2 on control DDS-glass to 15 μm^2 on Pluronic-grafted DDS-glass. Sun *et al.* [52] grafted PEG-methacrylates to

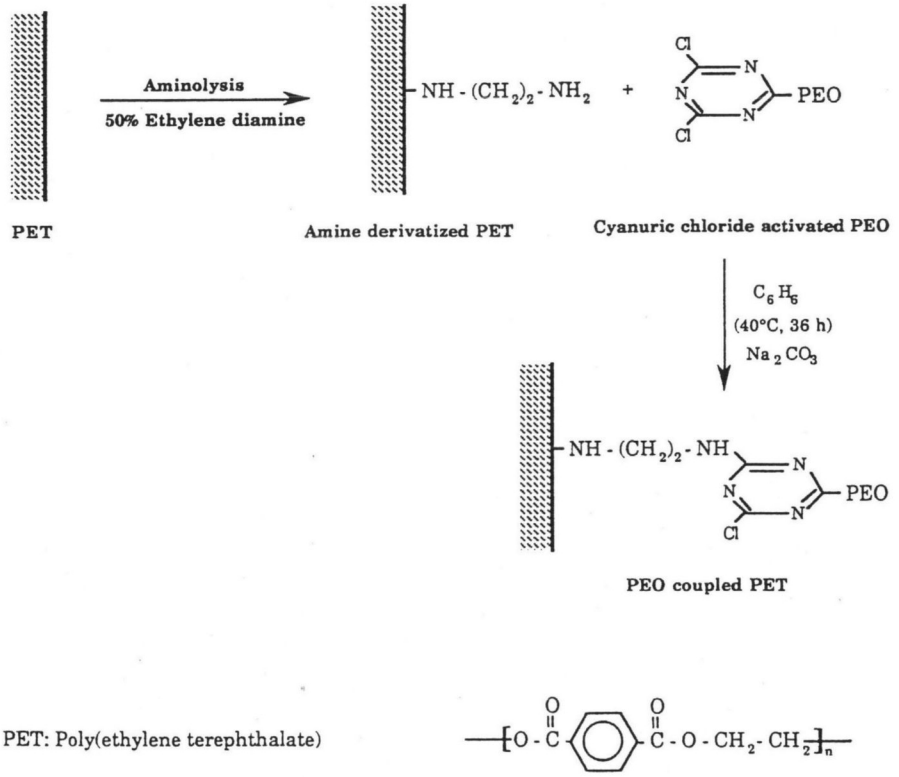


Figure 4. Covalent coupling reaction of cyanuric chloride activated PEO to amine-derivatized poly(ethylene terephthalate) (PET) film (from ref. 46).

Silastic® films by mutual irradiation in the prescence of Cu^{2+} ions to prevent homopolymer gelation. A 72% decrease in fibrinogen adsorption was observed when the number of EO residues of the grafted PEG were 100. However, gamma irradiation at high doses may alter the bulk properties of some polymers such as polypropylene.

ALBUMIN-TREATED SURFACES

Properties and functions of albumin

Albumin is the most abundant protein found in blood. The concentration of albumin in healthy adults varies from 35 mg/ml to 50 mg/ml [53]. There are 585 amino acid residues in human serum albumin, 181 of which have either acidic or basic side-chains. Albumin has some unique properties which distinguishes it from other globular proteins in the blood. It is an acidic protein with high aqueous solubility and stablity. Stability of albumin against thermal denaturation and low pH conditions is due to 17 disulfide bonds [54]. It has high aqueous solubility, attributed to the polar surface of the molecule. At pH 7, albumin molecule has a net charge of −15 [55].

Albumin serves three important functions in the body [56]. First, a sufficient amount of albumin is synthesized by the liver to serve as a nutritional source in

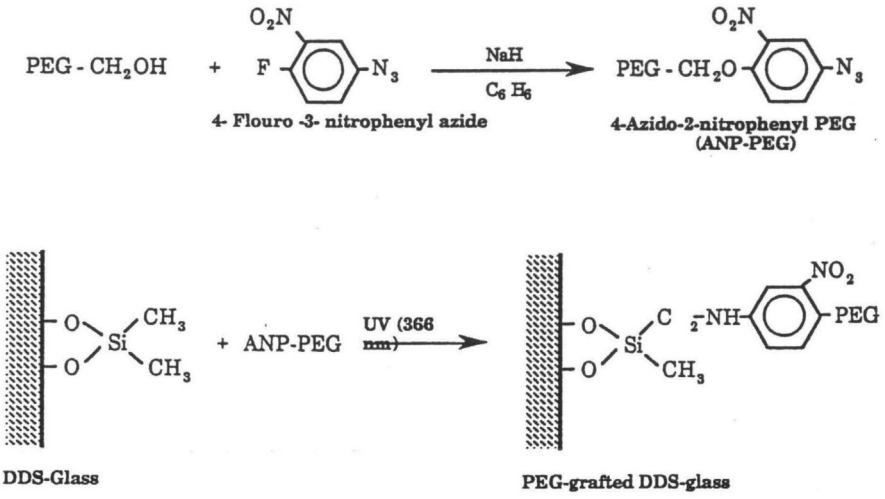


Figure 5. Synthesis and photoinduced grafting of PEG-phenylazide to dimethyldichlorosilane-treated glass (DDS-glass) (from ref. 49).

cellular metabolism. Second, albumin serves to transport small molecules such a steroids, ions, and fatty acids. Finally, albumin maintains the osmotic pressure of the blood.

Mechanism of surface passivation by albumin

Plasma protein adsorption and platelet adhesion and activation is significantly reduced on albumin-coated biomaterial surfaces [57, 58]. Albumin has poor affinity for hydrophilic surfaces. Previously, we have found that platelets were able to adhere and activate on albumin-coated hydrophilic glass even when the bulk albumin concentration was increased up to 50 mg/ml [59]. Albumin adsorbs tightly onto hydrophobic surfaces and the adsorption is usually irreversible [60]. Since albumin molecules do not have receptors on the platelet membrane, platelets do not specifically interact with albumin or adhere to albumin-coated surface [61].

Platelets were able to adhere and activate completely on albumin-coated DDS-glass, if the adsorbed albumin was crosslinked with glutaraldehyde, dried and rehydrated, or digested with trypsin, a proteolytic enzyme [62]. Crosslinking with glutaraldehyde is expected to reduce the motional freedom or flexibility of the surface accessible segments of adsorbed albumin molecules. Drying of absorbed albumin resulted in the exposure of bare surface sites. Tryptic digestion may have resulted in the exposure of bare surface sites and cleavage of flexible albumin segments. The three requirements of steric repulsion by water-soluble polymers are met by albumin molecules which are adsorbed on DDS-glass. First, albumin binds tightly to DDS-glass [63]. Second, the flexibility of surface accessible fragments of albumin is important in preventing platelet adhesion. Finally, prevention of platelet adhesion by albumin is effective only when the surface is fully covered. This suggests that steric repulsion is the predominant mechanism for the prevention of protein adsorption and platelet adhesion by the absorbed albumin.

The relationship between colloidal stabilization by steric repulsion and prevention of platelet adhesion on the albumin layer was first proposed by Morrissey [64].

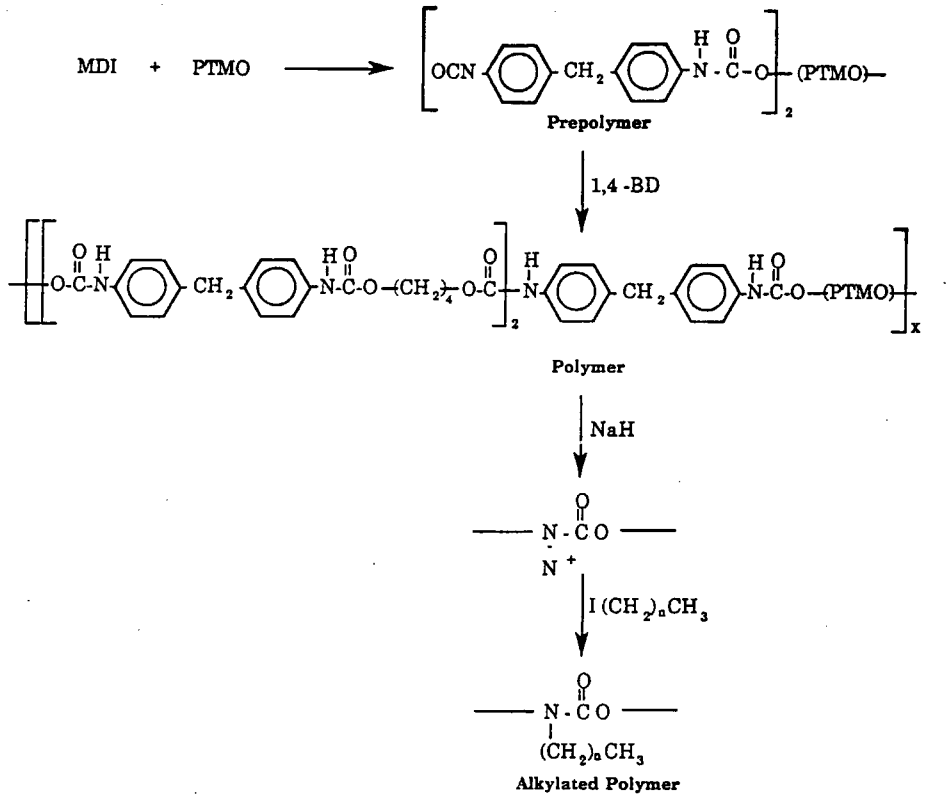
Recently, using the surface force technique, Blomberg *et al.* [65] concluded that short range repulsion between albumin-coated mica surfaces could be due to steric repulsion. It should also be noted that albumin can be used for the stabilization of colloidal gold particles [66] and negatively charged polystyrene latices [67]. Van der Scheer *et al.* [67] concluded that the optimum stability of polystyrene latices was obtained only when albumin molecules completely covered the surface of the latices. Further experimental evidence of colloidal stabilization with adsorbed albumin on polystyrene latices has been obtained by Tamai *et al.* [68]. In high ionic strength media, adsorbed albumin was effective in preventing coagulation of latices by steric repulsion.

Interfacial properties of albumin-treated biomaterials

Chang [69, 70] initially suggested the use of albumin coating as a method to prevent surface-induced thrombosis in clinical situations. Physically adsorbed albumin has been used for improving blood compatibility of clinical implants [71]. Physically adsorbed albumin, however, is effective in preventing platelet adhesion and activation only for a short period of time. For long-term use of blood-contacting biomaterials, adsorbed albumin is desorbed from the surface by other plasma proteins with high surface affinity.

In an attempt to improve the stability of albumin on the surface, Sigot-Luizard *et al.* [72] treated adsorbed albumin with glutaraldehyde. The results, however, showed that platelets were able to adhere and activate on the glutaraldehyde-crosslinked albumin layer. For permanent albumin immobilization, Hoffman *et al.* [73] covalently attached albumin to hydrogel-grafted polymer surface using ε-aminocaproic acid spacer group. Sharma and Kurian [74] attempted covalent grafting of albumin to polyurethane surface by γ-irradiation. After 1.0 Mrads of irradiation, the number of adherent platelets actually increased. It is unclear whether albumin was grafted at all or if adsorbed albumin was damaged by γ-irradiation. Matsuda and Inoue [75] synthesized photoreactive albumin for grafting to fabricated medical devices. Platelet reactivity on photografted albumin surface was found to decrease significantly. Unfortunately, no long-term stability studies have been done to suggest that albumin remains on the surface in the native, undenatured state.

Eberhart *et al.* [76–78] studied selective adsorption of albumin from blood onto polymeric surfaces. Long carbon chains (C-16 and C-18) grafted on polyurethane surfaces were found to have high affinity for albumin. Alkyl-grafted surfaces were found to have high affinity for albumin adsorbed from simple protein solution, from binary protein mixture, from plasma, and from whole blood [76]. Similar alkylated surfaces were later tested in canine *ex vivo* shunt model [79, 80]. Figure 6 shows the reaction scheme for synthesis of polyurethanes with alkyl chains for enhanced albumin affinity. Fibrin formation and platelet adhesion and aggregation were completely inhibited in short-term *in vivo* experiments. The alkyl-modified surfaces, however, could not achieve total specificity towards albumin [77]. In long-term applications, thrombogenic proteins in blood can displace albumin from the surface to create a favorable environment for platelet activation.



MDI : 4,4'-diphenylmethane di-isocyanate

1,4-BD : 1,4 Butanediol

PTMO : Poly(tetramethylene oxide)

Figure 6. Reaction scheme for synthesis of alkyl-derivatized polyurethanes with enhanced albumin affinity (from ref. 39).

HEPARIN-TREATED SURFACES

Properties and functions of heparin

Heparin is an anionic linear polysaccharide chemically known as glycosaminoglycans. The molecular weight of heparin ranges from 3000 to 40 000 Daltons with an average of 15 000 Daltons [81]. Commercially available heparin is isolated from bovine and porcine lung or intestinal mucosa. The chemical structure of heparin sodium is shown in Fig. 7, consisting of two repeating disaccharide units: D-glucosamine-L-iduronic acid and D-glucosamine-L-glucouronic acid. Heparin is strongly acidic because of its content of covalently bound sulfonate and carboxylic acid groups [82].

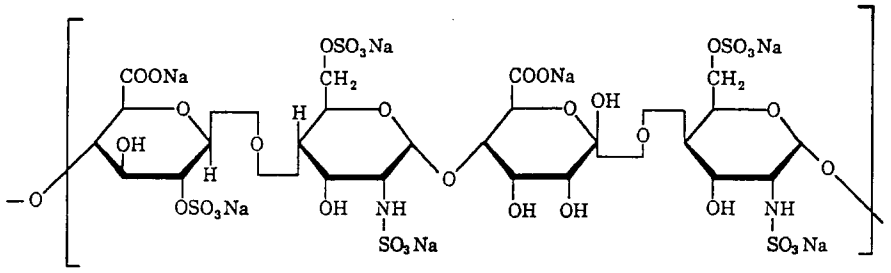


Figure 7. Proposed structure of heparin sodium.

Endogenous or intravenously injected heparin is known to inhibit blood coagulation. The anticoagulant activity of heparin is mediated through a co-factor, antithrombin III [83]. Heparin–antithrombin III complex inactivates serine proteases, most importantly thrombin, which catalyzes the conversion of fibrinogen to fibrin in the final step of the coagulation cascade reaction [84]. Heparin markedly accelerates the rate, but not the extent, of thrombin inactivation. Heparin-antithrombin III complex may also bind with the activated clotting factors (XII_a, kallikrein, XI_a, IX_a, X_a, II_a, and $XIII_a$) resulting in inactivation of these factors [83].

Mechanism of surface passivation by heparin

Heparinized materials, first described by Gott *et al.* in 1963 [85], have been extensively used as blood-contacting biomaterials. Several detailed review articles on the subject of heparinized biomaterials are available [86, 87]. Although heparinized surfaces have been studied extensively, the exact mechanism of surface passivation by heparin is still not well understood. In addition, the effect of heparin seems to be different *in vitro* and *in vivo*. When heparin is ionically immobilized, there is a gradual release of heparin into the biological environment. The released heparin can then interact with antithrombin III and mediate the anticoagulant effect [88]. The exact mechanism of thrombo-resistance of heparin grafted to the biomaterial surface is still unclear. Ebert and Kim [89] have shown that the activity of heparin grafted to the surface through alkyl spacer groups was dependent on the length of the spacer, while prevention of platelet activation occurred independent of the spacer length. These results suggest that the ability of heparin to prevent platelet activation is independent from the anticoagulant activity of heparin.

Larsson *et al.* [90–92] proposed a mechanism of surface passivation of heparin based on steric repulsion. Heparinized materials were tested by measuring the activity of thrombin after exposure to heparin–antithrombin III complex. Heparin was attached to the surface either by end-point or multi-point attachment. End-point attached heparin completely inhibited thrombin activity, whereas multi-point attached partially inhibited thrombin activity. End-point grafted heparin showed inactivation of thrombin similar to that of the vascular endothelium, since heparin was flexible and may extend into the bulk aqueous medium. These studies have shown that the flexibility of immobilized heparin is important for optimum surface passivation.

Heparin flexibility is also improved by the use of a hydrophilic spacer group, such as PEO or albumin, when immobilizing heparin onto a surface [93–97]. The anticoagulant properties of heparin can be improved by end-point attachment or

with the use of a hydrophilic spacer group. In addition, end-point grafted or the use of a hydrophilic spacer group would extend heparin into the bulk solution for effective steric repulsion. For effective surface passivation, heparin should be covalently or tightly bound to the surface, remain flexible and extend into the bulk solution, and cover the surface completely.

Interfacial properties of heparin-treated biomaterials

Gott *et al.* [85] ionically immobilized negatively charged heparin onto the positively charged benzalkonium chloride-treated graphite surface. Other more hydrophobic quaternary ammonium salts were also used, since they are soluble in organic solvents [98]. The organic solution can be spread on hydrophobic substrates and nonrigid hydrophobic polymers such as silicone rubber can be effectively coated. Although effective for short-term blood-compatibility, ionically bound heparin was easily displaced from the surface by an ion exchange mechanism when exposed to blood. In order to increase the stability of ionically-bound heparin, heparin-bound surfaces were treated with glutaraldehyde [99]. Treatment with glutaraldehyde significantly reduced the release of heparin from the surface.

Even with crosslinking by glutaraldehyde, surface-bound heparin is slowly released into the circulation upon exposure to blood. To further improve heparin stability on the surface, albumin-heparin conjugates were synthesized for preparing heparin-surfaces by simple coating on hydrophobic biomaterials [93, 94]. In contact with plasma, however, physically adsorbed albumin-heparin conjugates were gradually replaced by other plasma proteins.

Heparinized hydrogels combine two biologically important features: anti-coagulant properties of heparin and blood compatibility of hydrogels. Sefton [100] reviewed the work on heparinized hydrogels, especially the blood compatibility of heparin-poly(vinyl alcohol) (PVA). *In vitro* and short-term *in vivo* studies provided sufficient evidence of improved blood compatibility with heparinized-PVA [101, 102]. Recent studies by Cholakis *et al.* [103, 104], however, suggested that the number of adherent platelets as well as platelet reactivity remained similar to the control PVA surface. In addition, platelet consumption was found to increase on heparin-PVA surface when exposed to blood in an *ex vivo* shunt. On some hydrogel-grafted surfaces platelet adhesion is decreased, while platelet consumption increases [105]. The exact mechanism of increased platelet consumption on hydrogel-grafted surfaces is not understood [106]. Cholakis *et al.* [104] concluded that there was no correlation between the improved blood compatibilities of heparin-PVA surface obtained from *in vitro* and *ex vivo* tests.

Polyurethanes with covalently grafted heparin were synthesized and their blood compatibility reported [39]. Heparin linked with alkyl groups was reacted with the isocyanate-derivatized polyurethanes. Blood compatibility of heparin-grafted polyurethanes was much better compared to physically adsorbed or ionically bound heparin. Larsson *et al.* [90–92] immobilized heparin on polyethylene imine (PEI)-grafted polyethylene (PE) tubing. Heparin was immobilized either by end-point or by multi-point attachment procedure. Platelet adhesion was almost negligible when heparin was attached through end-point attachment.

In order to increase the flexibility of heparin, it was attached to surfaces through a hydrophilic PEO spacer. Grainger *et al.* [95, 96] prepared poly(dimethylsiloxane)–poly(ethylene oxide)–heparin (PDMS–PEO–heparin) block copolymers which were

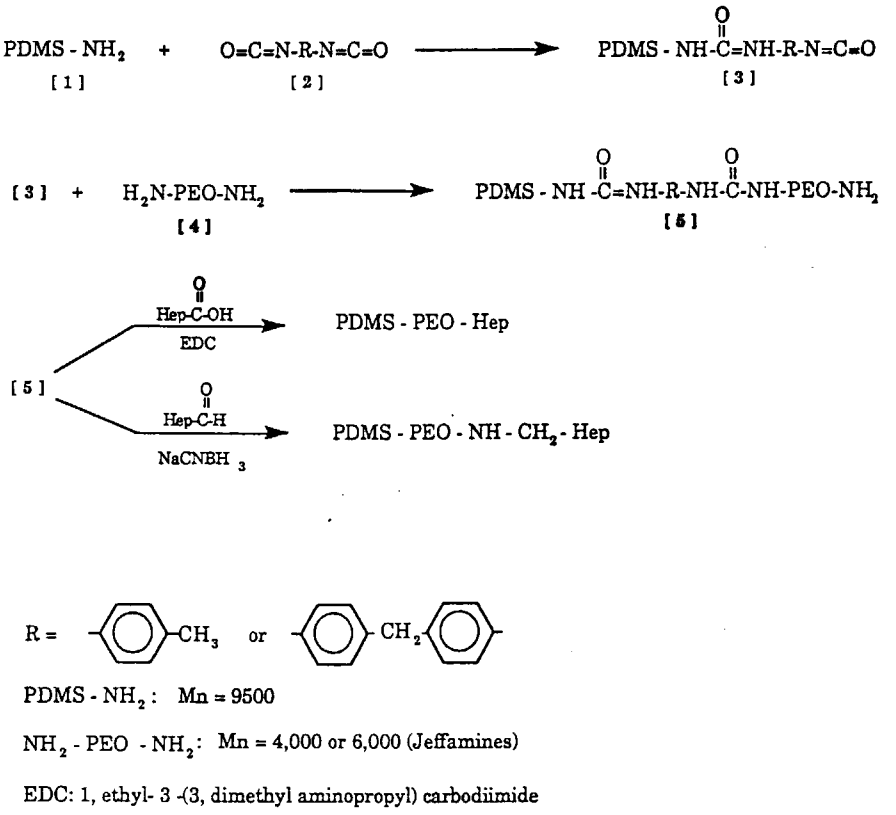


Figure 8. Covalent coupling of heparin to poly(dimethylsiloxane) (PDMS) through PEO spacer group (from ref. 95).

adsorbed onto hydrophobic surfaces. Figure 8 shows the reaction scheme for the synthesis of PDMS-PEO-heparin. Platelet adhesion and activation actually increased on surfaces treated with PDMS-PEO-heparin compared to PDMS alone. This is probably due to poor grafting efficiency of heparin to PDMS. Park *et al.* [97] synthesized segmented polyurethaneurea-PEO-heparin (Biomer®-PEO-Hep) copolymers using three different molecular weights of PEO. PEO was grafted to Biomer® through free isocyanate linkage and heparin was reacted with isocyanate-derivatized PEO. Figure 9 illustrates the reaction scheme for synthesis of Biomer®-PEO-Hep. Although the amount of heparin immobilized was highest with PEO-1000 (0.47 $\mu g/cm^2$), the heparin bioactivity ratio was highest with PEO-3400 (22.17%).

Copolymers containing tertiary amine groups which can be protonated at the physiological pH such as *N,N'*-dimethylaminoethyl or *N,N'*-diethylaminoethyl have been synthesized [107]. This approach avoids the toxicity of using quaternary ammonium salts; however, heparin is still bound ionically to the surface. Ferruti and co-workers [108–111] have synthesized poly(amido-amine) (PAA) which can be grafted to various biomaterial surfaces. PAA forms a strong complex with heparin prior to blood-polymer contact [109]. Figure 10 illustrates the reaction scheme for grafting PAA to polyurethane surface. Studies with PAA-grafted surfaces have

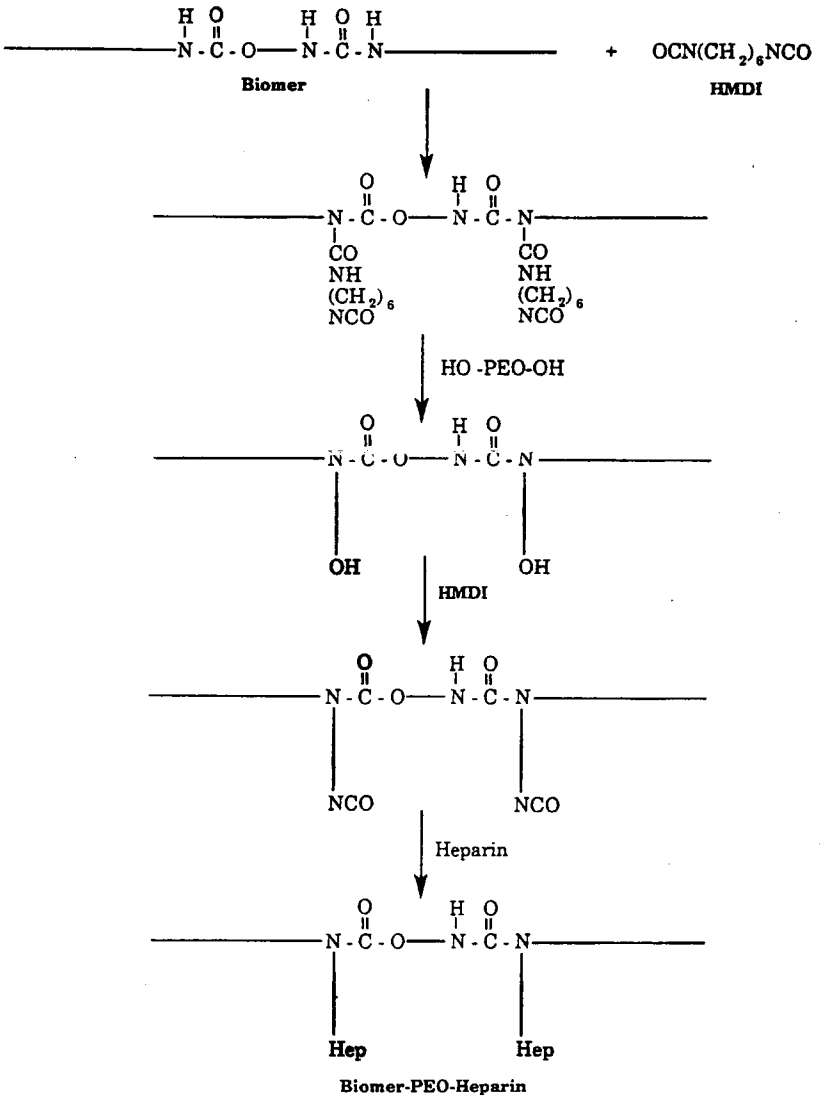


Figure 9. Covalent coupling of heparin to Biomer® through PEO spacer group (from ref. 97).

shown tight heparin binding. Upon long-term exposure to blood, even tightly bound heparin can be displaced by ion-exchange mechanism with endogenous cations and proteins.

CONCLUDING REMARKS

This paper reviewed the effectiveness of PEO, albumin, and heparin in reducing the surface-induced thrombosis in terms of the steric repulsion mechanism. PEO, albumin, and heparin are effective in preventing surface-induced thrombosis only when they meet the three requirements: (1) tight binding to the surface; (2) complete surface coverage; and (3) flexibility of the grafted polymers. The tight binding of the hydrophilic polymers to the surface is important, since the polymers can be

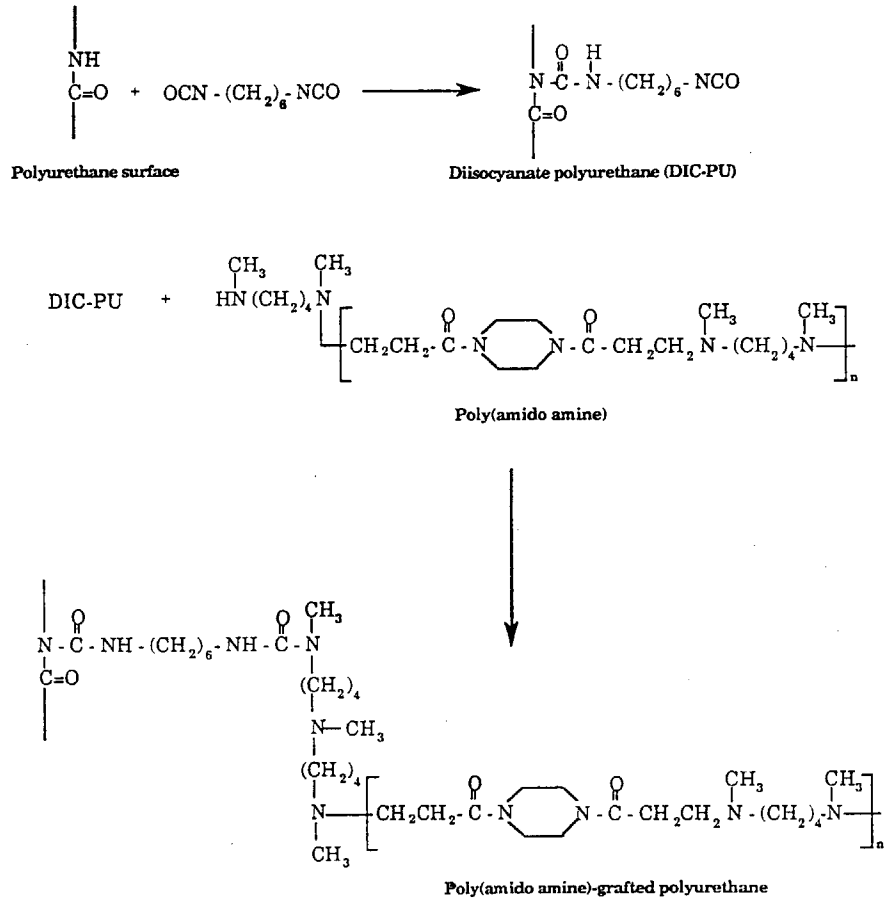


Figure 10. Reaction scheme for grafting poly(amido amide) to polyurethane (from ref. 111).

otherwise easily displaced from the surface by proteins and cells in blood. Thus, covalent grafting to the surface is most desirable. The grafted polymers have to cover the surface in such a way that no significant bare surface sites are exposed to blood proteins and cells. The beneficial effect of PEO, albumin, and heparin will not be observed if the surface coverage is not complete. The grafted hydrophilic polymers should remain flexible on the surface. Otherwise, it will simply create another surface on which protein adsorption and cell adhesion can occur. The steric repulsion mechanism can explain many of the observed phenomena described in the literature. The concept of the steric repulsion mechanism should be useful in the design of improved biomaterials.

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