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SCANNING ELECTRON MICROSCOPY ANALYSIS OF POLYETHYLENE OXIDE HYDROGELS FOR BLOOD CONTACT

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Abstract

Hydrogels are a class of synthetic material, composed of a polymer-water matrix and have been proposed as tissue substitutes and drug delivery vehicles. Polyethylene oxide (PEO) hydrogels were synthesized and used to produce coated wires and conduits for baboon blood compatibility studies. Blood-material interactions were studied both by Scanning Electron Microscopy (SEM) and ^{111}In labeled platelet deposition.

SEM processing modifications were first evaluated in order to reduce shrinkage and surface distortion incurred during sample preparation of these high water content materials. Pretreatment with 1% tannic acid reduced bulk shrinkage associated with critical point drying by 10 - 20%. This effect is small, nevertheless, it prevented major sample disruption.

Coated guidewires were exposed to baboon blood for one hour in the inferior vena cava and conduits were placed for either 30 or 60 minutes in an ex vivo femoral arteriovenous shunt. Reference materials included Gore-tex®, polyethylene and silica-free polydimethyl siloxane (PDMS). In the guidewire studies, ^{111}In labeled platelet levels were highest on Gore-tex® (6568.97 platelets/ 1000 μm^2) and large thrombotic deposits were well visualized by SEM. Formulations containing PEO had low levels of platelet deposition and little evidence of platelet activation was noted by SEM. Shunt studies demonstrated that materials of high PEO content and molecular weight had the lowest levels of platelet deposition. After 60 minutes of blood flow, mean platelet deposition on PDMS and Gore-tex® was 50 and 1000 fold higher than on a network composed of 65% PEO 20,000 ($p < 0.05$). SEM confirmed these findings.

Key Words: Hydrogel, Polymeric Biomaterials, Blood-Material Interactions, Arteriovenous Shunt, Platelets, Scanning Electron Microscopy.

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Introduction

The development of thromboresistant surfaces is a critical requirement for the fabrication of both small diameter vascular grafts and catheters for long term metabolic support and drug delivery. Blood compatible materials, however, have been difficult to develop because of the complex interaction of blood with foreign surfaces. The exposure of blood to an artificial surface can lead to coagulation, complement activation, as well as platelet adherence and activation. These events will induce thrombus formation which may, as in the case of a small diameter vascular graft, lead to occlusion or clot embolization, both potentially catastrophic events (Salzman and Merrill, 1982).

We are currently evaluating the blood contacting properties of polyethylene oxide (PEO) hydrogels (Chaikof, 1989; Chaikof, et al., 1988). PEO, whose structural repeat unit is [CH_2CH_2O], has the lowest levels of protein or cellular adsorption of any known polymer (Merrill and Salzman, 1983; Merrill, et al., 1985). This has been attributed, in part, to the presence in the polymer chain of only small hydrophobic, ethylene, and hydrophilic, ether oxygen, subunits. Theoretically, these structural repeat units provide only weak electrostatic binding sites for circulating blood elements (Golander, et al., 1986; Golander and Kiss, 1988; Hlady, et al., 1985; Nakao, et al., 1986).

PEO surfaces can also be formed from two phase block copolymers. 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. The most commonly studied class of two phase PEO based system has been the polyurethanes. Merrill (Merrill, et al., 1982a; Merrill, et al., 1982b) and his associates (Sa Da Costa, et al., 1981; Sa Da Costa, et al., 1980) noted that PEO containing polyurethanes adsorbed lower levels of thrombin and far fewer platelets in vitro than those composed of poly(propylene oxide) (PPO) or poly(tetramethylene) oxide (PTMO). These results have been confirmed in vitro by Brash (1987) and coworkers and by Grasel and Cooper (1986) using an ex vivo shunt.

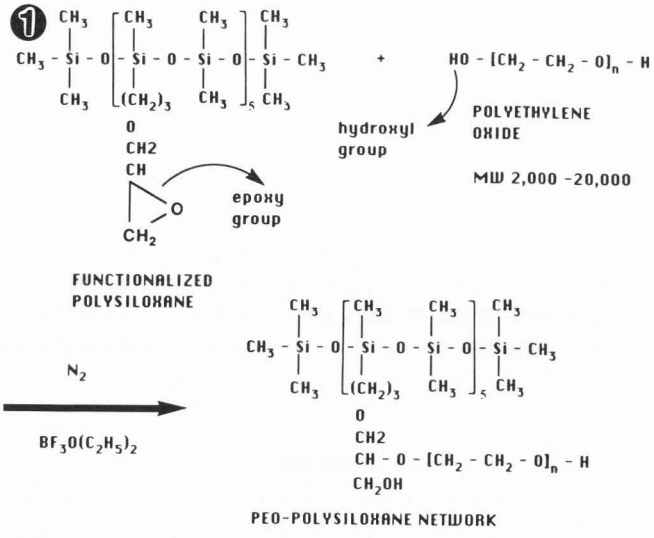


Figure 1: BF_3 catalyzed, epoxy/hydroxyl crosslinking reaction between a polysilozane oligomer, polyglycidoxy propylmethyl-dimethyl siloxane P(GMS/DMS), and PEO.

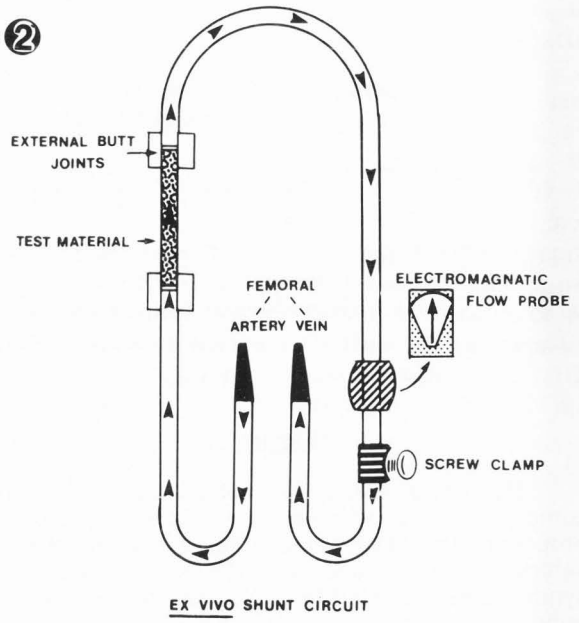


Figure 2: Femoral arteriovenous ex vivo shunt circuit used for exposing samples to baboon blood.

PEO block copolymers are promising biomaterials, but nevertheless, may be limited by the presence of significant levels of the thrombogenic non-PEO phase on the surface of these materials. We hypothesize that a polymer network in which one component consists of large PEO chains endlinked by small junctional units might provide enhanced PEO chain mobility and thereby improved PEO surface coverage and biocompatibility.

In this present study, the blood compatibility of a series of PEO hydrogels was evaluated in a baboon animal model. Radiolabeled platelet deposition was measured and the surfaces examined by scanning electron microscopy. Sample preparation of high water content materials is difficult due to shrinkage and surface distortion following dehydration. Bulk shrinkage associated with several processing techniques was evaluated.

Materials and Methods

Material preparation

Polymeric networks were produced via a BF_3 catalyzed, epoxy/hydroxyl crosslinking reaction between a polysiloxane oligomer, polyglycidoxy propylmethyl-dimethyl siloxane P(GMS/DMS), and PEO (Fig. 1). Detailed analysis of the physiochemical bulk and surface properties of these materials are provided elsewhere (Chaikof, 1989). PEO nominal molecular weights included: 2000, 8000, and 20,000 grams/mole and the PEO content ranged from 0 to 65 percent of the dry network weight. A polymer solution of equal proportions of PEO 8000 and P(GMS/DMS) was cast as a 1 to 2 millimeter thick film and sectioned into 1 centimeter circular discs for bulk shrinkage analysis. The average water content of this network is 50 %. For biocompatibility studies, the polymer solutions were either coated onto fiberglass covered stainless steel guidewires or cast as cylindrical conduits with a final internal diameter of 4 millimeters. Following network cure and solvent removal, the gels were swollen in deionized distilled water. The samples were coded on the basis of PEO molecular weight and content. For example, a gel composed of 50 percent PEO 8000 and 50 % P(GMS/DMS) was designated as 8K-50.

Platelet isolation and labeling

Baboon platelets were radiolabeled on the day prior to the shunt study. Forty-five milliliters of whole blood were withdrawn into syringes containing nine milliliters of acid citrate dextrose anticoagulant. The blood was centrifuged at 160 g for 15 minutes and the platelet rich plasma (PRP) removed and centrifuged at 1500 g for 15 minutes. The platelet pellet was removed, washed in normal saline, recentrifuged, and then resuspended in four milliliters of normal saline with 0.1 % (w/v) dextrose. Six hundred μCi of Indium-111-oxine (Amersham Co., Arlington Heights, IL) was added to the platelet suspension. Following a 10 minute incubation at room temperature, three milliliters of platelet poor plasma were added and the platelets incubated for an additional two minutes. The mix was centrifuged at 1500 g for five minutes to form a platelet pellet and resuspended in five milliliters of reserved plasma. Approximately 0.5 mCi of Indium-111-oxine (^{111}In) labeled platelets were injected into the baboon.

Biocompatibility testing

Testing was performed on adult male baboons (20-30kg) which were sedated with ketamine hydrochloride (200 to 250 mg IM) and maintained anesthetized with sodium pentobarbital (50-75 mg IV

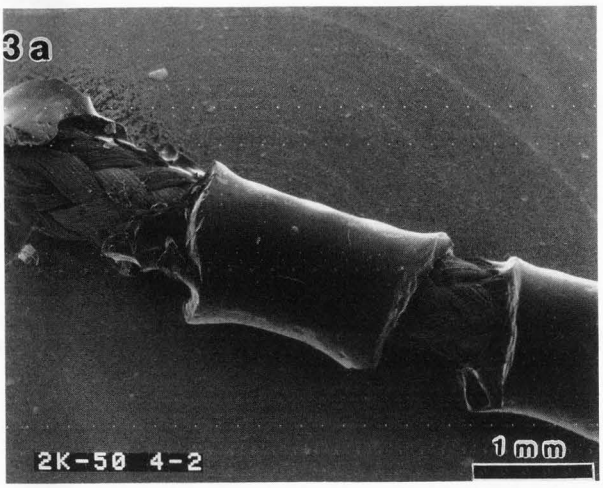


Figure 3a: Coated guidewire sample processed with traditional CPD procedure.

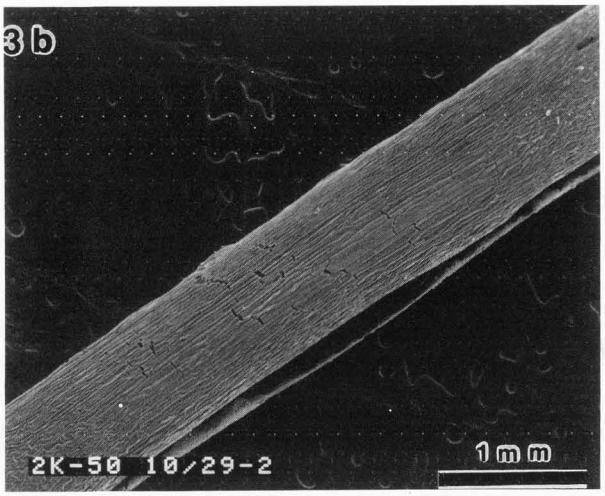


Figure 3b: Coated guidewire sample processed with CPD/TA procedure.

prn). Blood compatibility was assessed in two separate stages. In the first phase, polymer coated guidewires were placed percutaneously in the inferior vena cava (IVC) and ^{111}In-platelet deposition was measured following one hour of blood exposure. A total of nine different PEO network compositions were studied in triplicate, as was Gore-tex® and Intramedic Polyethylene®. The second phase investigated platelet uptake in a femoral arteriovenous ex vivo shunt (Fig. 2). The circuit was filled with lactated Ringer's solution prior to and following the blood contacting period. Three different formulations were studied, composed of 65 percent PEO of molecular weights 2000, 8000 or 20,000. Data was obtained at 30 and 60 minutes and at a shunt flow rate of 50 ml/min (wall shear rate of 133 sec^{-1}). These biomaterials were studied in triplicate, as was Gore-tex® and the National Heart, Lung and Blood Institute (NHLBI) reference material polydimethyl siloxane (PDMS). The data is reported as mean ± standard error and statistical analysis was performed by ANOVA and unpaired Student's t tests. Animal care complied with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1978) (Chaikof, 1989; Coleman, 1988).

Sample Preparation for Shrinkage Studies and SEM Examination

Fully hydrated discs of 8K-50 were processed for shrinkage studies by one of three techniques: (1) traditional critical point drying (CPD) ; (2) CPD following tannic acid (TA) incubation; and (3) air drying following TA and hexamethyldisilazane (HMDS) incubation. The disc diameter and thickness were measured, before and after processing, using a drop weight micrometer and venier calipers. The diameter, thickness, and volume of the processed samples were normalized with respect to the corresponding dimension of the vacuum dried disc prior to processing. The data is presented as mean ± standard error and statistical analysis was performed by ANOVA and unpaired Student's t tests.

Specimens were prepared for SEM, following blood exposure, by one of two methods. Polymer coated guidewires were processed by CPD with TA treatment (CPD/TA) and hydrogel conduits were air dried following TA and hexamethyldisilazane (HMDS) incubation. All samples prepared for SEM were coated with 20 nanometers of Au/Pd (60/40) and viewed using an Amray 1000B. The details of each technique follows.

Traditional Critical Point Drying

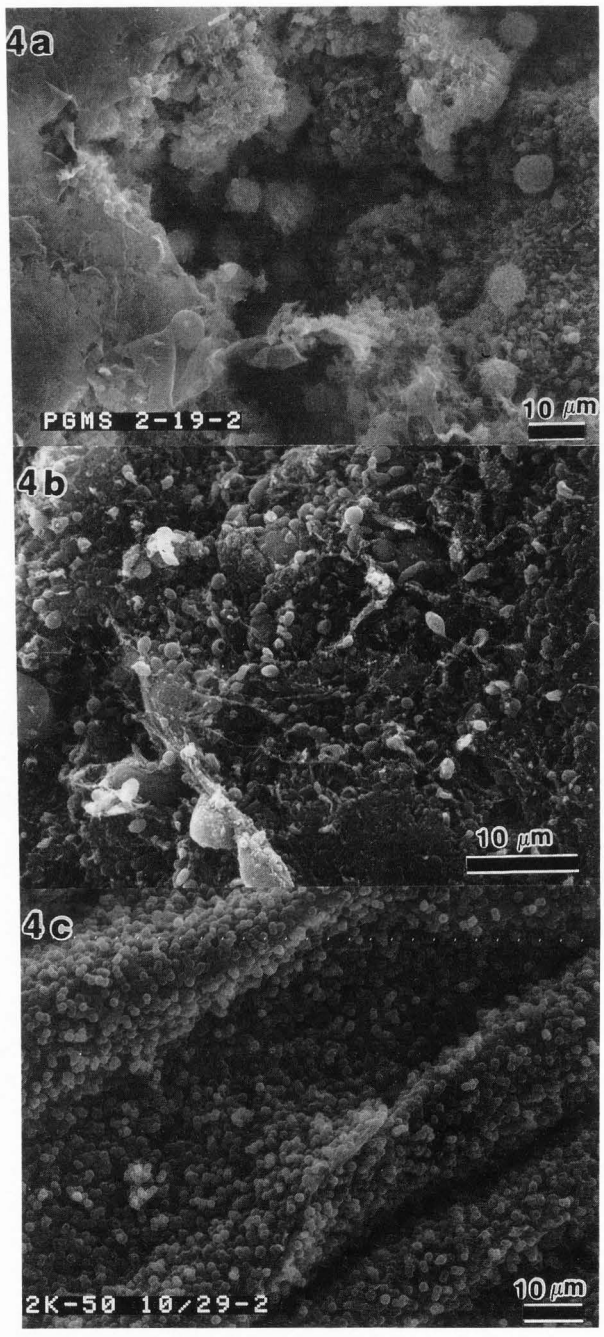
Samples underwent primary fixation in 0.1 M sodium cacodylate buffered 3 % glutaraldehyde and then postfixed in 0.1M sodium cacodylate buffered 1% osmium tetroxide with buffer washes between fixations. Samples were dehydrated through a graded series of ethanols, placed in a Samdri 790 critical point drier and critical point dried using liquid carbon dioxide (Anderson, 1951; Cohen, 1974).

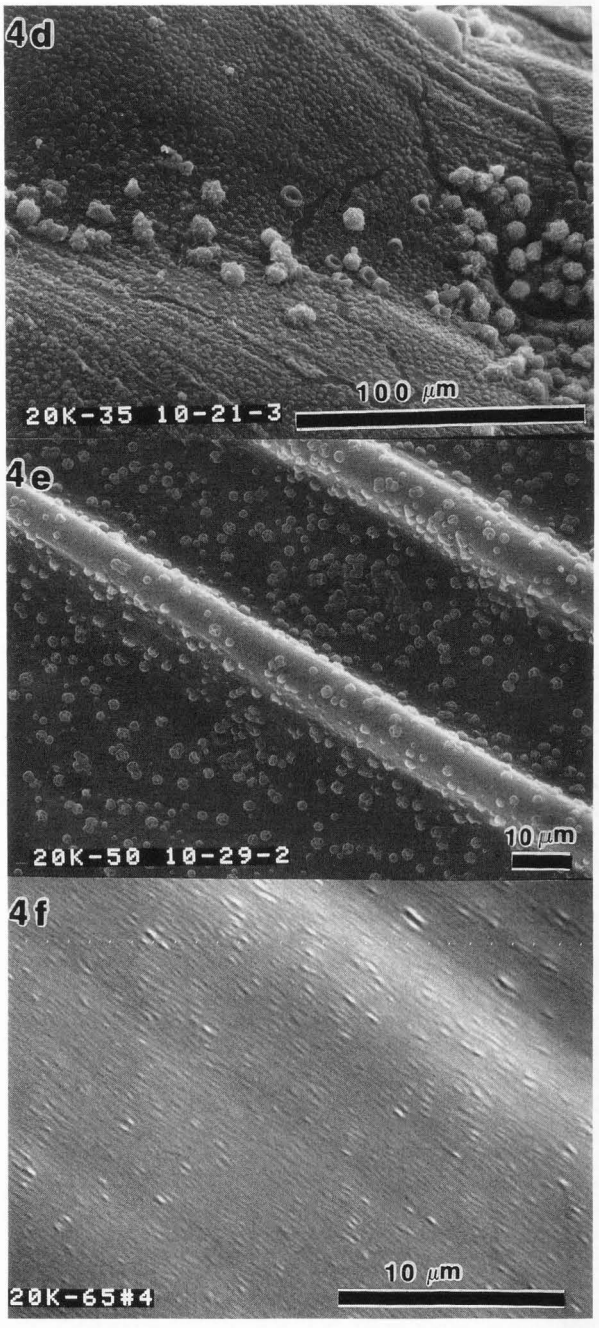
Critical Point Drying With Tannic Acid Pretreatment

Samples were fixed in 3% 0.1M sodium cacodylate buffered glutaraldehyde, then postfixed for 1h each in 0.1M sodium cacodylate buffered 1% osmium tetroxide and 1% tannic acid with buffer washes between fixations. Samples were dehydrated through a graded series of ethanols, placed in a Samdri 790 critical point drier and critical point dried using liquid carbon dioxide (Gamliel, 1985; Schroeter, et al., 1984).

Air Drying following Tannic Acid Treatment and HMDS Incubation

Samples went through primary fixation in 0.1M sodium cacodylate buffered 3% glutaraldehyde, then post- fixed for 1hr each in 0.1M sodium cacodylate buffered 1% osmium tetroxide and 1% tannic acid





with buffer washes between fixations. Samples were dehydrated through a graded series of ethanols, placed in HMDS for 10 minutes, and subsequently air dried (Adams, et al., 1987; Canby, et al., 1985; Nation, 1983)

Results

Shrinkage Study

During preliminary polymer coated guidewire studies, CPD was observed to produce significant hydrogel shrinkage, whereas pretreatment with TA, initially directed towards improved preservation of adsorbed cellular elements, was noted fortuitously, to reduce sample shrinkage (Figs. 3 a, b). In response to this observation, the diameter and thickness of 8K-50 hydrated discs was measured before and after one of three processing techniques described above. The shrinkage data are presented in Table 1. CPD produced a reduction in the original hydrated sample diameter and thickness by approximately 26 % and a volume reduction of 60 %. The addition of TA to the CPD protocol had little effect on sample diameter

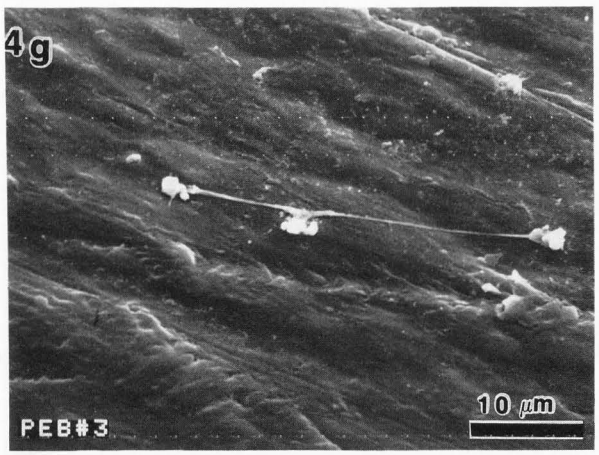


Figure 4a: Scanning electron micrographs of P(GMS/DMS) after one hour of blood exposure.

Figure 4b: Scanning electron micrograph of Gore-tex ® after 1 hr of blood exposure.

Figure 4c: Scanning electron micrograph of 2K-50 after one hour of blood exposure.

Figure 4d: Scanning electron micrograph of 20K-35 after one hour of blood exposure.

Figure 4e: Scanning electron micrograph of 20K-50 after one hour of blood exposure.

Figure 4f: Scanning electron micrograph of 20K-65 after one hour of blood exposure.

Figure 4g: Scanning electron micrograph of intramedic PE after one hour of blood exposure.

Table 2: Platelet Deposition On Polymer Coated Guidewires

Network Composition	Platelets/1000 μm^2
2K-35	5.10 ±2.12
2K-50	8.97 ± 1.81
8K-35	5.60 ± 2.37
8K-50	3.04 ± 1.00
8K-65	5.50 ± 1.42
20K-35	13.79 ± 4.46
20K-50	2.27 ± 0.47
20K-65	0.36 ± 0.11
P(GMS/DMS)	45.87 ± 17.06
Gore-Tex®	6568.97 ± 2244.76
Intramedic PE®	1.03 ± 0.39

Data is expressed as mean platelets/1000 μm^2 ± standard error

($p > 0.05$), but was associated with less shrinkage of sample thickness ($p < 0.01$). Sample fracturing, however, was observed and may have contributed to falsely elevated readings. Nevertheless, the TA step was retained and in order to further minimize dimensional changes, the effect of air drying was evaluated. Although fracturing was no longer noted, further improvement in the preservation of original sample size was not observed. The difference in measured thicknesses between the latter two techniques probably relates to the fracturing artifact discussed above.

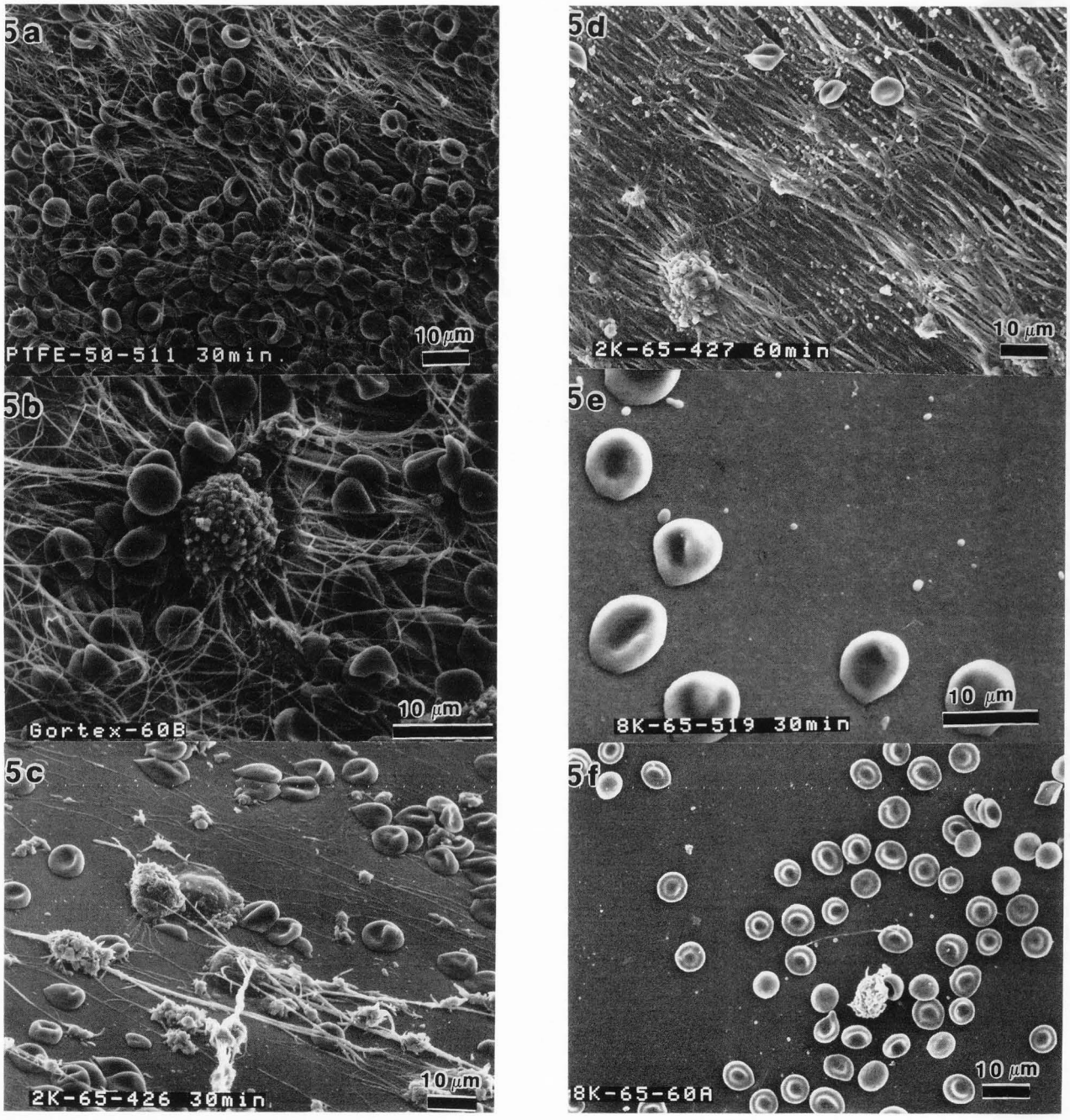
Biocompatibility Studies

Platelet deposition on coated guidewires is summarized in Table 2. Significantly lower levels of platelet deposition were demonstrated on all PEO/P(GMS/DMS) materials in comparison to Gore-tex® ($p < 0.001$). Formulations containing PEO, demonstrated platelet uptake that was 3 to 127 fold less when compared with pure P(GMS/DMS) ($p < 0.001$). Increasing the PEO content, at high PEO molecular weights (20,000), was associated with progressively lower levels of platelet deposition was

Table 1: Changes in 8K-50 Discs Diameter, Thickness, and Volume Following Sample Preparation

	Hydrated Sample	Critical Point Dried	Critical Point Dried/Tannic Acid	Air dried/ HMDS
	(n = 9)	(n = 3)	(n = 3)	(n = 3)
Sample Diameter / Dry Diameter	1.32 ± 0.02	0.98 ± 0.02	1.02 ± 0.01	1.04 ± 0.01
Sample Thickness / Dry Thickness	1.26 ± 0.08	0.87 ± 0.06	1.25 ± 0.06	1.08 ± 0.02
Sample Volume / Dry Volume	2.17 ± 0.11	0.83 ± 0.08	1.31 ± 0.07	1.16 ± 0.02

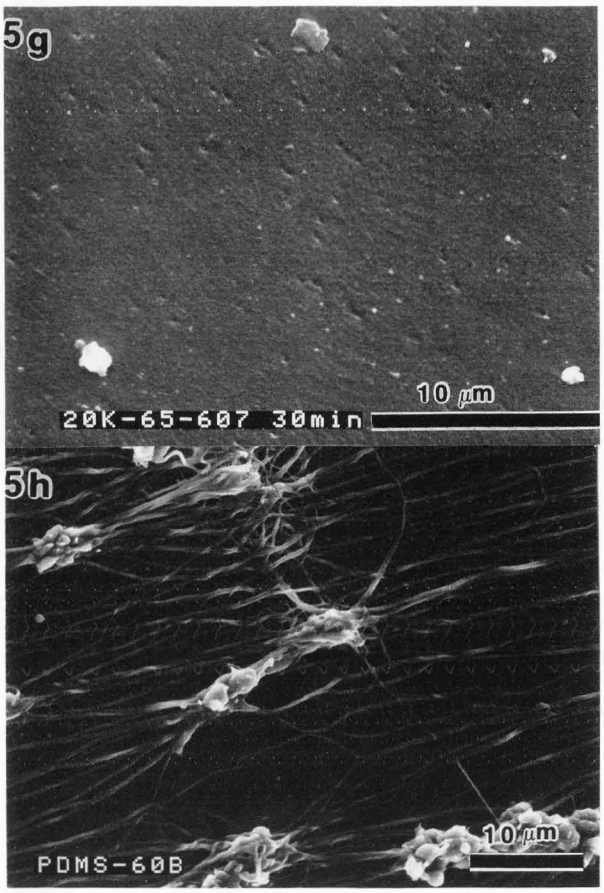
For comparative purposes, the data is presented in a dimensionless format. The results have been normalized with respect to the corresponding dimension of a pretreatment (vacuum dried) group.



found for a polymeric network (20K-65) composed of 65 weight percent PEO of molecular weight 20,000 and 35 weight percent P(GMS/DMS). When 20K-65 is compared to a commercially available smooth surface material, Intramedic Polyethylene®, a four fold difference exists, although this was not statistically significant ($p<0.1$).

SEM analysis confirmed the ^{111}In labeled platelet results. Significant levels of thrombus formation were noted on Gore-tex® and P(GMS/DMS) (Figs. 4 a, b). Activated platelets, white and red cells and proteinaceous strands are noted. The surface of 2K-50 networks was blanketed with platelets, but no morphological evidence of platelet activation was noted (Fig 4 c). Pseudopod formation, platelet flattening and surface spreading are absent. In PEO 20K networks, a noticeable reduction of platelet deposition is observed on increasing the PEO network content (Figs. 4 d, e, f). Again no platelet activation was observed. In contrast, activated platelets were seen on the surfaces of commercially available nonporous materials, such as Intramedic polyethylene® (Figs. 4 g).

Data from the ex vivo shunt phase of



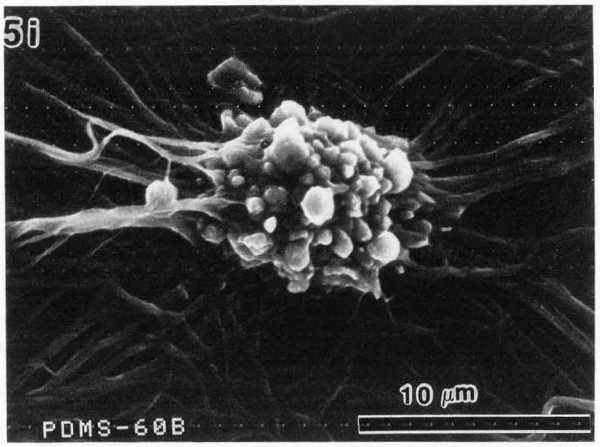


Figure 5a: Scanning electron micrograph of Gore-tex ® after 30 minutes of blood exposure.

Figure 5b: Scanning electron micrograph of Gore-tex® after 1 hour of blood exposure.

Figure 5c: Scanning electron micrograph of 2K-65 after 30 minutes of blood exposure.

Figure 5d: Scanning electron micrograph of 2K-65 after 60 minutes of blood exposure.

Figure 5e: Scanning electron micrograph of 8K-65 after 30 minutes of blood exposure.

Figure 5f: Scanning electron micrograph of 8K-65 after 60 minutes of blood exposure.

Figure 5g: Scanning electron micrograph of 20K-65 after 30 minutes of blood exposure.

Figure 5h. Scanning electron micrograph of PDMS after 60 minutes of blood exposure.

Figure 5i: A higher magnification scanning electron micrograph of PDMS after 60 minutes of blood exposure.

Table 3: Platelet Deposition In A Baboon Ex Vivo Shunt

Material	30 min	60 min
2K-65	23.21 ± 5.53	55.86 ±12.40
8K-65	6.35 ± 2.99	1.76 ± 0.31
20K-65	0.36 ± 0.10	0.60 ± 0.22
Gore-Tex ®	66.77 ± 12.6	6656.30±114.43
PDMS	9.39 ± 3.25	31.65 ± 8.24

Data is expressed as mean platelets/1000 μm^2 ± standard error

biomaterial testing is summarized in Table 3. Platelet deposition on PEO materials decreased as the molecular weight of PEO increased. At both time points, the low molecular weight PEO material, 2K-65, was statistically indistinguishable from PDMS, but significantly higher than the mid or high molecular weight PEO networks ($p < 0.05$). Platelet accumulation on the high molecular weight, 20K-65 network, remained at less than one platelet per 1000 μm^2 during the entire observation period and was significantly lower than PDMS after 60 minutes of blood contact ($p < 0.05$). Gore-tex® showed the greatest deposition of platelets. At 30 minutes, the deposition was seven times that of PDMS ($p < 0.05$) and at 60 minutes it had increased to 21 times the PDMS value ($p < 0.001$). When compared to the high molecular weight PEO network, 20K-65, the relative platelet depositions were 185 and 1094 times greater at 30 and 60 minutes, respectively ($p < 0.05$).

Additional insight into the patterns of platelet deposition was provided by SEM analysis. The formation of a fibrin network with red cells and individual platelets was noted on Gore-tex® after 30 minutes of blood contact (Fig. 5 a). By 60 minutes, well formed platelet aggregates were seen (Fig. 5 b).

At 30 minutes, scattered platelets, red cells and fibrin strands were seen on the surface of 2K-65 (Fig. 5 c) which progressed to a fibrin network with individual platelet aggregates following 60 minutes of blood exposure (Fig. 5 d). Individual red cells and platelets are seen on 8K-65 and very little is noted on 20K-65 at both time points (Figs 5 e, f, g, h). The surface of PDMS demonstrates features similar to those seen on 2K-65 after 60 minutes (Figs. 5 h, i).

Discussion

Hydrogels are hydrophilic polymeric materials. Their large volume fraction of water (> 30 % w/v) reduces surface frictional forces and adhesiveness, increases solute permeability, facilitates the design of compliant structures, and potentially improves tissue biocompatibility. Hydrogels have been used as contact lenses for nearly twenty-five years, (Tighe, 1987) and are actively being studied as artificial tendons (Migliaresi, et al., 1987), blood contacting surfaces, tissue substitutes and drug delivery vehicles (Peppas and Korsmeyer, 1987).

PEO hydrogels may have an important role in blood interfacing applications. A necessary component of biomaterial evaluation is the direct visualization of the biological-material interface. This has been particularly difficult with these materials because their high water content caused shrinkage and surface distortion during sample preparation. A number of processing modifications were observed to reduce sample shrinkage. Even though the effects were small, major sample disruption noted with CPD during the initial stages of this study, was reduced. Residual hydroxyl and epoxy groups are the only functional groups that may remain following the cure of this polymeric network.

The considerable shrinkage which followed primary fixation with glutaraldehyde, postfixation with OsO_4 and CPD precludes significant network cross-linking by these fixatives. However, it is tempting to speculate that the improvements following the addition of tannic acid may have been a result of an augmented reaction of OsO_4 with ethylene bonds in the PEO backbone (Hayat, 1970). Overall, we conclude that air drying with HMDS following tannic acid treatment is the procedure of choice for PEO hydrogels because of a reduction of bulk shrinkage, minimal surface fracturing, simplicity, and speed.

Blood compatibility testing included both the measurement of radiolabeled platelet deposition and surface visualization by scanning electron microscopy. In both guidewire and shunt studies, ^{111}In labeled platelet levels were highest on Gore-tex® and large thrombotic deposits were well visualized by SEM. Further guidewire tests suggested that biocompatibity, as judged by SEM and ^{111}In uptake, varied among different PEO formulations with 20K-65 demonstrating the lowest levels of platelet uptake. It was also notable that morphologically activated platelets were seen on polyethylene, but not on PEO coated surfaces. Evaluating the extent of surface activated platelets could only have been made by direct surface examination. Shunt studies demonstrated, in particular, that among PEO networks, materials of high PEO content and molecular weight had the lowest levels of platelet deposition and thus, appear to be highly thromboresistant.

In addition to the chemical and physical nature of the surface, the test configuration appeared to influence platelet deposition. Platelets were uniformly scattered over the surface of PEO coated guidewires, but were deposited as well localized aggregates on 2K-65 conduit surfaces, despite equivalent blood exposure periods. These differences may have been due to changes in the flow regime. The exact location of the guidewire in the IVC lumen is unknown, but if suspended centrally, shear rates would be lower than at the vessel wall and may have contributed to reduced platelet deposition and activation. Additionally, the continuous flow of platelets through the shunt circuit may have increased their level of activation and induced aggregate formation.

In conclusion, several PEO/polysiloxane network formulations demonstrate low platelet binding and activation; characteristics which are necessary for thromboresistance. Ultimately, the adequacy of this biomaterial in blood contacting applications will depend upon the results of future experimental work to geometry, surface characteristics, degradation behavior, implant size, and potential drug pretreatment in the patient population. Additional in vivo testing, including patency studies of small diameter vascular grafts, will need to be performed before considering these materials for use in clinical applications.

Acknowledgements

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Discussion with Reviewers

G. J. Stewart: Did washing in saline cause any clumping of baboon platelets? Please cite the reference for the platelet washing method or comment on validation of the method (clumping, function) if the method was developed by your group.

Reviewer III: With regard to the ^{111}In platelets the authors should establish that these platelets act as a tracer for normal platelets in terms of adhesion on these materials? Do the labeled platelets adhere the same as unlabeled? Perhaps they are de-activated with respect to adhesion on these gels.

Authors: Platelet isolation and labelling is associated with a 50 percent platelet recovery and 75 percent labeling efficiency. Typically, two hours postinjection, 85 percent of the label is cell associated and 15 percent is free in the plasma. The majority of the circulating cell-associated radioactivity is localized to the platelet fraction. Furthermore, aggregation was absent as evident by the relatively narrow distribution of the platelet peak on a continuous albumin gradient. Platelet function is not altered by the isolation, incubation or labeling techniques, when studied by either thrombin stimulated platelet release of ^{14}C serotonin or by morphological studies of electron dense body

distribution. Platelets treated with thrombin released virtually no ^{111}In (Vecchione, et al., 1980).

G. J. Stewart: In the biocompatibility testing were all three tests on each material from the same fabrication and were all done on the same animal? In how many animals was each material tested?
Authors: Each material was fabricated individually and every effort was made to distribute the three tests among different baboons within the laboratory colony. Each material was studied in triplicate.

G. J. Stewart: Were Gore-tex ® and PE tested as coatings for guidewires? If so, are Gore-tex® and PE coated guidewires commercially available or were they prepared in your laboratory? If the configuration of Gore-tex® and PE was not that of guidewires, would the difference be expected to influence blood element deposition?
Reviewer III: Since Gore-tex ® is a textured material one may ask what is the nature of the "area" used to calculate platelet density? Nominal area could of course be much less than true area making the reported data erroneously high. I do not believe it is meaningful to compare Gore-tex®, which is designed to trap cells and other elements from the circulating blood via microporosity, with smooth-surfaced materials. I would imagine most smooth materials would show much less platelet deposition than Gore-tex® without necessarily implying non-thrombogenicity.
Authors: Gore-tex® (W. L. Gore, Flagstaff AZ) and Intramedic Polyethylene® tubing were obtained from commercial suppliers. The tubing was passed over stainless steel guidewires for biomaterial testing. The surfaces of Intramedic Polyethylene® and the synthesized hydrogels were smooth. Gore-tex® has a textured surface. Its higher surface area could potentially augment platelet deposition. The magnitude of this effect, however, is difficult to characterize. We reported the data on Gore-tex®, so as to compare our experimental data with a representative commercial material.

E. Wintermantel: What could be done to quantify the SEM results? Would image processing and analysis systematically applied to the surfaces,.i.e. scanning a significant number of areas on the surface, be a valuable tool when correlated with the ^{111}In labeled platelet results?
Authors: Yes, but sample shrinkage would need to be corrected for and we do not have a system available to us.

Reviewer III: The word electrostatic seems inappropriate with respect to PEO since there is no charge on this molecule.
Authors: Three electrostatic interactions between a surface and adjacent proteins can be identified: 1) ionic interactions, due to ionization of the surface groups and/or specific adsorption of ions from the solution; 2) lewis acid-base or electron donor/acceptor interactions, which include hydrogen bonding as a subset; and 3) charge transfer effects, which are mainly due to π-π orbital interactions. Although ionic interactions and charge transfer effects do not participate in protein - PEO surface interactions, hydrogen bonding may occur between the acceptor oxygen in the PEO ether linkage and an appropriate donor group ($-NH_2$, -OH, etc.) on the surface of plasma proteins or cells.

Additional Reference

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