Platelet Interaction with Poly(ethylene Oxide) Networks

Interpenetrating polymer networks (IPNs) of poly(ethylene oxide) (PEO) and a polyether substituted polysiloxane were synthesized containing variations of PEO content (35 and 65% dry network weight) and PEO systematically varied from low to high molecular weight (2,000, 8,000, and 20,000). Biocompatibility was assessed by measureing ^{111}In platelet deposition in a baboon femoral *ex vivo* shunt. Mass transfer analysis revealed that platelet transport to PEO/polysiloxane surfaces was kinetically limited and correlated with both PEO content and molecular weight. Differences in material performance were noted particularly after the initial 30 min blood contact period. In mid and high PEO molecular weight networks (8,000 and 20,000), reduced platelet deposition was noted with increased PEO content. In materials of high PEO content (65%), mid and high PEO molecular weight samples had significantly lower levels of platelet adsorption than PDMS (60 min; $p < 0.05$) or networks of low PEO molecular weight, coded 2K-65 (30 and 60 min; $p < 0.05$). The lowest level of platelet deposition was noted on 20K-65 networks, less than one platelet per 1,000 μm^2.

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Introduction

Poly(ethylene oxide) (PEO) based biomaterials are recognized as having low levels of plasma protein and cellular adsorption and consequently have generated considerable interest as candidate thromboresistant materials. Poly(ethylene oxide)–polyethylene terephthalate block copolymers have been studied as dialysis membranes (Lyman, 1964) and as tissue implants (Annis and Jones, 1972). Furusawa et al. (1977), as well as Kim and coworkers (Grainger et al., 1987), have produced block copolymers of PEO and polystyrene and noted minimal levels of *in vitro* plasma protein adsorption. Merrill and Salzman (1983) studied poly(ethylene oxide)–polybutylene terephthalate block copolymers in a bead column and reported very low platelet retention.

The polyurethanes have been the most commonly studied class of PEO-based material. Merrill et al. (1982 a,b) and associates (Sa Da Costa et al., 1980, 1981) studied the effect of the soft segment component on platelet adsorption. *In vitro* tests revealed that PEO-containing polyurethanes adsorbed lower levels of thrombin and far fewer platelets than those composed of poly(propylene oxide) (PPO) or poly(tetramethylene oxide) (PMTO). Furthermore, platelet retention was inversely related to the surface concentration of the PEO phase, as measured by the fraction of ether carbons detected by x-ray photoelectron spectroscopy. These results were confirmed *in vitro* by Brash (1987) and coworkers (Whicher and Brash, 1978; Brash and Uniyal, 1979; Hudson et al., 1986) and by Grasel and Cooper (1986) using a canine *ex vivo* shunt.

PEO block copolymers and polyurethanes, nevertheless, have several limitations. First, significant levels of the "hard" non-PEO phase, which is usually highly thrombogenic, may appear on the surface and thereby limit blood compatibility. Second, although the PEO component in block copolymers is usually amorphous, as demonstrated by differential scanning calorimetry, this does not guarantee that on a molecular level the structural organization resembles a liquidlike state. Specific molecular configurations might be required for optimized biocompatibility. We hypothesize that interpenetrating polymer networks (IPNs) in which one component consists of long PEO chains end-linked by small junctional units might provide a material with improved PEO chain mobility and surface coverage.

In this study, platelet deposition on a series of IPNs composed

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of polyethylene oxide and a polyether substituted polysiloxane is analyzed and related to network surface characteristics. These networks are composed of:

1. Variations of PEO content
2. PEO systematically varied from low to high molecular weight
3. Pentafunctional siloxane oligomers—poly(glycidoxy propyl methyl-dimethyl siloxane), P(GMS/DMS)—which acted as crosslinking units for both polymer networks

Such networks have also been evaluated in this laboratory as drug delivery vehicles (Sung, 1988) and detailed bulk and surface characterization studies have been reported (Chaikof, 1989).

During the past two decades, a number of mathematical models of platelet deposition onto blood exposed surfaces have been presented (Friedman et al., 1970; Grabowski et al., 1972; Turrito and Baumgartner, 1975; Marmur and Cooper, 1982; Wilson et al., 1986; Stubley et al., 1987; Strong et al., 1987). The assumption that the platelet is the limiting species in surface-induced thrombosis has allowed for both quantitative *in vivo* experimental observation through the use of radiolabeled tracers and modeling simplification. These models, based on a conservation equation approach, have been used to evaluate platelet mass transfer limitations in a variety of flow cell configurations. Theoretically, in a kinetically limited regime a unique surface reaction rate constant can be determined which ideally would quantitatively characterize a surface's thromboresistant capacity as a consequence of its overall chemical and physical properties.

A baboon *ex vivo* shunt was used to evaluate platelet deposition on PEO/polysiloxane IPNs in a well-defined flow regime. The mass transfer and kinetic limitations of platelet deposition were examined and in view of this analysis, physiochemical network properties were related to biomaterial platelet reactivity.

Materials and Methods

Network precursors

Hydroxy-terminated poly(ethylene oxides) of nominal molecular weights 2,000 (Fluka), 8,000 (Fisher Scientific Co.), and 20,000 (Fluka) g/mol were used in this study. Number and weight average molecular weights, *Mn* and *Mw*, were determined by size exclusion chromatography (SEC) using PEO standards. The hydroxyl equivalent weight was calculated by dividing the polyether number average molecular weight *Mn* by two, as listed in Table 1.

A pentafunctional glycidoxy-derivitized siloxane oligomer, poly(glycidoxy propyl methyl-dimethyl siloxane), P(GMS/DMS), was synthesized from poly(hydromethyl-dimethyl siloxane) (Petrarch Systems) and allyl glycidyl ether (Aldrich Chemical Co.), as previously described (Chaikof, 1989). The catalyst for the hydrosilation reaction was hydrogen hexachloroplatinate (IV) hydrate, $H_2PtCl_6 \cdot H_2O$ (Aldrich Chemical Co.), present at a concentration of 2×10^{-7} M. Toluene (MCB Manufacturing Chemists, Inc.) was distilled over calcium hydride (Aldrich Chemical Co.) and used as the solvent in each synthesis. This was reaction scheme 1, Figure 1. SEC using PDMS standards was used to determine number and weight average molecular weights of 2,317 and 2,849, respectively, and a polydispersity of 1.23.

Table 1. Polyethylene Oxide Molecular Weights

Nominal Molec. Wt.	*Mn*	*Mw*	Polydispersity	Hydroxy Equiv. Wt. g/OH
2,000	1,687	1,908	1.13	844
8,000	8,618	9,703	1.13	4,309
20,000	17,667	22,885	1.30	8,833

Network synthesis and coated conduit production

Polymer components were dispensed to a final concentration of approximately 17.2 g/dL in an 80/20 (v/v) mixture of carbon tetrachloride and chloroform. IPNs were synthesized by a boron trifluoride etherate, $BF_3 \cdot O(C_2H_5)_2$ (Aldrich Chemical Co.), catalyzed reaction, which resulted in ring opening of the P(GMS/DMS) epoxy groups, producing both endlinking of PEO hydroxyls and simultaneous polymerization to form polyether-substituted polysiloxane sequences. These were schemes 2a and 2b, shown in Figures 2 and 3. The catalyst was added dropwise with frequent mixing to a final concentration of 12 mM.

Composite vascular conduits with 4 mm ID were produced for *ex vivo* shunt studies by centrifugal casting of an appropriate volume of polymer reaction mix at 2,700 rpm. Materials of low tensile strength (65% PEO, w/w, of the dry network) were cast onto a woven fiberglass substrate (Varflex Corp.); gels of higher tensile strength (35% PEO) were cast as solid tubes without an underlying substrate. Following a 24 h cure, the solvent and other extractable components were removed by a series of gradient washes in ethanol/water solutions and a 24 to 48 h purge with humidified nitrogen. The gel-coated conduits were swollen in ultrapure water and sonicated for 20 min prior to use. All conduits were used within 1–2 weeks of synthesis.

Networks were coded throughout this study on the basis of PEO molecular weight and content. Networks composed of PEO of molecular weights 2,000, 8,000, and 20,000 were respectively designated as 2K, 8K, and 20K networks. The PEO content was noted by following the first portion of this coding

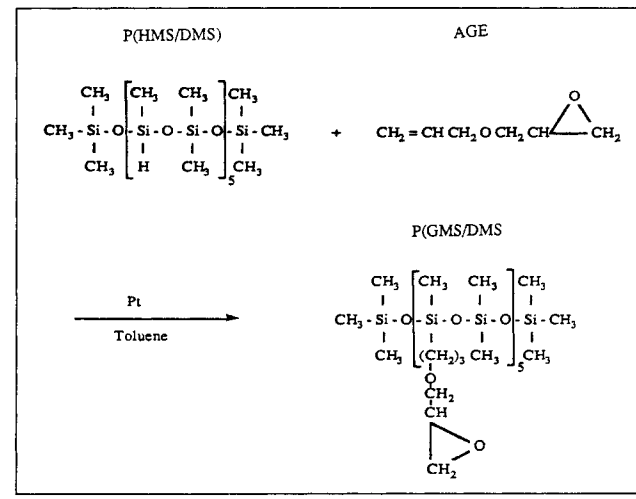

Figure 1. Scheme 1: Hydrosilation reaction for expoxy derivitization of siloxane oligomers.

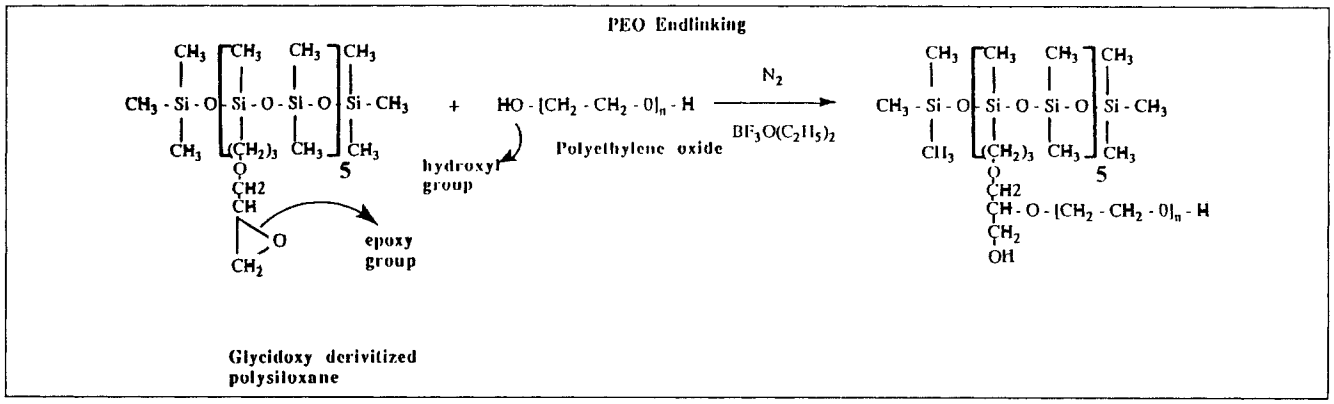

Figure 2. Scheme 2a: Endlinking reaction of PEO hydroxyls.

with either 35 or 65, indicating the weight percent of PEO in the dry network. In addition, reference to low, mid, or high molecular weight materials refers to networks composed of PEO 2,000, 8,000, or 20,000, respectively.

Experimental protocol

Baboon platelets were radiolabeled on the day prior to the shunt study. Forty-five mL of whole blood were withdrawn into syringes containing 9 mL of acid citrate dextrose anticoagulant. The blood was centrifuged at 160 g for 15 min and the platelet-rich plasma (PRP) removed and centrifuged at 1,500 g for 15 min. The platelet pellet was removed, washed in normal saline solution, recentrifuged, and then resuspended in 4 mL of normal saline solution with 0.1% (w/v) dextrose. Six hundred μCi of Indium-111-oxine (Amersham Co.) was added to the platelet suspension. Following a 10 min incubation at room temperature, 3 mL of platelet-poor plasma were added and the platelets incubated for an additional 2 min. The mix was centrifuged at 1,500 g for 5 min to form a platelet pellet, the supernatant and excess In-111-oxine was removed, and the platelets were resuspended in 5 mL of reserved plasma. Approximately 0.5 mCi of Indium-111-oxine labeled platelets were reinjected into the baboon. Platelet function is not altered by this technique, when studied by either thrombin-stimulated platelet release of ^{14}C serotonin or by morphological studies of electron dense body distribution (Vecchione et al., 1980).

Adult male baboons (20–30 kg) were sedated with ketamine hydrochloride (200 to 250 mg IM) and maintained anesthetized throughout the study with sodium pentobarbital (50–75 mg IV prn). The *ex vivo* shunt was created by the percutaneous insertion of a 5F. introducer catheter (KMA Inc.) and a 16 gauge angiocatheter into the femoral vein and femoral artery, respectively. Hematological parameters were measured during the course of these studies, including: hematocrit 38.95 ±3.57, platelet count 209.31 ±41.05 $10^3/mm^3$, fibrinogen 150.82 ±30.55 mg/dL, and clotting time 184.77 ±57.41 s (mean ± SD).

The tubular flow cell for biomaterial analysis consisted of a single conduit, 4 mm ID and 8 cm long, which was inserted into the proximal limb of the circuit with external screw-type butt joints, Figure 4. The inlet and outlet tubing were composed of medical grade Silastic (3.3 mm ID, Dow Corning) and were 6 and 10 in long, respectively. Prior to the initiation of blood flow, the circuit was filled with lactated Ringer's solution.

The flow rate was measured with an in-line square-wave electromagnetic blood flow meter (model 501, Carolina Medical Electronics Inc.) and adjusted by varying the downstream resistance with a screw clamp. A flow rate of 50 mL/min (wall shear rate 133 s^{-1}) was maintained throughout these studies.

Test materials were studied in triplicate as were the NHLBI reference materials, polydimethylsiloxane (PDMS) (Mercor) and low-density polyethylene (LDPE) (Abiomed). All conduits were 4 mm ID and 8 cm long. Blood exposure times included 2, 5, 10, 15, 30, and 60 min. Following the test period, the circuit was flushed for approximately 2.5 min with lactated Ringer's solution at a flow rate of 30 mL/min and the study material placed in 0.1 M sodium cacodylate buffered (pH 7.3), 2.5%

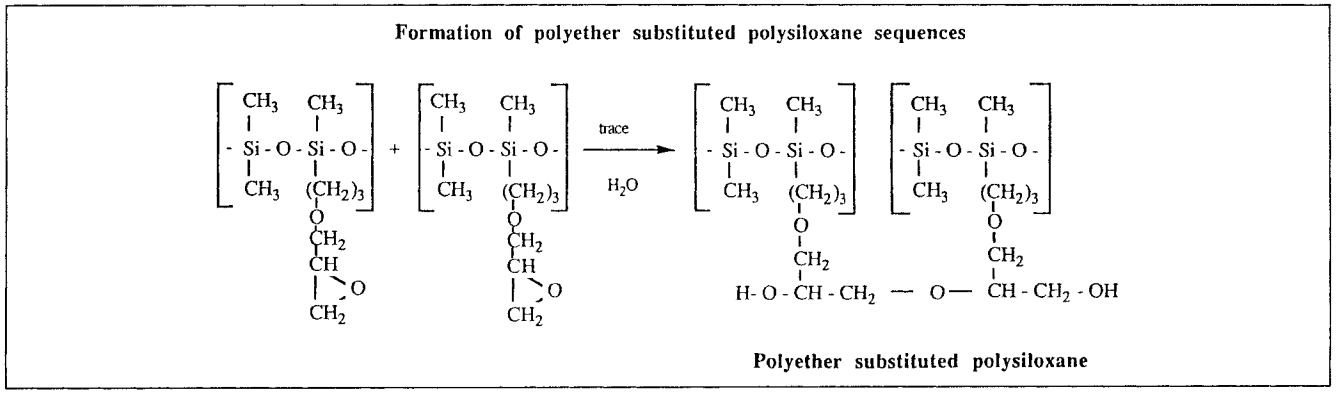

Figure 3. Scheme 2b: Cationic polymerization of epoxy groups to form polyether-substituted polysiloxane sequences.

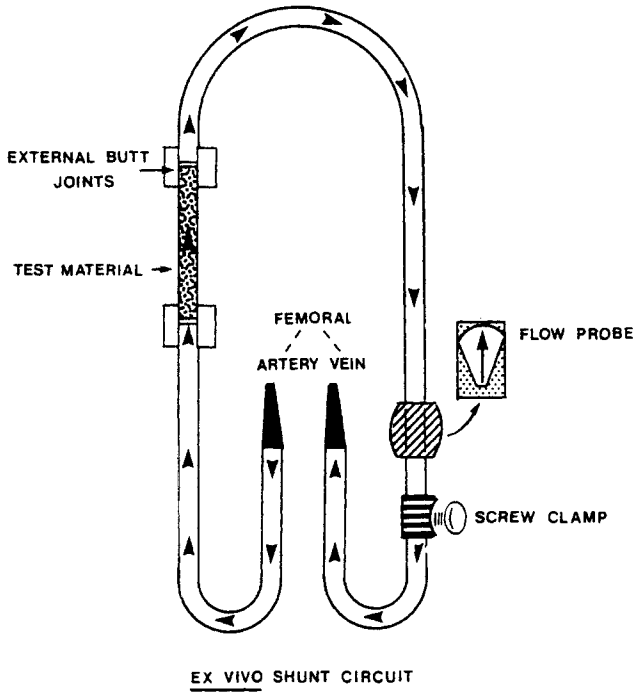

Figure 4. Tubular flow cell for biomaterial analysis.

glutaraldehyde. A section of the tubing was removed for measurement of ^{111}In uptake. The specimens were obtained at a distance 3 cm downstream from the inlet. Experimental parameters and related dimensionless groups are summarized in Table 2. Data were expressed as the number of platelets per 1,000 μm^2. Statistical analysis was performed using the Duncan multiple range test.

Animal care compiled with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, rev. 1978).

Theoretical Approach

The governing species conservation equation for platelet transport in a cylindrical conduit is

$$\frac{\partial c}{\partial t} + u\frac{\partial c}{\partial x} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD\frac{\partial c}{\partial r}\right) + \frac{\partial}{\partial x}\left(D\frac{\partial c}{\partial x}\right) \tag{1}$$

Terms are defined in the Notation.

A model of the velocity field in the test chamber is required to characterize the convective component of platelet transport. In this system, laminar flow is assumed on the basis of a Reynolds number of approximately 80 and axisymmetric and fully developed flow are expected because of the cylindrical design of the biomaterial conduit and test sampling beyond the hydrodynamic entrance region, respectively. Although an *ex vivo* system is inherently oscillatory, a steady flow approximation is justified by experimental and theoretical studies, which have concluded that pulsatile flow has a negligible effect on mass transfer (Fagela-Alabastro and Hellums, 1969; McMichael and Hellums, 1975; Gupta et al., 1982). In fact, Turitto et al. (1977) studied the effect of pulsatile flow on subendothelial platelet deposition. No effect was seen at any of the tested pulsation frequencies, ranging from 0 to 214 min^{-1}. Grabowski et al.

Table 2. Experimental Parameters and Dimensionless Groups

Q, flow rate	50 mL/min
a, conduit radius	0.2 cm
U, bulk velocity	6.63 cm/s
γ_w, wall shear rate	132.6 s^{-1}
τ_w, wall shear stress	4.7 dyn/cm^2
μ, blood viscosity	0.035 poise
ρ, blood density	1.06 gm/cm^3
ν, kinematic viscosity	0.033 cm^2/s
c_o, bulk platelet concentration	2×10^8 platelets/cm^3
D, effective platelet diffusivity	10^{-7} cm^2/s
Re, Reynolds no. ($2aU/\nu$)	80.36
Pe, Peclet no. (Ua/D)	1.33×10^7
Sc, Schmidt no. (ν/D)	3.3×10^5
L_h, hydrodynamic entrance length	1.13 cm
Lm, mass transfer entrance length	5.83×10^5 cm

(1977) found that the presence or absence of pulsatile flow did not influence dog platelet adhesion onto biomaterials. Finally, blood acts as a Newtonian fluid at shear rates above 100 s^{-1} (Merrill, 1969), which were obtained in the experimental flow cell. The velocity profile of a steady, laminar, axisymmetric, and fully developed flow regime can then be expressed simply as

$$u = 2U\left[1 - \left(\frac{r}{a}\right)^2\right] \tag{2}$$

where a is the tube radius and U is the bulk velocity.

Although convective transport is responsible for platelet flow along parallel streamlines, platelet interaction with the test surface is due to transport in the radial direction secondary to dispersive effects induced by the movements of red cells in a shear field. Karino and Goldsmith (1979) studied the effect of red cell concentration and reported platelet diffusivities ranging from 0.1 to 2.0 $\times$ 10^{-7} cm^2/s for hematocrits between 0 and 40. Turrito and Weiss (1983) observed that inceasing the shear rate from 50 to 10,000 s^{-1}, produced an increase in the radial diffusivity from 2.2 to 28.8 $\times$ 10^{-7} cm^2/s. Under most physiological conditions, associated with clinically relevant small-diameter arterial substitutes, effective platelet diffusivity is approximately 10^{-7} cm^2/s (Stubley et al., 1987).

The boundary conditions for this problem assume constant platelet concentration at the inlet of the test region and a finite concentration within the conduit. Grabowski et al. (1972) and Friedman et al. (1970) postulated that platelet flux to the surface is equal to the rate of platelet adsorption, and formulated a second-order rate equation in terms of bulk concentration, c_o, a surface rate constant, k, and surface availability, s. The surface availability at time t is a function of the limiting platelet surface density, ρ_p, and the accumulated surface flux. The boundary conditions are represented as

$$c = c_o \qquad \text{at } x = 0 \tag{3}$$

$$c \text{ is finite} \qquad \text{at } x \geq 0,\ t \geq 0,\ 0 \leq r \leq a \tag{4}$$

$$-D\frac{\partial c}{\partial r} = kcs \qquad \text{at } r = a \tag{5}$$

$$\text{where } s = 1 - \frac{1}{\rho_p}\int_0^t \left(-D\frac{\partial c}{\partial r}\right)\Big|_{r=a} dt \tag{6}$$

The simplified problem, in which s is constant and equal to one, was solved by Solbrig and Gidaspow (1967). The dimensionless platelet flux and surface-averaged platelet accumulation can be expressed as:

$$\left.\frac{\partial \bar{c}}{\partial \bar{\eta}}\right|_{\bar{\eta}=0} = \frac{(2/3)\,(2/\bar{x})^{1/3}}{1 + (2/3)\,(2/\bar{x})^{1/3}\,(1/DaPe^{-1/3})} = \frac{DaPe^{-1/3}}{(3/2)\,(\bar{x}/2)^{1/3}\,DaPe^{-1/3} + 1} \quad (7a)$$

and

$$\bar{\rho}_{av} = \frac{\int_{\bar{x}_1}^{\bar{x}_2} \frac{DaPe^{-1/3}\,\bar{t}}{(3/2)\,(\bar{x}/2)^{1/3}\,DaPe^{-1/3} + 1}\,d\bar{x}}{\int_{\bar{x}_1}^{\bar{x}_2} d\bar{x}} \quad (7b)$$

where Da and Pe are the Damköhler and Peclet numbers, respectively. The dimensionless group, $DaPe^{-1/3}$, which appears after rescaling in the boundary layer, can be considered a generalized Damköhler number. As illustrated:

$$DaPe^{-1/3} = \frac{kaPe^{-1/3}}{D} = \frac{k\delta}{D} = \frac{k}{(D/\delta)} = \frac{1/(D/\delta)}{1/k} \quad (8)$$

which is the ratio of platelet mass transfer resistance within the concentration boundary layer to kinetic reaction resistance to platelet adhesion at the biomaterial surface. The former includes the effect of species diffusivity and boundary layer thickness. This dimensionless group suggests three possible conditions of platelet transport to a biomaterial surface

$$\begin{aligned} DaPe^{-1/3} &\ll 1 \\ DaPe^{-1/3} &\sim 1 \\ DaPe^{-1/3} &\gg 1 \end{aligned} \quad (9)$$

which are respectively the kinetically limited, intermediate, and mass transfer limited regimes. In practice, regimes in which $DaPe^{-1/3} \geq 10$ can be considered predominantly mass transfer limited, while values less than 0.1 suggests that surface kinetics dominates transport. A mixed process exists between these two limits.

The case of limited surface availability ($s \neq 1$) has been approached by others numerically and analytically. Grabowski et al. (1972) used an approximate integral technique combined with numerical integration. Stubley (Stubley et al., 1987; Strong et al. 1987) has recently solved Grabowski's model using a finite-difference technique.

Turitto and Baumgartner (1975) and Turitto et al. (1977) have suggested that the effects of surface coverage and limiting surface density can be incorporated by assuming that a finite number of surface sites are occupied exponentially with respect to time and initial platelet flux ($s = 1.0$):

$$\bar{\rho} = 1 - \exp\left[-\frac{\partial \bar{\rho}}{\partial \bar{t}}(s = 1.0)^{*}\,\bar{t}\right] \quad (10)$$

This treatment implies that both the number of free binding sites and the platelet flux decrease exponentially with time at a rate dependent upon the initial platelet flux. There is little evidence to suggest that Turrito's semiempirical approach has a physical basis; nevertheless, the results generated by Eq. 10 are closely approximated by Stubley's numerical solution (Stubley et al., 1987). The most significant differences were noted at low shear rates (20 s^{-1}) well below that utilized in the present study. Although both the numerical solution and the Turitto model arrived at the same limiting surface platelet density at long exposure times, platelet accumulation was underestimated by as much as 20% during the assumed exponential approach. This effect would produce a lower level of platelet deposition for any given flow system and reactive surface. As a consequence, a more conservative estimate of the extent of the kinetically limited regime ($DaPe^{-1/3} < 0.1$) would be provided. The utilization of the Turrito model furnishes a simple means of assessing the mass transfer and kinetic limitations of a test configuration designed for platelet deposition measurement. Theoretically, in a regime dominated by the chemical nature of the biomaterial surface, kinetic expressions can be used to provide a quantitative means of evaluating surface reactivity and hence, thrombogenicity.

Results and Discussion

Theoretical profiles of dimensionless surface-averaged platelet accumulations as a function of time are presented in Figure 5 along with the experimental data. The data were nondimensionalized assuming that the maximum surface coverage was the observed experimental plateau for each biomaterial surface. These values probably represent a lower bound for ρ_p. Theoretical and observed platelet fluxes are presented in Figure 6. Experimental platelet fluxes were calculated and nondimensionalized as follows:

$$\left.\frac{\partial \bar{c}}{\partial \bar{\eta}}\right|_{\bar{\eta}=0} = \frac{\rho(t_2) - \rho(t_1)}{t_2 - t_1}\left(\frac{1}{c_o\,D/\delta}\right) \quad \text{at } t = t_1 + (t_2 - t_1)/2 \quad (11)$$

Both platelet fluxes and accumulation fall, for the most part, in a kinetically limited region. These results suggest that the inher-

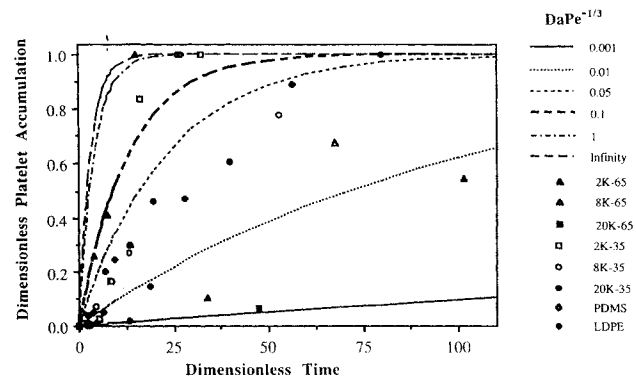

Figure 5. Dimensionless surface-averaged platelet accumulation as a function of time.

Theoretical profiles and experimental data
Maximum platelet surface densities estimated on the basis of observed platelet plateaus for each biomaterial

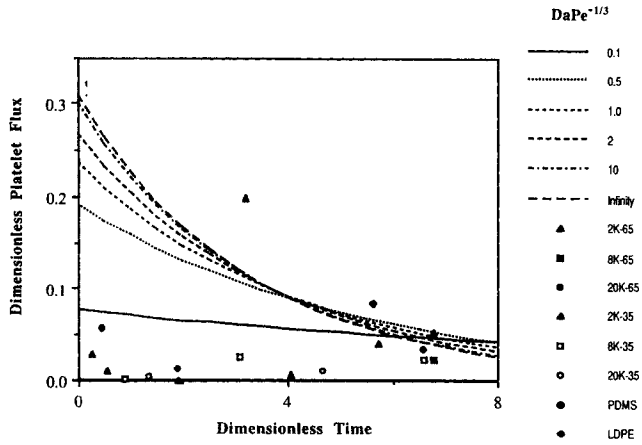

Figure 6. Dimensionless surface-averaged platelet flux as a function of time.
Theoretical profiles and experimental data

ent physical and chemical nature of these surfaces are the predominant factors influencing platelet adherence.

Blood compatibility correlated with both PEO content and molecular weight, particularly after 30 min of blood contact, Figures 7, 8. Platelet deposition on PEO 2,000 networks, was higher on 2K-65 when compared with 2K-35 after 60 min ($p < 0.05$). Otherwise, on mid and high PEO molecular weight networks (8,000 and 20,000), a trend toward reduced platelet deposition was observed with increased PEO content. In materials of high PEO content (65%), mid and high PEO molecular weight samples had lower levels of platelet adsorption than those networks of low PEO molecular weight (2K-65), at 30 ($p < 0.1$) and 60 min ($p < 0.001$). The lowest level of platelet deposition was noted on 20K-65 networks. During the observation period, platelet accumulation on this surface was less than one platelet per 1,000 μm^2. Platelet adsorption of PDMS was statistically indistinguishable from 2K-65 and LDPE during the entire observation period. However, after 60 min of blood exposure, levels on 8K-65 and 20K-65 were significantly lower than on PDMS ($p < 0.05$).

A second-order rate equation was derived to provide a quantitative means of evaluating platelet deposition. Irreversible binding is assumed and the expression postulates that the rate of platelet deposition is directly proportional to:

1. the free platelet concentration near the surface, taken to be identical to the bulk value of 2×10^8 platelets per milliliter, and
2. the number of surface sites available for platelet binding

There are two adjustable parameters, the second-order rate constant, k'', and the maximum number of sites, ρ_p. The equation can be expressed as

$$\frac{d\rho}{dt} = k'' c_o(\rho_p - \rho) \tag{12}$$

where

$$\rho = \rho_p [1 - \exp(-k'' c_o t)] \tag{13}$$

Equation 13 was fitted to the experimental data using a Levenberg-Marquandt nonlinear least-squares routine. The calculated parameters and statistical analysis are summarized in Table 3.

Although regression analysis could account for most of the data variability, as is evident from the high values of the multiple correlation coefficients and the statistical significance of the analysis of variance for the overall regression, the calculated coefficients were not statistically unique. Consequently, it is difficult to justify the use of the derived coefficients as "material-specific" parameters for relative biomaterial comparison.

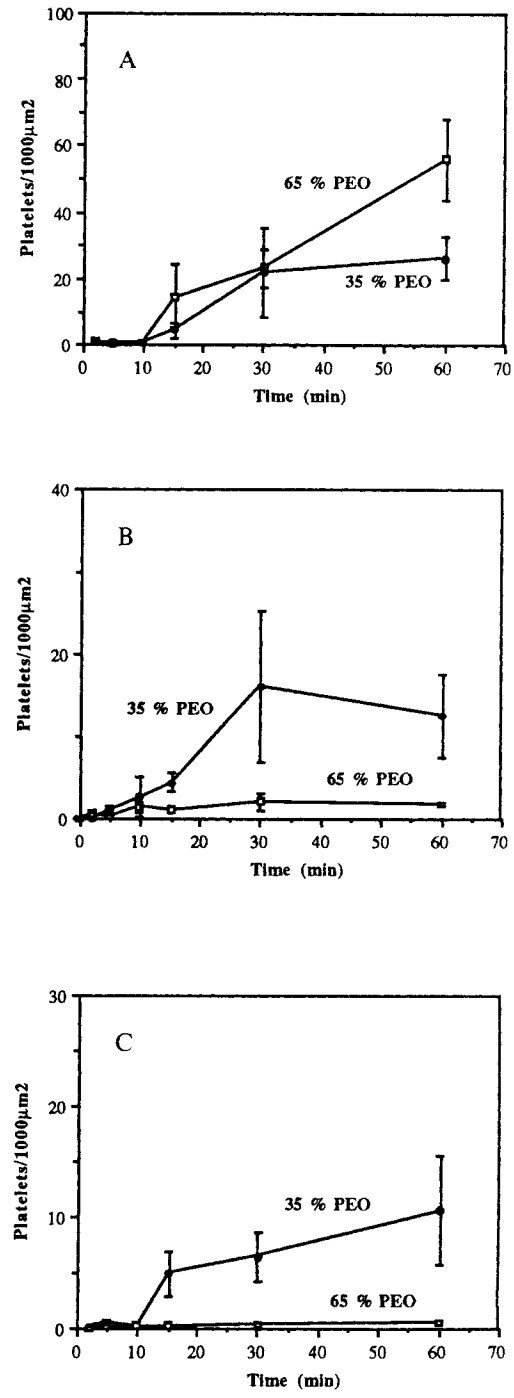

Figure 7. Platelet deposition on three networks.
A. 2,000 PEO; B. 8,000 PEO; C. 20,000 PEO

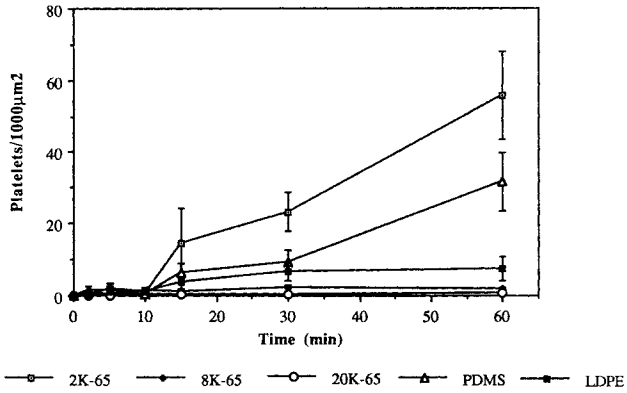

Figure 8. Platelet deposition on high PEO content materials (65%), PDMS, and LDPE.
Increasing PEO molecular weight decreases platelet deposition

In view of this analysis, an estimated average platelet deposition rate was determined for each biomaterial using a first-order rate equation:

$$\frac{\partial \rho}{\partial t} = k' c_o \tag{14}$$

where

$$\rho = K_1 t + K_2 \tag{15}$$

The regression coefficients and statistical analysis are summarized in Table 4. K_1 is the rate of platelet deposition and K_2 is a constant of integration, which regression analysis confirmed was not significantly different from zero. The regression coefficients were often low due to the observed nonlinear behavior for platelet deposition onto most test surfaces. Nonetheless, K_1 can be utilized as an estimated average platelet deposition rate during the 60 min blood exposure period and the results plotted as a function of the surface atomic concentration of ether carbons or silicon, as previously determined by ESCA (Chaikof and Merrill, 1990), Figure 9. Surface reactivity as represented by the average platelet deposition rate is inversely proportional to the PEO surface content while directly proportional to the surface silicon content. One exception, which is not depicted, is 2K-65. The platelet deposition rate was high, despite high surface ether carbon content (29.98%). Clearly, surface ethylene oxide content is only a single determinant of these relatively complicated interactions; PEO domain size, relative chain mobility, and residual surface hydroxyl groups are presumably other important determinants that influence material biocompatibility.

The difficulty in achieving statistical significance is attributable to the approximations and assumptions, which limit the ability of a simple kinetic expression to model the data of this complex system. First, embolization effects are not considered. Second, the rate question for platelet adhesion assumes that both the number of effective binding sites (ρ_p) and the platelet–surface reactivity (k) remain unchanged. In fact, events following blood contact, including protein deposition and conformational changes, dynamic plasma–surface protein exchange, and the release of platelet alpha and dense granule proteins, dramatically alter the reactive state of both the surface and the platelet, as well as the number of effective platelet binding sites. Finally, as other have discussed, a continuum assumption underlies this analysis (Grabowski et al., 1972). A continuum can be defined as a medium in which the statistically averaged characteristics of the smallest volume of interest can be accurately extrapolated to the system as a whole. The regions of interest are the boundary layer adjacent to the surface and the surface itself. The boundary layer thickness in this experimental system is approximately 40 μm (Chaikof, 1989). Platelets are small enough in size that the fluid layer adjacent to the surface can be considered a continuum. However, the visualization of localized platelet surface aggregates is common and thus the absence of random surface deposition limits the appropriateness of this assumption. Although Grabowski et al. (1972) and Strong et al. (1987) have reported reaction rate constants, the difficulty in obtaining statistically unique parameters is apparent in both reports.

Attempts at developing more sophisticated models of platelet deposition have met with little success. Marmur and Cooper (1982) and Wilson et al. (1986) formulated an eight-parameter model that included the effects of thrombus embolization. Regression analysis of experimental data has not been reported and statistical significance will probably be difficult to achieve because of the large number of adjustable parameters.

The ability to use a mathematical model to derive biomaterial-specific surface reaction parameters is hampered by the biological complexity and temporal variability of this system. The possibility of deriving rate equations that more accurately reflect the biological reaction mechanism is appealing, yet it is

Table 3. Regression Coefficients and Statistical Analysis of Second-order Kinetic Model

Network	Max Binding Sites ρ_p, platelets/1,000 μm^2	Rate Constant k, μm^3/platelet · s	$r_{\rho k}^2$	Multiple R^2	Std. Dev. of Regression
2K-65	285.80 ± 1070.26 ($p = 0.793$)†	0.27 ± 1.27 ($p = 0.836$)	0.99	0.79 ($p = 0.0001$)††	11.50*
8K-65	1.94 ± 0.49 ($p = 0.001$)	7.05 ± 4.70 ($p = 0.152$)	0.567	0.69 ($p = 0.001$)	0.93
20K-65	0.65 ± 0.28 ($p = 0.03$)	2.88 ± 2.31 ($p = 0.23$)	0.85	0.69 ($p = 0.0001$)	0.22
2K-35	86.59 ± 308.52 ($p = 0.782$)	0.53 ± 2.22 ($p = 0.815$)	0.997	0.60 ($p = 0.001$)	12.10
8K-35	16.54 ± 6.58 ($p = 0.053$)	2.87 ± 2.21 ($p = 0.25$)	0.85	0.89 ($p = 0.0037$)	3.07
20K-35	23.87 ± 23.04 ($p = 0.348$)	0.83 ± 1.03 ($p = 0.454$)	0.99	0.96 ($p = 0.001$)	1.23
PDMS	213.52 ± 1057.55 ($p = 0.842$)	0.22 ± 1.35 ($p = 0.871$)	1.0	0.80 ($p = 0.0001$)	7.46
LDPE	9.27 ± 5.45 ($p = 0.113$)	2.88 ± 3.38 ($p = 0.409$)	0.876	0.64 ($p = 0.001$)	3.76

†Significance level, based upon the t statistic, representing significance of the difference of the parameter from zero
††Significance level of the F-value; that is, whether the equation produces a significant reduction in the total sum of squares
*Estimate of standard deviation of the data about the fitted line

Table 4. Linear Regression Coefficients and Statistical Analysis

Network	K_1 platelets/1,000 $\mu m^2 \cdot s$	K_2 platelets/1,000 μm^2	$r_{K_1K_2}{}^{2*}$	Multiple R^2	Std. Dev. of Regression
2K-65	1.61E-02 ± 2.41E-03 ($p = 0.0001$)†	−3.63 ± 3.57 ($p = 3.240$)	0.48	0.74 ($p = 0.0001$)††	10.98**
8K-65	4.14E-04 ± 1.93E-04 ($p = 0.046$)	0.67 ± 0.32 ($p = 0.052$)	0.48	0.21 ($p = 0.046$)	1.00
20K-65	1.31E-04 ± 4.10E-05 ($p = 0.005$)	0.12 ± 0.07 ($p = 0.08$)	0.48	0.38 ($p = 0.005$)	0.21
2K-35	8.28E-03 ± 2.12E-03 ($p = 0.001$)	−1.24 ± 3.83 ($p = 0.75$)	0.50	0.46 ($p = 0.001$)	12.07
8K-35	4.22E-03 ± 1.24E-03 ($p = 0.019$)	0.85 ± 1.96 ($p = 0.68$)	0.44	0.70 ($p = 0.019$)	3.89
20K-35	4.35E-03 ± 8.37E-04 ($p = 0.001$)	−0.58 ± 1.24 ($p = 0.643$)	0.48	0.64 ($p = 0.001$)	13.68
PDMS	9.55E-03 ± 1.40E-03 ($p = 0.0001$)	−1.29 ± 2.23 ($p = 0.571$)	0.48	0.71 ($p = 0.0001$)	7.35
LDPE	2.29E-03 ± 8.89E-04 ($p = 0.0231$)	0.94 ± 1.48 ($p = 0.53$)	0.54	0.34 ($p = 0.02$)	3.90

†Significance level, based upon the t statistic, representing the significance of the difference of the parameter from zero
††Significance level of the F-value; that is, whether the equation produces a significant reduction in the total sum of squares
*Dependency factor for the two adjustable parameters
**Estimate of standard deviation of the data about the fitted line

generally impossible to distinguish between alternative mechanisms associated with large numbers of adjustable parameters. Nonetheless, relatively simple mathematical models can be used to examine platelet mass transfer limitations. In a kinetically limited regime, the comparison of mean platelet deposition or the analysis of average platelet deposition rates can then be used for quantitative material comparison.

In this study, such an analysis revealed that PEO molecular weight and content in PEO/polysiloxane IPNs dictate short-term blood compatibility. Networks of both high PEO content and molecular weight (20K-65) had negligible levels of platelet binding. Furthermore, the average rate of short-term platelet deposition correlated directly with PEO surface content as measured by ESCA.

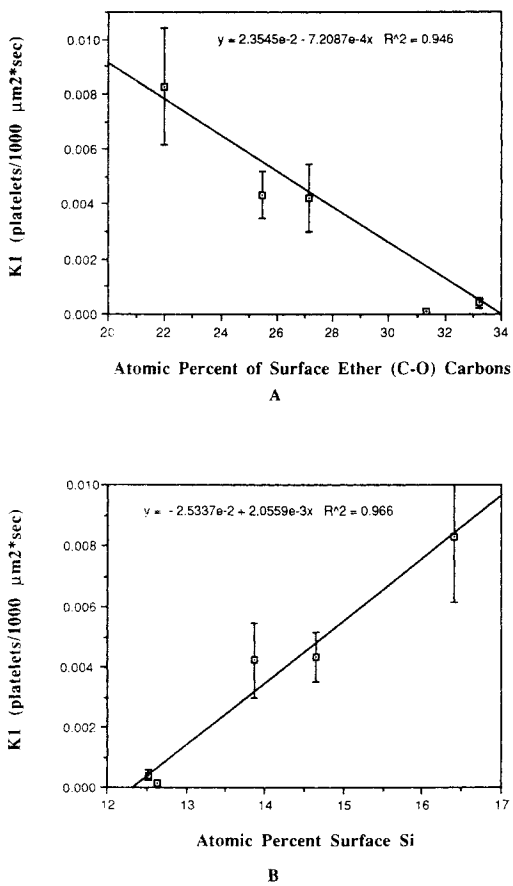

Figure 9. Platelet deposition rate.
A. Rate vs. surface ether carbon content
B. Rate vs. atomic percent silicon content, determined by ESCA

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Notation

a = conduit radius, cm
c = $c(r, x, t)$ bulk platelet concentration, platelets/cm^3
c_o = steady state bulk platelet concentration, platelets/cm^3
$\bar{c}$ = dimensionless platelet concentration, c/c_o
D = effective platelet diffusion coefficient, cm^2/s
Da = Damkohler number
k = surface reaction rate constant, μm^3/platelet · s
k' = first-order rate constant
k'' = second-order rate constant
K_1 = global platelet deposition rate, platelets/1,000 $\mu m^2 \cdot s$
K_2 = ordinate intercept, platelets/1,000 μm^2
Mn = number-average molecular weight
Mw = weight-average molecular weight
Pe = Peclet number
r = dimensional radial coordinate, cm
s = fractional surface availability
t = time, s
$\bar{t}$ = dimensionless time, t/τ
u = blood velocity in x direction, cm/s
U = bulk velocity, cm/s
x = dimensional axial coordinate, cm
$\bar{x}$ = dimensionless axial coordinate, x/a

Greek letters

δ = boundary layer thickness, $\sim aPe^{-1/3}$
$\bar{\eta}$ = stretched radial coordinate, $(1 - \bar{r})\, Pe^{1/3}$
ρ_{av} = spatially averaged dimensionless platelet accumulation
ρ = platelet accumulation
$\bar{\rho}$ = dimensionless platelet accumulation, ρ/ρ_p
ρ_p = maximum number of bound platelets, platelets/1,000 μm^2
τ = Characteristic time, $\rho_p/[c_o\,(D/\delta)]$

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